IN VITRO CLONAL PROPAGATION OF TWO PROMISING GLADIOLUS (*Gladiolus grandiflorus* L.) VARIETIES

BY

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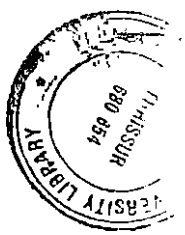

THESIS
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DECLARATION

I hereby declare that this thesis entitled **"*In vitro* clonal propagation of two promising gladiolus (*Gladiolus grandiflorus* L.) varieties"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled "***In vitro* clonal propagation of two promising gladiolus (*Gladiolus grandiflorus* L.) varieties"** is a record of research work done independently by Ms. Priyakumari. I. (99-12-11) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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CONTENTS

LIST OF TABLES

LIST OF TABLES Contd...

LIST OF FIGURES

LIST OF PLATES

LIST OF PLATES Contd...

LIST OF ABBREVIATIONS

AC	Activated charcoal
BA	Benzyl adenine
BAP	Benzylaminopurine
CD	Critical difference
CV	Cultivar
CW	Coconut water
GA3	Gibberellic acid
IAA	3- indole acetic acid
IBA	3- indole butyric acid
KIN	Kinetin
MS	Murashige and Skoog (1962)
NAA	1-napthyl acetic acid
pH	Per hydrogen
SH	Schenk and Hildebrandt (1972)
B5	Gamborg *et al.* (1968)
1/2 MS	Half strength Murashige and Skoog
2, 4 - D	2,4-dichlorophenoxy acetic acid

Introduction

1. INTRODUCTION

Gladiolus is one of the most important bulbous ornamental belonging to the family Iridaceae. Gladiolus is popular for its attractive spikes having florets of different forms, dazzling colours, varying size and long keeping quality. The magnificent inflorescence makes gladiolus suitable for use in herbaceous border, bedding and as pot plants. It is ideal for floral arrangements, for table and interior decoration as well as for making high quality bouquets.

In India, gladiolus has established itself as a commercial proportion with an area of about 6000 ha. This flower crop has got much export potential also. It occupies fifth place in the international cut flower trade (Sharma and Sharga, 1994).

In Kerala, cultivation of gladiolus is limited by their low rate of multiplication as well as by the severe problem of corm rot during storage. The crop has dormancy of four to five months. Further it can be cultivated during a single season only. Availability of sufficient quality planting material at reasonable price and in time is the other major problem.

Evolving protocols for the *in vitro* propagation of gladiolus varieties becomes relevant in this context. *In vitro* propagation is the only viable technology that enables rapid mass multiplication of novel cultivars on a commercial scale. There are several reports pertaining to *in vitro* propagation of different gladiolus varieties (Ziv *et al.*, 1970; Hussey, 1977; Bajaj *et al.*, 1983; Hussain, 1995; Misra and Singh, 1999; Pathania *et al.*, 2001).

The protocol for *in vitro* propagation cannot be applied uniformly to all the varieties. Difference in response to *in vitro* culture is noticed from variety to variety. Peach Blossom and Tropic Seas are two varieties found suitable for cut flower production in the southern region of Kerala (KAU, 1996). The present study was undertaken with the objective of evolving protocols for the *in vitro* propagation of these promising gladiolus varieties.

Review of Literature

2. REVIEW OF LITERATURE

The conventional methods of asexual propagation of tropical ornamental plants are inadequate to meet the increasing demand of quality planting materials. *In vitro* propagation has proved to be a better alternative to the conventional vegetative propagation methods. Morel (1960) was the pioneer in clonal multiplication through *in vitro* techniques. He successfully cloned the orchid *Cymbidium* through meristem culture. Since then *in vitro* clonal multiplication of the ornamental plants has gained momentum.

There are three possible routes available for *in vitro* propagule multiplication (Murashige, 1974) *viz.*, (a) enhanced release of axillary buds (b) production of adventitious shoots through organogenesis and (c) somatic embryogenesis. Primary meristem like shoot tips and axillary buds are mainly used as explants for the enhanced release of axillary buds. 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).

Several studies have been conducted in the rapid *in vitro* propagation of *Gladiolus* spp. This review highlights the research on the various techniques of *in vitro* propagation of gladiolus with special emphasis on enhanced release of axillary buds.

2.1 Enhanced release of axillary buds

Axillary and apical shoots contain quiescent or active meristems depending on the physiological state of the plant. Due to apical dominance only a limited number of axillary meristems can develop into shoots. Since the mechanism of apical dominance has been demonstrated to be under control of various growth regulators, (Phillips, 1975) the proportion of these substances in the media can be so manipulated as to induce each meristem to regenerate a shoot in cultures.

Cytokinin is utilized to overcome the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils, there by inducing axillary shoot proliferation. This enhanced release of axillary buds with cytokinins was discovered by Wickson and Thimann (1958).

Shoot tips cultured on a basal medium containing no growth regulators typically develop into single seedling–like shoots with strong apical dominance. On the contrary, when the shoots of the same plant material are grown on culture media containing cytokinin, axillary shoots develop precociously which proliferate to form clusters of secondary and tertiary shoots (Pierik, 1989). These clusters can further be subdivided into smaller clumps of shoots, which will form similar clusters when subcultured on a fresh medium. About 5-10 multiplication rates can be achieved on a regular 4-8 week micropropagation cycle and can thus lead to rapid clonal propagation levels in the range of 0.1-3.0 x 10^6 within one year (Mantell *et al.*, 1985).

Proliferating shoots of gladiolus was maintained for 11 years with no apparent physiological deterioration (Hussey, 1977). In general, the technique of proliferation by axillary shoots is applicable to any plant that produces regular axillary shoots and responds to an available cytokinin (Hussey, 1983).

Factors influencing *in vitro* clonal propagation through enhanced release of axillary buds

The key factors that influence *in vitro* clonal propagation include the explant, culture medium, plant growth substances, media supplements added, mode of culture and culture conditions.

2.1.1 Explant

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

Clonal propagation *via* enhanced release of axillary buds was successful in many bulbous ornamental plants belonging to the family Iridaceae and Liliaceae (Hussey, 1976a and 1976b). In gladiolus, Hussey (1977) reported precocious shoot formation using axillary buds from corms as explant material. Apical buds from corm (Takatsu, 1982; Lilien-Kipnis and Kochba, 1987; Ziv, 1989) and cormel (Lilien-Kipnis and Kochba, 1987) and axillary buds from corm (Ziv, 1979; Takatsu, 1982) were also reported as explants. The buds obtained from corm produced the highest frequency of shoots compared to other explants (Zakutsakaya and Murin, 1990; Rumynin *et al.*, 1990; Ziv, 1990; Steinitz *et al.*, 1991; De Bruyn and Ferreira, 1992).

Cormel shoot tip was identified as excellent explant in many economically important varieties (Rao *et al.*, 1991; Nagaraju and Parthasarathy, 1995; Misra and Singh, 1999; Pathania *et al.*, 2001).

Dehusked intact cormels were also tried as explant for the successful *in vitro* regeneration of plantlets. (Nagaraju and Parthasarathy, 1995; Remotti and Loffler, 1995; Kumar *et al.*, 1999).

Apart from *in vivo* raised cormels, *in vitro* raised cormlets, cormlet-tips and axillary buds were also effective for axillary bud proliferation (Bajaj *et al.*, 1983; Remotti and Loffler, 1995).

2.1.2 Culture media

Growth and morphogenesis of plant tissues *in vitro* are largely governed by the composition of the culture media. A proper tissue culture medium should contain not only adequate quantity of major plant nutrients like salts of nitrogen, phosphorous, potassium, calcium, magnesium and sulphur and micronutrients like salts of iron, manganese, zinc, boron, copper, molybdenum and cobalt but also carbohydrates, usually sucrose, less weight organic compounds like vitamins, amino acids and plant growth regulators. In some cases, complex organic additives like yeast extract, coconut milk, casein hydrolysate, corn milk, malt extract and tomato juice are used to support plant tissue growth. Murashige and Skoog's (1962) medium developed for tobacco had been used for *in vitro* culture of plant species (Bonga, 1980).

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.

Hussey (1977) used half strength MS medium in which iron was added as ferrous ethylene diamine sulphate (25.00 mg l^{-1}) for the *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. Alper *et al.* (1985) successfully obtained virus-free plantlets from meristems of gladiolus cultivars cultured on half strength MS medium.

MS medium as the ideal medium for *in vitro* propagation of gladiolus was also reported by various workers (Sutton, 1978; Logan and Zettler, 1985; Dickens *et al.*, 1986; Lilien-Kipnis and Kochba, 1987; Dantu and Bhojwani, 1987; Rao *et al.*, 1991; Gosal and Grewal, 1991; Steinitz *et al.*, 1991; De Bruyn and Ferreira, 1992; Prasad *et al.*, 1993, Hussain, 1995; Misra and Singh, 1999; Babu and Chawla, 2000; Pathania *et al.*, 2001).

According to Ziv (1989), agitated liquid media containing growth retardants were effective for bud proliferation in gladiolus. Liquid shake cultures supplemented with adequate cytokinin and high sugar concentration produced early and rapid corm regeneration in *in vitro* raised shoots (Steinitz *et al.*, 1991).

2.1.3 Plant growth substances

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. Shoots were induced when cytokinin level was higher than the

auxin. Increasing auxin level relatively over cytokinin level induced roots. Intermediate concentrations of these produced unorganized 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 the effectiveness of the cytokinin varied with the plant species and type of growth regulator used.

The role of exogenous auxin in culture proliferation medium is to nullify the suppressive effect of high cytokinin concentrations on axillary shoot elongation thereby resorting normal shoot growth in apple shoot cultures (Lundergan and Janick, 1980).

The use of auxins (NAA, IBA, IAA and 2,4-D) and cytokinins (kinetin and BAP) have been reported in gladiolus for *in vitro* culture (Hussey, 1976 a and b; Ziv, 1979; Konoshima, 1980; Bajaj *et al.*, 1983; Dickens *et al.*, 1986; Lilien-Kipnis and Kochba, 1987; Rao *et al.*, 1991; Steinitz *et al.*, 1991; Gosal and Grewal, 1991; De Bruyn and Ferreira, 1992; Prasad *et al.*, 1993; Hussain, 1995; Nagaraju and Parthasarathy, 1995; Misra and Singh, 1999; Anandhi and Sekhar, 2000; Pathania *et al.*, 2001).

Arora and Grewal (1990) reported the influence of GA on shoot elongation. The addition of GA3 caused earlier sprouting and faster increase in sprout length than BAP alone in the culture establishment medium of cvs. Eurovision and Wild Roses (Pathania *et al.*, 2001).

The effects of growth retardants on shoot proliferation and morphogenesis in liquid cultured gladiolus plants were also reported (Ziv, 1990 and 1991).

In cv. American Beauty, BAP at 3.00 mg l^{-1} added to basal medium took 8.4 days for explant establishment, produced more number (4.40) of initial shoots and earliest (14.20 days) to shoot elongation. BAP 1.00 mg l^{-1} + NAA 0.50 mg l^{-1} was found optimum for further shoot multiplication (Misra and Singh, 1999).

The absence of growth substances in the basal media induced long single shoots with roots (Nagaraju and Parthasarathy, 1995; Nagaraju *et al.*, 1998).

2.1.4 Carbon source

Plant cells and tissues in the culture medium lack autotrophic ability and therefore need external carbon for energy. The addition of an external carbon source to the medium enhances proliferation of cells and regeneration of green shoots. The most preferred carbon source in plant tissue culture is sucrose. It also acts as an osmoticum that can stimulate and regulate morphogenesis (Wethrell, 1984).

Sucrose 3.00 per cent was best for shoot proliferation than glucose 3.00 per cent in *Anthurium andreanum* var Dragon's Tongue (Thomas, 1996).

In gladiolus, growth of all parts of the plant benefited from high sugar concentration (Steinitz *et al.*, 1991). De Bruyn and Ferreira (1992) obtained best shoot production at 15 ^{0}C in *Gladiolus tristis* in a medium with high sucrose (6.00 and 9.00 per cent) content. MS medium supplemented with 3.00 per cent sucrose was optimum for *in vitro* culture of gladiolus

(Hussain, 1995; Misra and Singh, 1999; Pathania *et al.*, 2001). In cvs. Her Majesty and Aldebaran, growth and proliferation of shoots increased at higher sucrose concentrations (8.00, 10.00 and 12.00 per cent) (Kumar *et al.*, 1999).

2.1.5 Other media supplements

Culture media are often supplemented with a variety of complex organic extracts having constituents of an undefined nature. These organic additives influence the growth of *in vitro* cultures. Adenine, adenine sulphate, casein hydrolysate, yeast extract, peptones, coconut water, ground banana, orange juice, grape juice, activated charcoal etc. are some of the complex substances added to the media.

In gladiolus, the beneficial effects of coconut water on cormel shoot tip explants (Simonsen and Hildebrandt, 1971) and for anther culture (Bajaj *et al.*, 1983) have been reported.

2.1.6 Amino acids

Cultured tissues are normally capable of synthesizing the amino acids necessary for various metabolic processes. The addition of amino acids to media is important for stimulating cell growth in cultures. Unlike inorganic nitrogen, amino acids are taken up more rapidly by plant cells. Casein hydrolysate (0.05-0.1 per cent), *L.* glutamine (8 mmol l^{-1}), *L.* asparagine (100 mmol l^{-1}), *L.* glycine (2 mmol l^{-1}), *L.* arginine and *L.* cysteine (10 mmol l^{-1}) are common sources of organic nitrogen used in culture media (Razdan, 1993).

2.1.7 Solidifying agents

Gelling or solidifying agents are commonly used for preparing semi-solid or solid tissue culture media. They support the tissues growing in static

conditions. A change in the agar concentration affects the nutrients in it as well as the overall nutrient concentration in the experiment. Whether explants grow better on agar or other supporting agents depend on the tissue and the species (Razdan, 1993).

Agar and gelrite are the commonly used solidifying agents for the micropropagation of gladiolus. Agar 0.80 per cent was beneficial in many cultivars (Steinitz *et al.*, 1991; Jager *et al.*, 1998; Nagaraju *et al.*, 2000; Anandhi and Sekhar, 2000). Agar concentration of 0.70 per cent was reported for the *in vitro* propagation of cv. American Beauty (Misra and Singh, 1999).

Gelrite 0.20 per cent was also found optimum for *in vitro* propagation of various gladiolus cvs. (De Bruyn and Ferreira, 1992; Stefaniak, 1994; Pathania *et al.*, 2001). Remotti and Loffler (1995) used 0.25 per cent gelrite for the *in vitro* plant regeneration of cv Peter Pears.

2.1.8 Culture conditions

Murashige (1974 and 1977) observed that light intensity, quality and duration affect the growth of *in vitro* cultures. 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. Yeoman (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. Light had significantly influenced organogenesis of gladiolus under *in vitro* culture.

Ziv (1979) reported 24 ^{0}C day temperature and 20 ^{0}C night temperature and a light intensity of 10 W/M^2 during initial culture period and 28 W/M^2 during hardening stage as ideal. The light was provided by mixture of grow lux WS fluorescent and incandescent lamp for 14 hours of photoperiods. Buds excised from the corms of gladiolus were cultured at 22-25^{0}C temperature and 400-600 lux and then newly developed buds were incubated at 1000 lux (Rumynin *et al.*, 1990). A room temperature requirement of 15-24 ^{0}C for the *in vitro* clonal propagation of gladiolus was reported by De Bruyn and Ferreira (1992). Misra and Singh (1999) reported a room temperature of 25±2^{0}C and 1.5K lux light intensity for 16h and 8h of dark period.

2.2 Somatic organogenesis

Somatic organogenesis can be direct or callus mediated (Evans *et al.*, 1981). This is useful in inducing genetic variability or to recover pre-existing natural genetic variability. In direct somatic organogenesis, adventitious shoots arise directly from the tissues of the explant followed by root formation. Indirect somatic organogenesis requires the re-determination of the differentiated cells leading to callus formation. Separate shoot and root initials are characteristically formed in callus cultures (George and Sherrington, 1984).

Ziv *et al.* (1970) observed direct organogenesis on inflorescence stem tissue when the explants cultured initially on high NAA medium were subdivided and subcultured to media with 0.50 or 1.00 mg l^{-1} kinetin. The polarity of the inflorescence stem explant affected organogenesis (Ziv *et al.*, 1970; Bajaj *et al.*, 1983). Adventitious buds have been induced on inflorescence stem explants, and both axillary and adventitious buds can be

induced on small axillary bud or shoot tip explants. Small dormant corms cut transversely developed a large number of buds at the cut edge of the upper half of the corm (Sutter, 1986). Successful regeneration from gladiolus callus has been reported when *in vitro* grown cormels or whole intact plants were used as explant source (Kim *et al.*, 1988; Kamo, 1994; Stefaniak, 1994). The rate of regeneration depends on the combination of growth regulators in the medium and on the cultivar.

Explants from inflorescence stem and the shoot tip produced callus within 1-3 weeks on media with 5.00 mg l^{-1} or 10.00 mg l^{-1} NAA and 0.50 mg l^{-1} kinetin (Ziv *et al.*, 1970; Wilfret, 1971; Bajaj *et al.*, 1983). Induction of callus was made possible from inflorescence stem, bracts and flower stalks (Bajaj *et al.*, 1983) of gladiolus. Best callus production was induced on MS medium with high levels of NAA or 2,4-D.

Explants from the corm, leaf, stem and ovary wall tissue also produced callus in the presence of 2,4-D alone (Hussey, 1975; Kim *et al.*, 1988; Li and Wang, 1989).

Plantlets have been successfully regenerated from callus obtained from meristem tips, buds or the inflorescence stem. Shoots differentiate in the presence of KIN, whereas roots develop in the presence of NAA. When both are added to the medium, roots always precede shoot formation (Ziv *et al.*, 1970).

Callus mediated organogenesis and subsequent plantlet regeneration was obtained from roots of gladiolus cv. Friendship raised under *in vitro* and *in vivo* conditions (Hussain *et al.*, 1994).

Misuk *et al.* (1998) induced callus from cormel tissues of gladiolus cv. Topaz on MS medium supplemented with 10.00 mg l^{-1} 2,4-D. Adventitious bud formation from calluses occurred on media supplemented with low levels of BA (0.01 – 0.10 mg l^{-1}) or without BA.

Simonsen and Hildebrandt (1971) found that MS medium with 23.00 μM kinetin induced the best callus and that agar-solidified medium was superior to liquid medium. Wilfret (1971) proliferated callus aggregates in liquid medium in the presence of NAA as long as the cultures were agitated.

Normal plantlets were regenerated from the cryopreserved callus (treated in cryoprotectant for 30 minutes at 0^0C and then preserved in liquid nitrogen at -196^0C) within 20-25 days of culture period (Li and Wang, 1989).

2.3 Somatic embryogenesis

The application of somatic embryogenesis for crop improvement has been reported first by Sharp *et al.* (1982). Somatic embryogenesis is the development of embryos from somatic cells (Mascarenhas, 1989).

Direct embryogenesis proceeds from the pre-embryogenically determined cells, while indirect embryogenesis requires the re-determination of differentiated cells, callus proliferation and differentiation of embryogenic determined cells (Kato and Takeuchi, 1966).

Level of growth regulators in the culture medium particularly lower levels of auxin was ideal for embryo formation or somatic embryogenesis (Skoog and Miller, 1957; Hussey, 1986). It required an auxin medium for the induction of embryo and medium devoid of growth regulator for embryogenesis (Ammirato, 1983).

Kamo *et al.* (1990) successfully induced compact non-friable callus from inflorescence stalks of greenhouse grown plants of gladiolus cvs. Blue Isle and Hunting Song in MS medium containing NAA 53.60 µM, which could be regenerated into plantlets. Friable embryogenic callus and somatic embryos of cultivars Blue Isle, Jenny Lee, Peter Pears and Rosa Supreme were cultured by Stefaniak (1994). She used MS medium with various concentration of auxin and corm slices, young leaf base and whole intact plantlets as explants. Successful regeneration of plantlets was also obtained on MS hormone-free medium.

2.4 *In vitro* rooting

The plantlets produced *in vitro* should have a strong and functional root system. There are three stages for rhizogenesis, *viz.*, induction, initiation and elongation.

2.4.1 Plant growth substances

Among auxins, IBA and NAA are most effective for root induction (Ancora *et al.*, 1981). NAA concentration affected the number, length and morphology of roots developing on subcultured plantlets of gladiolus. Root number increased with rising concentration of NAA up to an optimum, whereas root length diminished with rising NAA levels (Lilien-Kipnis and Kochba, 1987).

Incorporation of NAA at 0.50 mg l^{-1} in the hardening off medium stimulated the root production (Dickens *et al.*, 1986) and produced maximum root number (Hussain, 1995) in gladiolus. NAA 0.50 mg l^{-1} proved to be the best for rooting in cv. American Beauty (Misra and Singh, 1999). A high

concentration of NAA (4.00 mg l^{-1}) gave highest root regeneration in cv. Friendship (Anandhi and Sekhar, 2000).

MS medium containing IBA 4.00 mg l^{-1} was best for rooting of separated shoots (Arora and Grewal, 1990). IBA 1.00 mg l^{-1} resulted in high rate of root production when light was excluded (Hussain, 1995). MS medium supplemented with IBA 4.90 μM was used for rooting of shoots of cvs. American Beauty and Yellow Topaz (Babu and Chawla, 2000).

The combination of two different auxins such as NAA 0.50 mg l^{-1} and IBA 0.50 mg l^{-1} was found effective for *in vitro* rooting of gladiolus cultivars (Dohare, 1991).

The deleterious effect of high cytokinin concentration as well as residual cytokinin in suppressing the root induction, even after transferring the shoots to cytokinin free medium was reported in *in vitro* cultures (Lo *et al.*, 1980; Ancora *et al.*, 1981; Yeoman, 1986). Hussey (1976a and 1977) reported inhibition of root growth in gladiolus when BAP concentration increased above 0.12 mg l^{-1}. However, cytokinins at low concentration promoted rooting of shoots. MS media supplemented with IAA 2.00 mg l^{-1} along with kinetin 0.50 mg l^{-1} enhanced rooting of shoots derived from axillary buds and cormel tips (Bajaj *et al.*, 1983).

Roots along with shoots were formed from callus in the MS medium supplemented with kinetin 1.00 mg l^{-1}, sucrose 2.00-3.00 per cent and agar 0.50-1.00 per cent on 30th day under culture (Kim *et al.*, 1988). Root regeneration from callus occurred within seven days in the presence of growth

regulators like BAP and kinetin at lower levels and within 10 days in the absence of growth regulators in the medium (Kim *et al.*, 1991).

2.4.2 Basal media

Half strength MS medium supplemented with NAA 0.50 mg l^{-1} and sucrose 15.00 g l^{-1} affected rooting of elongated shoots of gladiolus (Ziv, 1979). Profuse and quick rooting occurred in filter paper bridges in liquid media as compared to agar-solidified media (Arora and Grewal, 1990).

2.4.3 Other media supplements and culture conditions

Improved root growth was observed under high light intensities when 0.30 per cent activated charcoal (AC) was added to the medium (Ziv, 1979). The inhibition of root growth under rising levels of auxin was not observed in the presence of AC (Lilien-Kipnis and Kochba, 1987). The survival rate of transplanted plantlets also enhanced on AC containing media. Activated charcoal at 5.00 g l^{-1} promoted rooting in cv. Bellarina, with the production of long thin white roots and secondary roots (Nagaraju *et al.*, 1998).

Higher sucrose concentrations (8.00, 10.00 and 12.00 per cent) in the medium increased the number of roots in cvs. Her Majesty and Aldebaran (Kumar *et al.*, 1999).

2.5 Hardening and planting out

The environment in a tissue culture container is that of very high humidity, low light levels and usually a constant temperature. Leaves or shoots or plantlets leaving the environment are as a result, very poorly adapted to resist the low relative humidity, high light levels and more variable temperature found *in vivo* (Wainwright, 1988).

Physiologically the leaves grown *in vitro* are incapable of significant photosynthesis. The stomata are unable to close and as cuticular wax in the leaf surface is minimal, are unable to control water loss. Improper development of vascular connections between the shoots and roots may cause poor establishment of the plantlets (Langford and Wainwright, 1987).

Light, temperature and relative humidity are the major factors to be controlled during acclimatization. A period of humidity acclimatization is required for newly transferred plantlets (Hu and Wang, 1983). These methods of controlling relative humidity are polythene tent, misting and fogging. Standardized rhizosphere environment is also necessary for settling better growth of plantlets (Zimmerman and Fordham, 1985). In polythene tent, as the aerial environment is closed, it is possible to take advantage of CO_2 enrichment during hardening (Lakso *et al.*, 1986).

Direct transplanting of regenerated plants to non-aseptic conditions without prior hardening resulted in poor survival (Ziv *et al.*, 1970). Ziv (1979) transferred the *in vitro* proliferated shoots into a pre-transplanting medium with half MS salt mixture supplemented with 0.50 mg l^{-1} NAA and 0.30 per cent AC and cultured under high light intensities. The plantlets continued their growth and developed longer leaves, without signs of senescence and subsequently produced non-dormant cormels after eight weeks.

Under a low temperature of 17^0C during the period of acclimatization, 100 per cent survival rate of plantlets was achieved. At 27^0C the survival rate was zero (Lilien-Kipnis and Kochba, 1987).

Hussain (1995) obtained 50 per cent survival rate of *in vitro* plantlets, treated with Carbendazim (0.10 %) for five minutes and transferred to mud pots containing coarse sand. Post planting treatment with 0.10 MS salt solution on alternate days and drenching with Triazol (Triadimefon) 20.00 mg l^{-1} at three days interval and an improvised mist chamber for high relative humidity were also provided.

Rao *et al.* (1991) successfully transferred the *in vitro* raised plantlets in pots containing FYM, sand and soil (2: 1: 1, v/v). Using the same planting media, Misra and Singh (1999) achieved 60 per cent survival rate after 15 days of transplanting.

Successful hardening of *in vitro* plantlets in sand: soil (2: 1) mix was obtained by Jager *et al.* (1998).

The survival rate (90.00 per cent) of the rooted plantlets produced on high sucrose (8.00 per cent) medium was better than that of (40.00 per cent) plants produced on normal sucrose concentrations of 3.00 per cent (Kumar *et al.*, 1999).

Materials and Methods

3. MATERIALS AND METHODS

Investigations were carried out at Department of Pomology and Floriculture and Plant Molecular Biology and Biotechnology Centre, College of Agriculture, Vellayani with the objective of evolving protocols for the *in vitro* propagation of two promising varieties of gladiolus during 2000-2001.

The materials and methods tried for the micropropagation *via* enhanced release of axillary buds are described in this chapter.

3.1 Varieties

Two promising gladiolus varieties found suitable for the southern region of Kerala *viz.*, Peach Blossom and Tropic Seas were tried for the study.

3.2 Explant

Cormels of both the cultivars were used as explant source.

3.2.1 Cormel

Cormels are miniature corms arising in the region between mother and daughter corms, attached at the base of the daughter corm with short stolons. These vary in size, each one enclosed in a hard shell or tunica which is less permeable to water, prolonging dormancy.

3.3 Collection and preparation

The cormels were collected from the gladiolus plants raised in the field of Department of Pomology and Floriculture.

Corms and cormels were harvested after the whole plant had dried up. They were lifted from the soil and washed free of soil and dirt particles and dried under shade. After proper drying they were treated with 0.4 per cent Mancozeb (Indofil M 45) for 30 minutes and again dried under shade. The fungicide treated dry cormels were stored in moisture free place with proper ventilation at room temperature.

Cormels were dehusked and only healthy cormels of uniform size (0.8 – 1.2 cm) without malformations or necrotic spots were selected for *in vitro* culture. They were immersed in 1000 times diluted 'Labolene' solution for 30 minutes, washed thoroughly in running tap water for five minutes and then in glass distilled water. They were kept in open beakers with proper aeration until surface sterilization and inoculation were carried out.

3.4 Surface sterilization

Surface sterilization of the cormels was carried out inside a laminar air flow chamber just before inoculation. The cormels were transferred to a sterilized beaker and surface sterilized with 0.08 per cent mercuric chloride for 10 minutes with intermittent shaking. The solution was drained and the cormels were washed four to five times with sterile distilled water. The cormels were transferred carefully into a sterile petri plate.

3.5 Inoculation and incubation

All the inoculation operations were carried out inside a laminar air flow chamber (Klenzaids, model 1104).

The vessels and tools (beakers, petri plates, blades, forceps etc.) required for inoculation were washed thoroughly, rinsed with glass distilled water, covered air tight with aluminium foil and autoclaved at 121^0C temperature and 1.06 $kgcm^{-2}$ pressure for 45 minutes. They were further flame sterilized just before inoculation using a spirit lamp inside the laminar air flow chamber. To inoculate the explants on the culture medium, the cotton plugs of the culture vessels were removed and the mouth was flamed. The cormels were inoculated into the medium using sterile forceps. The mouth of the culture vessels was flamed again and cotton plugs were replaced.

The cultures were then transferred to the culture rooms provided with light or darkness at 26 ± 1^0C. Subculturing was carried out as and when required.

3.6 Media

The basal media used for the study were MS (Murashige and Skoog, 1962), B5 (Gamborg *et al.*, 1968) and SH (Schenk and Hildebrandt, 1972). The chemicals used for the preparation of the culture media were of analytical grade obtained from British Drug House (Bombay), Sisco Research Laboratory (Bombay) and Merck (Bombay).

Standard procedures were followed for the preparation of media (Thorpe, 1980). Stock solutions of major and minor nutrients were prepared by dissolving the required quantity of chemicals in specified volume of

double glass-distilled water. Plant growth substances were first dissolved in dilute acid/alcohol and volume made up with double glass-distilled water. The stock solutions were stored under refrigerated condition (+4^{0}C).

The culture vessels used were 'Borosil' brand test tubes (25 x 150 mm) and Erlenmeyer flasks (100 ml). They were washed with 1000 times diluted 'Labolene' and tap water, rinsed with glass-distilled water and kept overnight in a hot air oven (60^{0}C) for drying and pre-sterilization.

All items of glassware and vessels used for the preparation of culture media were washed thoroughly in 1000 times diluted 'Labolene' and tap water and rinsed with glass-distilled water. Specific quantities of stock solutions were pipetted out into a 1000 ml beaker. Sucrose and myo-inositol were added fresh and dissolved. For specific treatment requirements, glucose, glycine and arginine were added fresh. Coconut water (CW) when used was collected from freshly harvested tender coconut (eight months old). The volume was made up to 1000 ml using glass-distilled water. The pH of the medium was adjusted between 5.6 and 5.8 using 0.1 N NaOH or 0.1 N HCl with the aid of an electronic pH meter (Global Electronic, model DPH 500). Agar (in the case of solid medium) was then added to the medium and stirred thoroughly.

For the preparation of SH and B5 media, the chemicals were taken in required quantity and dissolved in glass distilled water. Growth regulators, sucrose and inositol were added fresh and the volume was made up to 1000 ml.

The medium was heated by placing the vessel on a heating mantle with constant stirring using a glass rod till the agar melted. Activated charcoal (AC) when used in the medium was added at this stage and stirred well for uniform distribution. The medium was then poured into the pre-sterilized culture vessels at the rate of 15 ml in the case of test tubes, and 40 ml in the case of Erlenmeyer flasks. The mouth of the culture vessels were plugged tightly with sterilized cotton, covered with aluminium foil or paper, labelled and autoclaved at 121^0C temperature and 1.06 $kgcm^{-2}$ pressure for 20 minutes. After sterilization, the culture vessels were transferred to the culture room.

3.7 Enhanced release of axillary buds

3.7.1 Culture establishment

Establishment of both the varieties was subjected to different treatments for culture establishment. The treatments involved different levels of cytokinins, *viz.*, BA and kinetin (1.00 mg l^{-1}, 2.00 mg l^{-1} and 4.00 mg l^{-1}) alone and in combination with auxins, *viz.*, NAA (0.10 mg l^{-1} and 0.50 mg l^{-1}) and IAA (1.00 mg l^{-1} and 2.00 mg l^{-1}) (Table 1). The treatments were replicated three times for each variety.

Observations were recorded on the number of surviving cultures (%), number of days for bud initiation and number of shoots per culture after four weeks of culturing.

Table 1 Treatments tried to assess the effect of plant growth substances on culture establishment of cormels of gladiolus varieties

Medium : MS + inositol 100.00 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 8.00 g l^{-1}.

Treatment No.	Plant growth substances (mg l^{-1})
CEA1	BA 0.00 + NAA 0.10
CEA2	BA 0.00 + NAA 0.50
CEA3	BA 0.00 + IAA 1.00
CEA4	BA 0.00 + IAA 2.00
CEB1	BA 1.00
CEB2	BA 1.00 + NAA 0.10
CEB3	BA 1.00 + NAA 0.50
CEB4	BA 1.00 + IAA 1.00
CEB5	BA 1.00 + IAA 2.00
CEB6	BA 2.00
CEB7	BA 2.00 + NAA 0.10
CEB8	BA 2.00 + NAA 0.50
CEB9	BA 2.00 + IAA 1.00
CEB10	BA 2.00 + IAA 2.00
CEB11	BA 4.00
CEB12	BA 4.00 + NAA 0.10
CEB13	BA 4.00 + NAA 0.50
CEB14	BA 4.00 + IAA 1.00
CEB15	BA 4.00 + IAA 2.00
CEK1	Kinetin 1.00
CEK2	Kinetin 1.00 + NAA 0.10
CEK3	Kinetin 1.00 + NAA 0.50
CEK4	Kinetin 1.00 + IAA 1.00
CEK5	Kinetin 1.00 + IAA 2.00
CEK6	Kinetin 2.00
CEK7	Kinetin 2.00 + NAA 0.10
CEK8	Kinetin 2.00 + NAA 0.50
CEK9	Kinetin 2.00 + IAA 1.00
CEK10	Kinetin 2.00 + IAA 2.00
CEK11	Kinetin 4.00
CEK12	Kinetin 4.00 + NAA 0.10
CEK13	Kinetin 4.00 + NAA 0.50
CEK14	Kinetin 4.00 + IAA 1.00
CEK 15	Kinetin 4.00 + IAA 2.00
CEG1	GA3 1.00
CEG2	GA3 2.00

3.7.2 Shoot proliferation

The elongated shoots and roots of sprouted cormels from culture establishment medium were cut off using sterile blade. A longitudinal cut was given exactly through the middle of the apical bud portion. The cut halves of cormel tips were transferred to shoot proliferation medium. The basal medium initially tried was full MS. Different combinations of cytokinins and auxins were tried. Of these, the best treatment combination was tried for the standardization of other media components.

For the different shoot proliferation treatments except for the treatments involving plant growth substances, multiple auxillary bud aggregates (6-8 buds) were used.

The number of cultures survived (%), number of shoots per culture and length of the longest shoot were recorded after six weeks of culturing.

3.7.2.1 Plant growth substances

The effects of plant growth substances like cytokinins, *viz.*, BA and kinetin (1.00 mg l^{-1}, 2.00 mg l^{-1} and 4.00 mg l^{-1}) alone and in combination with auxins, *viz.*, NAA (0.10 mg l^{-1} and 0.50 mg l^{-1}) and IAA (1.00 mg l^{-1} and 2.00 mg l^{-1}) were studied by incorporating them in shoot proliferation medium (Table 2).

3.7.2.2 Basal media

The basal medium used for multiple shoot proliferation was full MS. The best treatment involving plant growth substances in full MS medium was later tried in Schenk and Hildebrandt (SH) and Gamborg *et al.* (B5) basal medium to compare their effect on shoot proliferation (Table 3).

Table 2 Treatments tried to assess the effect of plant growth substances on multiple shoot proliferation from cormel tips of gladiolus varieties.

Medium : MS + inositol 100.00 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 8.00 g l^{-1}

Treatment No.	Plant growth substances (mg l^{-1})
MSA1	BA 0.00 + NAA 0.10
MSA2	BA 0.00 + NAA 0.50
MSA3	BA 0.00 + IAA 1.00
MSA4	BA 0.00 + IAA 2.00
MSB1	BA 1.00
MSB2	BA 1.00 + NAA 0.10
MSB3	BA 1.00 + NAA 0.50
MSB4	BA 1.00 + IAA 1.00
MSB5	BA 1.00 + IAA 2.00
MSB6	BA 2.00
MSB7	BA 2.00 + NAA 0.10
MSB8	BA 2.00 + NAA 0.50
MSB9	BA 2.00 + IAA 1.00
MSB10	BA 2.00 + IAA 2.00
MSB11	BA 4.00
MSB12	BA 4.00 + NAA 0.10
MSB13	BA 4.00 + NAA 0.50
MSB14	BA 4.00 + IAA 1.00
MSB15	BA 4.00 + IAA 2.00
MSK1	Kinetin 1.00
MSK2	Kinetin 1.00 + NAA 0.10
MSK3	Kinetin 1.00 + NAA 0.50
MSK4	Kinetin 1.00 + IAA 1.00
MSK5	Kinetin 1.00 + IAA 2.00
MSK6	Kinetin 2.00
MSK7	Kinetin 2.00 + NAA 0.10
MSK8	Kinetin 2.00 + NAA 0.50
MSK9	Kinetin 2.00 + IAA 1.00
MSK10	Kinetin 2.00 + IAA 2.00
MSK11	Kinetin 4.00
MSK12	Kinetin 4.00 + NAA 0.10
MSK13	Kinetin 4.00 + NAA 0.50
MSK14	Kinetin 4.00 + IAA 1.00
MSK15	Kinetin 4.00 + IAA 2.00

Table 3 Treatments tried to assess the effect of basal media on shoot proliferation in gladiolus varieties

Medium : As given in Appendix 1

Treatment No.	Basal media
SMS	MS
SSH	SH
SB5	B5

3.7.2.3 Strength of MS basal medium

Different strength of MS medium (full, ¾, ½, ¼) was tried for shoot proliferation (Table 4).

Table 4 Treatments tried to assess the effect of MS basal media on shoot proliferation in gladiolus varieties

Medium : MS minor salts + inositol 100.00 mg l^{-1} + sucrose 30.00 g l^{-1} + BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1} + agar 8.00 g l^{-1}

Treatment No.	MS major stock solution (ml l^{-1})
MS1	Stock A 25.00 + Stock B 5.00
MS2	Stock A 18.75 + Stock B 3.75
MS3	Stock A 12.50 + Stock B 2.50
MS4	Stock A 6.25 + Stock B 1.25

3.7.2.4 Mode of culture

Liquid MS medium as well as solid MS medium were tried in order to assess the effect of mode of culture on multiple shoot proliferation (Table 5).

Table 5 Treatments tried to assess the effect of mode of culture on multiple shoot proliferation in gladiolus varieties

Medium : MS + inositol 100.00 mg l^{-1} + sucrose 30.00 g l^{-1} + BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1}

Treatment No.	Mode of culture
SS	Solid (Agar 8.00 mg l^{-1})
SL	Liquid (No agar)

3.7.2.5 Carbon source

Sucrose and glucose at different levels (10.00, 20.00, 30.00, 40.00 g l^{-1}) were tried to study their effect on shoot proliferation (Table 6).

Table 6 Treatments tried to assess the effect of carbon source on shoot proliferation in gladiolus varieties

Medium : MS + inositol 100.00 mg l^{-1} + BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1} + agar 8.00 g l^{-1}

Treatment No.	Carbon source (g l^{-1})
SPS1	Sucrose 10.00
SPS2	Sucrose 20.00
SPS3	Sucrose 30.00
SPS4	Sucrose 40.00
SPG1	Glucose 10.00
SPG2	Glucose 20.00
SPG3	Glucose 30.00
SPG4	Glucose 40.00

3.7.2.6 Other media supplements

Studies were conducted to find out the effect of different media supplements on shoot proliferation. Three treatments each were tried to assess the effect of aminoacids (glycine and arginine, Table 7), three for coconut water (Table 8) and three for activated charcoal (Table 9).

Table 7 Treatments tried to assess the effect of aminoacids (glycine and arginine) on shoot proliferation in gladiolus varieties

Medium : MS (*without glycine*) + *inositol* 100.00 mg l^{-1} + *sucrose* 30.00 g l^{-1} + BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1} + agar 8.00 g l^{-1}

Treatment No.	Amino acids (mg l^{-1})
SGL0	Glycine 0.00
SGL1	Glycine 25.00
SGL2	Glycine 50.00
SGL3	Glycine 100.00
SAR1	Arginine 25.00
SAR2	Arginine 50.00
SAR3	Arginine 100.00

Table 8 *Treatments tried to assess the effect of coconut water on shoot proliferation in gladiolus varieties*

Medium : MS + inositol 100.00 mg l^{-1} + sucrose 30.00 g l^{1} + BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1} + agar 8.00 g l^{-1}

Treatment No.	Coconut water (ml l^{-1})
SCW1	50.00
SCW2	100.00
SCW3	200.00

Table 9 Treatments tried to assess the effect of activated charcoal on shoot proliferation in gladiolus varieties

Medium : MS + inositol 100.00 mg l^{-1} + sucrose 30.00 g l^{-1} + BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1} + agar 8.00 g l^{-1}

Treatment No.	Activated charcoal (g l^{-1})
SAC1	0.50
SAC2	1.00
SAC3	2.00

3.7.2.7 Solidifying agent

Different levels of agar were tried to study their effect on shoot proliferation (Table 10).

Table 10 Treatments tried to assess the effect of agar on shoot proliferation in gladiolus varieties

Medium : MS + inositol 100.00 mg l^{-1} + sucrose 30.00 g l^{-1} + BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1}

Treatment No.	Agar (g l^{-1})
SAG1	5.00
SAG2	6.00
SAG3	7.00

3.7.2.8 Culture conditions

The cultures were kept in light or in darkness in order to assess the effect of light on multiple shoot proliferation. Light (3000 lux, 16 hours photoperiod) was provided using cool white fluorescent tubes. Darkness was provided by keeping the cultures in a temperature controlled darkroom (26 ± 2^0C).

3.8.1 *In vitro* rooting

Trials on the *in vitro* rooting were conducted in full MS medium. Individual shoots measuring 2.50-3.50 cm length excised from the shoot proliferating cultures were used as explants.

Thirteen treatments were tried to assess the effect of the auxins (IBA, NAA, IAA and 2,4-D) on rooting (Table 11). The effect of carbon sources was assessed with six treatments by providing different levels of sucrose and glucose in the basal medium (Table 12).

Three to seven replications were provided for the treatments. Observations on number of days for root initiation, number of roots, root length and abnormalities in root growth, if any, were recorded four weeks after culturing.

Table 11 Treatments tried to assess the effect of auxins on *in vitro* rooting in gladiolus varieties

Medium : MS + inositol 100.00 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 8.00 g l^{-1}

Treatment No.	Auxin (mg l^{-1})
R1	IBA 0.50
R2	IBA 1.00
R3	IBA 2.00
R4	NAA 0.50
R5	NAA 1.00
R6	NAA 2.00
R7	IAA 0.50
R8	IAA 1.00
R9	IAA 2.00
R10	2,4-D 0.10
R11	2,4-D 0.20
R12	2,4-D 0.40
R13	2,4-D 0.80

Table 12 Treatments tried to assess the effect of carbon sources on *in vitro* rooting in gladiolus varieties

Medium : MS + inositol 100.00 mg l^{-1} + agar 8.00 g l^{-1} + IBA 2.00 mg l^{-1} (Peach Blossom)

MS + inositol 100.00 mg l^{-1} + agar 8.00 g l^{-1} + IAA 2.00 mg l^{-1} (Tropic Seas)

Treatment No.	Carbon source (mg l^{-1})
RS1	Sucrose 2.00
RS2	Sucrose 3.00
RS3	Sucrose 4.00
RG1	Glucose 2.00
RG2	Glucose 3.00
RG3	Glucose 4.00

3.8.2 *Ex vitro* rooting

The elongated shoots with 2-4 leaves and 10-13 cm length were subjected to treatments with IBA 50 ppm and 100 ppm overnight. The treated plantlets were planted out in a suitable medium. The percentage survival of plantlets was recorded after two weeks and four weeks.

3.9 Planting out and acclimatization

The cotton plugs 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 carefully from culture vessels with the help of forceps. The agar adhering to the roots were completely removed by thorough washing with running tap water.

The plantlets were treated with Carbendazim (Bavistin 50 WP), 0.10 % solution for five minutes before planting out. The effects of media on the

growth of plantlets were tried by providing different media (sand, soilrite and sand : soil in the ratio 2 : 1) (Table 13) which were sterilized before the planting of plantlets.

Post-planting treatment with 0.1 MS on every three days was given. The plantlets were kept inside a polythene tent, where mist spraying of water was done twice a day, to maintain humidity.

Observations were made on percent plantlets survived after fifteen days.

Table 13 Treatments tried to assess the effect of media on the survival of *in vitro* raised plantlets of gladiolus varieties

Container : Plastic pots

Treatment No.	Media
GSD	Sand
GSR	Soilrite
GSS	Sand : Soil (2 : 1)

3.10 Statistical analysis

Completely randomised design (CRD) was followed for statistical analysis wherever necessary as per Panse and Sukhathme (1985).

Results

4. RESULTS

The results of the trials conducted on the *in vitro* clonal propagation of gladiolus varieties Peach Blossom and Tropic Seas are presented in this chapter.

4.1 Enhanced release of axillary buds

4.1.1 Culture establishment

The response of both the varieties Peach Blossom and Tropic Seas were good in the initial culture establishment medium. Survival rates of 79.41 per cent and 80.39 per cent were obtained for Peach Blossom and Tropic Seas, respectively. With respect to bud initiation in the medium, 100 per cent of the survived cultures of both the varieties showed positive response.

4.1.1.1 Plant growth substances

The culture establishment trials were carried out using cormels as explant in MS medium supplemented with cytokinins (BA and kinetin) alone and in combination with auxins (NAA and IAA). The effect of GA3 in breaking dormancy was also tried. The results are shown in Table 14.

Thirty-six treatments were tried to assess the effect of plant growth substances on culture establishment of gladiolus varieties. Significant difference was noticed among these treatments. The average number of days for bud initiation varied from 3.67 to 35.00 in Peach Blossom and 4.00 to 32.33 in Tropic Seas. The minimum number of days for bud initiation in Peach Blossom (3.67) was recorded in the treatment CEK7 with kinetin 2.00 mg l^{-1} +

NAA 0.10 mg l^{-1}. This treatment was on par with CEK14, CEK8, CEK6, CEB1, CEB12, CEK9, CEK15, CEB15, CEK10, CEB5, CEB10, CEK1, CEK4 and CEB11 (Plate 1) (Table 14). The maximum number of days was recorded in CEA4 (35.00) and was found on par with CEA2, control and CEK11. In the variety Tropic Seas, the earliest bud initiation (4.00) was observed in the treatment CEK7 (Plate 2) with kinetin 2.00 mg l^{-1} + NAA 0.10 mg l^{-1}. This was found to be on par with treatments *viz.*, CEK9, CEK10, CEB12, CEK8, CEK3, CEK6, CEB1, CEK14, CEK4, CEB7, CEB5, CEK2, CEK13, CEB10, CEK5, CEK1, CEK15, CEG1 and CEB15. The maximum number of days (32.33) for bud initiation was noticed in control. This was found to be on par with CEA2, CEA4, CEK11 and CEA1.

With respect to the number of shoots produced in Peach Blossom, the treatment CEB13 (BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1}) (Plate 3) produced highest number of shoots (3.67) and was superior to all other treatments. In Tropic Seas three treatments (CEB11, CEB13 (Plate 4) and CEK13) produced highest number of shoots (1.67) and was on par with CEB8 and CEK6 (1.33).

4.1.2 Multiple shoot proliferation

In the second stage i.e., multiple shoot proliferation, the longitudinally cut cormel halves of both the varieties were subjected to various treatments of plant growth substances. Multiple bud aggregates were further used for the shoot proliferation treatments involving different concentration of basal media, sucrose, amino acids, coconut water, activated charcoal, solidifying agent and culture conditions.

Table 14 Effect of plant growth substances on culture establishment of gladiolus varieties

Culture period : 4 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Days for bud initiation	Shoots per culture	Survival (%)	Days for bud initiation	Shoots per culture
CEA1	100.00	23.00	1.00	66.67	26.00	1.00
CEA2	100.00	33.00	1.00	66.67	32.00	1.00
CEA3	100.00	19.67	1.00	100.00	23.33	1.00
CEA4	100.00	35.00	1.00	100.00	31.33	1.00
CEB1	100.00	7.00	1.00	33.33	9.00	1.00
CEB2	100.00	17.00	1.00	100.00	15.00	1.00
CEB3	100.00	12.33	1.00	100.00	15.33	1.00
CEB4	100.00	12.00	1.00	100.00	15.67	1.00
CEB5	66.67	8.33	1.33	100.00	9.33	1.00
CEB6	66.67	20.67	1.00	66.67	21.67	1.00
CEB7	100.00	10.67	1.67	66.67	9.33	1.00
CEB8	100.00	19.33	1.33	66.67	20.00	1.33
CEB9	66.67	13.33	1.00	100.00	14.00	1.00
CEB10	66.67	9.00	1.00	66.67	10.67	1.00
CEB11	100.00	10.00	1.00	100.00	12.33	1.67
CEB12	66.67	7.00	1.00	100.00	7.67	1.00
CEB13	33.33	10.33	3.67	100.00	14.00	1.67
CEB14	100.00	17.33	1.00	100.00	17.67	1.00
CEB15	66.67	8.00	1.00	66.67	11.00	1.00

Table 14 Contd...

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Days for bud initiation	Shoots per culture	Survival (%)	Days for bud initiation	Shoots per culture
CEK1	100.00	9.33	1.00	33.33	11.00	1.00
CEK2	66.67	15.00	1.00	66.67	10.33	1.00
CEK3	100.00	11.33	1.00	100.00	8.00	1.00
CEK4	66.67	9.33	1.00	100.00	9.00	1.00
CEK5	66.67	14.00	1.00	66.67	10.67	1.00
CEK6	100.00	6.00	1.00	100.00	8.33	1.33
CEK7	33.33	3.67	1.00	33.33	4.00	1.00
CEK8	33.33	5.67	1.00	66.67	8.00	1.00
CEK9	66.67	7.67	1.00	66.67	5.00	1.00
CEK10	33.33	8.00	1.00	66.67	5.67	1.00
CEK11	100.00	30.00	1.00	66.67	31.00	1.00
CEK12	66.67	16.33	1.00	33.33	12.33	1.00
CEK13	100.00	13.33	1.00	66.67	10.33	1.67
CEK14	66.67	4.33	1.00	100.00	9.00	1.00
CEK15	66.67	7.67	1.00	100.00	11.00	1.00
CEG1	66.67	12.00	1.00	100.00	11.00	1.00
CEG2	100.00	16.33	1.00	100.00	17.00	1.00
Control	100.00	32.00	1.00	100.00	32.33	1.00
F	-	11.34*	2.79*	-	7.57*	1.18*
CD (0.05)	-	6.35	0.77	-	7.50	0.51

◈Treatment combinations are given in Table 1.

The data represents mean value of three replications

* Significant at 5 % level

Plate 1. **Cormel of the cv. Peach Blossom showing bud initiation in the culture establishment medium supplemented with BA 4.00 mg l^{-1}**

Plate 2. **Cormel of the cv. Tropic Seas showing bud initiation in the culture establishment medium supplemented with kinetin 2.00 mg l^{-1} + NAA 0.10 mg l^{-1}**

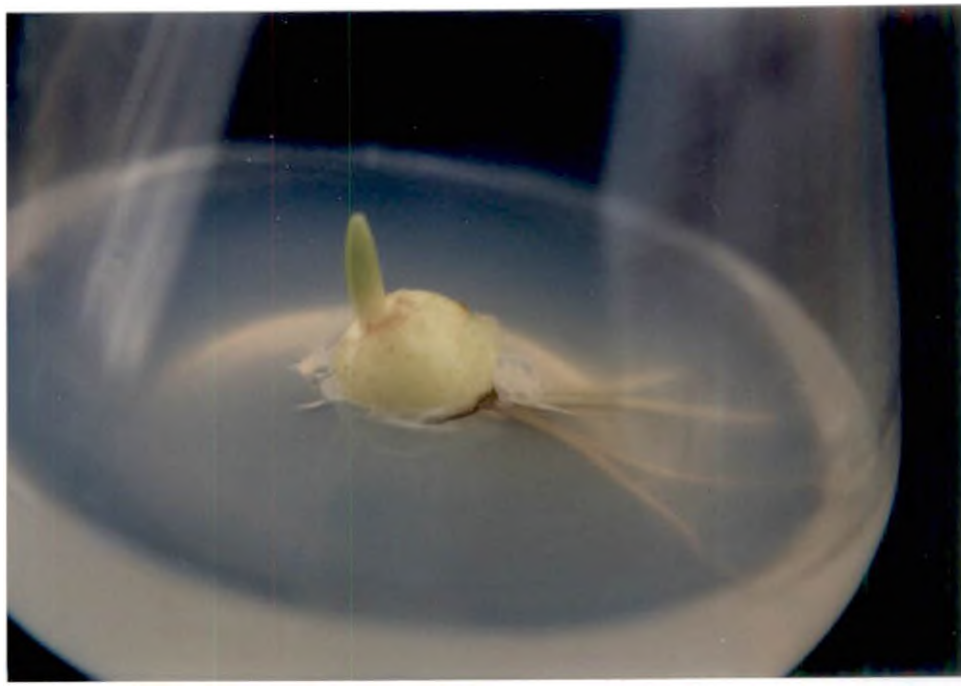
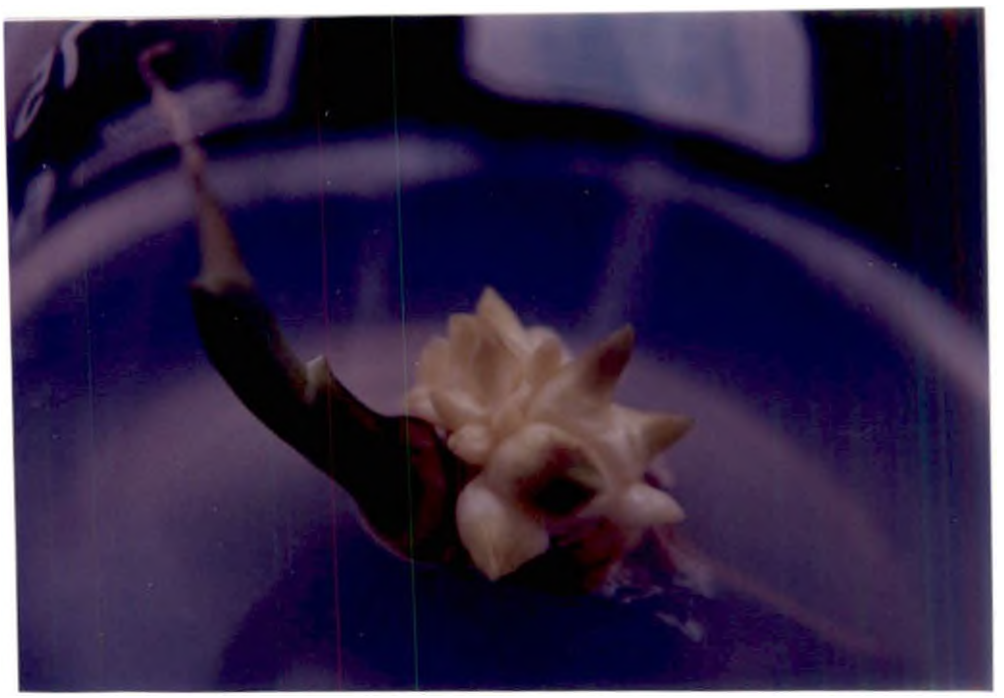


Plate 3. Cormel of the cv. Peach Blossom showing multiple shoot initiation in the culture establishment medium supplemented with BA 4.00 mg l^{-1} + NAA 0.50 mg 1^{-1}

Plate 4. Cormel of the cv. Tropic Seas showing multiple shoot initiation in the culture establishment medium supplemented with BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1}



4.1.2.1 Plant growth substances

All the cultures responded well and showed growth and multiple shoot production. The result of the treatments involving plant growth substances is presented in Table 15a and Table 15b.

The earliest bud initiation (2.00 days) in Peach Blossom was observed in the treatment MSB9 (BA 2.00 mg l^{-1} + IAA 1.00 mg l^{-1}) (Plate 5). This was followed by treatments MSB15, MSK15, MSB13, MSB12 and MSB14 (Table 15a). The utmost number of days (15.00) for bud initiation were noticed in the treatment MSB4. In the variety Tropic Seas, the treatment MSK15 recorded earliest shoot proliferation (2.33 days). This was followed by the treatments MSB15, MSK13, MSK7, MSB14, MSB10, MSB6 (Plate 6) and MSB13. The highest number of days for bud initiation was recorded in control (17.33), which was on par with treatments MSB4 (15.00) and MSA1 (13.33).

Significant difference was noticed among the treatments with respect to the number of shoots (Table 15b) in both the varieties. The highest number of shoots (33.67) having length greater than 0.10 cm was obtained in treatment MSB13 (BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1}) for the variety Peach Blossom. This was on par with MSB15 (BA 4.00 mg l^{-1} +IAA 2.00 mg l^{-1}), which recorded an average of 27.00 shoots. The next best response was noticed in MSB14 with an average of 26.33 shoots. The treatments MSB11 and MSB12 (Plate 7) produced same number of shoots (14.33). The highest shoot number in Tropic Seas was also obtained in MSB13 with an average of 27.67 shoots. Similarly, as in Peach Blossom the treatment MSB13 was on

Table 15a Effect of plant growth substances on multiple shoot proliferation of gladiolus varieties

◈Treatment	Peach Blossom		Tropic Seas	
	Survival (%)	Days for bud initiation	Survival (%)	Days for bud initiation
MSA1	100.00	13.00	33.33	13.33
MSA2	100.00	12.00	100.00	11.33
MSA3	100.00	8.67	66.67	4.67
MSA4	66.67	8.00	100.00	7.33
MSB1	33.33	9.67	100.00	5.00
MSB2	66.67	5.00	100.00	4.67
MSB3	100.00	9.00	66.67	8.33
MSB4	66.67	15.00	66.67	15.00
MSB5	100.00	11.00	100.00	12.33
MSB6	100.00	8.33	100.00	4.00
MSB7	100.00	5.33	100.00	6.00
MSB8	100.00	9.00	100.00	8.00
MSB9	100.00	2.00	66.67	9.00
MSB10	100.00	5.00	66.67	3.33
MSB11	33.33	7.33	100.00	7.67
MSB12	100.00	3.67	100.00	9.67
MSB13	66.67	3.33	100.00	4.00
MSB14	100.00	4.00	33.30	3.33
MSB15	66.67	2.67	100.00	2.67

Table 15a Contd...

◈Treatment	Peach Blossom		Tropic Seas	
	Survival (%)	Days for bud initiation	Survival (%)	Days for bud initiation
MSK1	66.67	10.33	100.00	5.00
MSK2	66.67	7.00	100.00	4.67
MSK3	33.33	6.00	100.00	6.00
MSK4	100.00	9.67	100.00	7.67
MSK5	66.67	4.00	33.33	4.33
MSK6	66.67	5.00	100.00	4.33
MSK7	66.67	4.00	100.00	3.33
MSK8	33.33	5.00	100.00	6.00
MSK9	33.33	6.00	66.67	9.33
MSK10	33.33	4.00	33.33	4.33
MSK11	33.33	4.67	100.00	4.33
MSK12	33.33	10.33	66.67	7.33
MSK13	66.67	6.67	100.00	3.00
MSK14	66.67	5.67	100.00	5.00
MSK15	100.00	3.00	100.00	2.33
Control	100.00	14.33	100.00	17.33
F	-	2.95*	-	3.31*
CD (0.05)	-	5.27	-	4.93

◈Treatment combinations are given in Table 2.

The data represents mean value of three replications

* Significant at 5 % level

Plate 5. **Multiple shoots formed *via* enhanced release of axillary buds from the cormel tips of cv. Peach Blossom, 3 weeks after culture in multiple shoot proliferation medium supplemented with BA 2.00 mg l^{-1} + IAA 1.00 mg l^{-1}**

Plate 6. **Multiple shoots formed *via* enhanced release of axillary buds from the cormel tips of cv. Tropic Seas, 3 weeks after culture in multiple shoot proliferation medium supplemented with BA 2.00 mg l^{-1}**

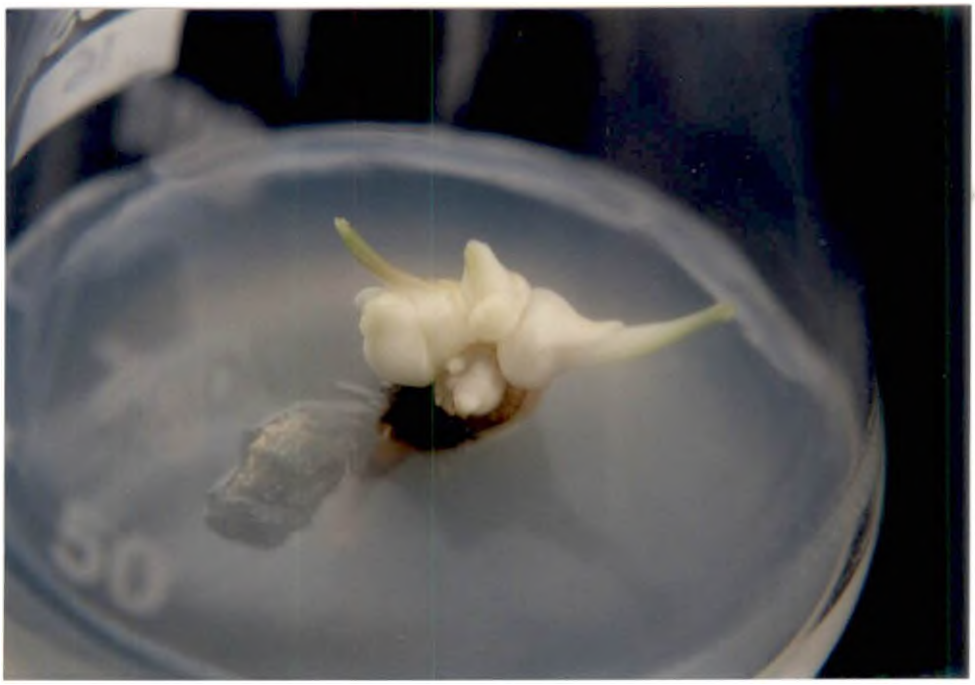


Table 15b Effect of plant growth substances on multiple shoot proliferation of gladiolus varieties

◈Treatment	Peach Blossom		Tropic Seas	
	Shoots per culture	Length of longest shoot (cm)	Shoots per culture	Length of longest shoot (cm)
MSA1	1.00	17.67	1.00	21.67
MSA2	1.00	19.63	1.33	19.33
MSA3	1.67	20.60	1.00	19.67
MSA4	1.00	19.43	1.00	14.67
MSB1	1.00	4.47	4.33	0.60
MSB2	4.33	6.17	2.33	6.83
MSB3	4.33	2.10	8.00	2.26
MSB4	2.67	5.83	2.67	8.33
MSB5	7.33	3.83	9.00	3.00
MSB6	1.33	2.67	5.00	2.53
MSB7	4.00	3.17	2.67	2.03
MSB8	13.33	2.77	11.33	0.40
MSB9	8.67	2.70	10.33	2.33
MSB10	8.33	0.60	17.33	1.07
MSB11	14.33	0.30	13.67	1.27
MSB12	14.33	3.93	15.00	0.43
MSB13	33.67	0.17	27.67	1.27
MSB14	26.33	1.10	19.67	0.37
MSB15	27.00	0.20	22.33	1.83

Table 15b Contd...

◈Treatment	Peach Blossom		Tropic Seas	
	Shoots per culture	Length of longest shoot (cm)	Shoots per culture	Length of longest shoot (cm)
MSK1	1.33	1.70	3.00	4.63
MSK2	2.33	9.27	2.00	6.17
MSK3	1.33	6.00	2.67	5.87
MSK4	2.00	3.60	4.00	3.80
MSK5	7.33	4.30	6.33	0.37
MSK6	8.00	1.40	6.67	5.07
MSK7	8.67	2.60	12.67	2.50
MSK8	7.00	8.70	3.67	0.57
MSK9	8.33	1.93	7.00	2.27
MSK10	7.33	5.67	6.33	3.20
MSK11	7.00	2.07	10.00	1.83
MSK12	5.33	5.33	3.67	0.53
MSK13	8.00	3.80	7.67	1.90
MSK14	6.33	2.27	9.67	1.33
MSK15	2.00	2.47	2.67	1.50
Control	1.00	11.23	1.00	22.67
F	10.03*	12.32*	12.57*	15.39*
CD (0.05)	6.97	4.55	5.23	4.13

◈Treatment combinations are given in Table 2.

The data represents mean value of three replications

* Significant at 5 % level

par with MSB15 (22.33 shoots). This treatment was followed by MSB14 (19.67), MSB10 (17.33) (Plate 8) and MSB12 (15.00).

With regard to shoot length, the longer shoots were produced in treatments with auxin alone. In Peach Blossom longest shoot (20.60 cm) was observed in treatment MSA3 followed by MSA2 (19.63 cm), MSA4 (19.43 cm), MSA1 (17.67 cm) and control (11.23 cm). The shortest shoot was observed in treatment MSB13 (0.17 cm). The treatments MSB15 (0.20 cm), MSB11 (0.30 cm) and MSB10 (0.60 cm) also produced very small shoots. The longest shoot in Tropic Seas was noticed in control (22.67 cm). This was found to be on par with MSA1 (21.67 cm), MSA3 (19.67 cm) and MSA2 (19.33 cm).

4.1.2.2 Basal media

There was significant difference among the different basal media tried for shoot proliferation in both the varieties. MS media was superior to SH and B5 media (Table 16). The treatment SMS produced an average number of 21.86 shoots in Peach Blossom. SSH and SB5 were found to be on par, with 12.29 and 15.00 number of shoots respectively. For the variety Tropic Seas also, highest shoot number was recorded in SMS (22.28) (Plate 9). The other two treatments SB5 and SSH found to be on par, produced 16.14 and 15.43 shoot numbers, respectively.

With regard to shoot length the opposite trend was observed in both the varieties (Table 16). In Peach Blossom, the longest shoot (1.41 cm) was noticed in SB5, which was on par with SSH (1.24 cm). The shoots produced in SMS were very small in size with an average size of 0.16 cm. In Tropic Seas also, similar response was noticed (Plate 10). Here also longest shoot

Plate 7. Multiple shoot proliferation from the cormel tips of cv. Peach Blossom, 6 weeks after culture in multiple shoot proliferation medium supplemented with BA 4.00 mg l^{-1} + NAA 0.10 mg l^{-1}

Plate 8. Multiple shoot proliferation from the cormel tips of cv. Tropic Seas, 6 weeks after culture in multiple shoot proliferation medium supplemented with BA 2.00 mg l^{-1} + IAA 2.00 mg l^{-1}

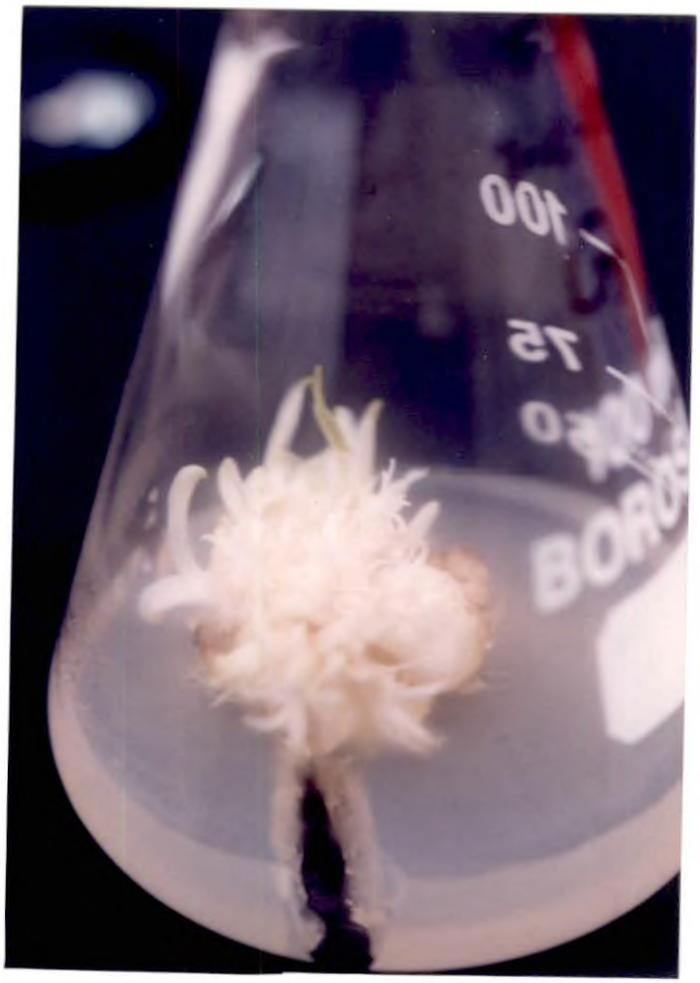

100
75

Plate 9. Multiple shoot production in cv. Tropic Seas on MS basal medium

Plate 10. Multiple shoot production in cv. Tropic Seas on B5 basal medium

was obtained in SB5 (1.26 cm) followed by SSH (1.23 cm). The shortest shoot (0.19 cm) was recorded in SMS.

In the cv. Peach Blossom, in one of the cultures supplemented with B5 medium, abnormal occurrence of pigmented tissues was noticed (Plate 11). These tissues did not survive on further subculture to the same media.

4.1.2.3 Strength of MS basal media

The effect of four different levels of MS major nutrients on multiple shoot proliferation was tried (Table 17). Significant difference was noted among the four different treatments in both the varieties.

The highest number of shoots produced (28.80) in Peach Blossom was recorded in the treatment MS4 (full MS), which was found superior to other treatments. The lowest number of shoots (12.20) was produced in MS1, which was on par with MS2 and MS3 with 13.60 and 15.20 shoots, respectively. The highest number of shoots (26.80) obtained for Tropic Seas was in the treatment MS4. An average number of 18.60 and 16.80 shoots were recorded in MS3 and MS1 respectively. The lowest number of shoot production was observed in MS2 (14.40).

On comparing the length of shoots in different treatments, it was observed that longest shoots (1.44 cm) in Peach Blossom were produced in MS2 and shortest (0.12 cm) in MS1 and MS3. The treatment MS4 was on par with MS1 and MS3. In Tropic Seas also, highest shoot length (1.26 cm) was observed in MS2 (Plate 12) and lowest (0.16 cm) was observed in MS1.

Table 16 Effect of basal media on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
SMS	100.00	21.86	0.16	100.00	22.28	0.19
SSH	100.00	12.29	1.24	100.00	15.43	1.23
SB5	100.00	15.00	1.41	100.00	16.14	1.26
F	-	11.96*	23.59*	-	4.71**	16.74*
CD (0.05)	-	4.24	0.42	-	5.16	0.45

◈Treatment combinations are given in Table 3.

The data represents mean value of seven replications

* Significant at 5 % level

** Significant at 1 % level

Table 17 Effect of MS basal media on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
MS1	80.00	12.20	0.12	100.00	16.80	0.16
MS2	100.00	13.60	1.44	100.00	14.40	1.26
MS3	80.00	15.20	0.12	100.00	18.60	0.18
MS4	100.00	28.80	0.13	100.00	26.80	0.22
F	-	10.50*	9.58*	-	3.94*	16.26*
CD (0.05)	-	7.09	0.63	-	8.13	0.17

◈Treatment combinations are given in Table 4.

The data represents mean value of five replications

* Significant at 5 % level

Plate 11. Pigmentation of tissues of cv Peach Blossom cultured in B5 basal medium

Plate 12. Multiple shoot production in cv. Tropic Seas on half- strength MS medium


100
50

4.1.2.4 Mode of culture

The effect of solid and liquid media on multiple shoot proliferation was significantly different (Table 18). With respect to the number of shoots produced, solid MS media was better than liquid media in both the varieties. Higher rate of shoot proliferation was observed in solid media (24.46) over the liquid media (13.31) in Peach Blossom. In Tropic Seas also, solid media produced more number of shoots (25.92) than that in liquid media (20.15).

With respect to the length of the shoot, the liquid media recorded good response in Peach Blossom and Tropic Seas (Table 18). In the former, liquid culture and solid culture recorded shoot length of 0.57 cm and 0.14 cm respectively, which were significantly different from each other. In the latter, the liquid culture recorded 0.69 cm shoot length, which was significantly different from that of solid culture with an average shoot length of 0.12 cm.

4.1.2.5 Carbon source

Among the four different levels of two carbon sources *viz.*, sucrose and glucose, tried, the treatment SPS4 having sucrose 4.00 per cent recorded highest shoot number (40.00) in Peach Blossom. This was followed by the treatments SPS3 and SPG4 (Table 19) with average shoot numbers of 33.67 and 27.00, respectively. SPS1 and SPG1 resulted in lowest number of shoots of 7.00 and 6.00, respectively. In the case of Tropic Seas also, the highest shoot number was obtained in SPS4 followed by SPS3. These treatments produced 33.33 and 27.67 shoots, respectively.

Table 18 Effect of mode of culture on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
SS	100.00	24.46	0.14	100.00	25.92	0.12
SL	84.60	13.31	0.57	100.00	20.15	0.69
F	-	53.32*	12.93*	-	8.39*	15.08*
CD (0.05)	-	3.15	0.25	-	4.34	0.30

◈Treatment combinations are given in Table 5.

The data represents mean value of thirteen replications

* Significant at 5 % level

Table 19 Effect of carbon sources on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
SPS1	100.00	7.00	0.33	100.00	11.67	0.20
SPS2	100.00	10.33	0.27	100.00	16.33	0.23
SPS3	100.00	33.67	0.17	100.00	27.67	0.80
SPS4	100.00	40.00	0.27	100.00	33.33	0.47
SPG1	100.00	6.00	0.33	100.00	8.67	0.17
SPG2	100.00	10.33	0.63	100.00	11.67	0.20
SPG3	100.00	19.00	0.13	100.00	16.33	0.50
SPG4	100.00	27.00	0.40	100.00	17.33	1.27
F	-	69.94*	NS	29.83*	29.83*	3.89**
CD (0.05)	-	4.66	-	4.65	4.65	0.59

◈Treatment combinations are given in Table 6.

The data represents mean value of three replications

*Significant at 5 % level

** Significant at 1 % level

With regard to shoot length, no significant difference was noticed among the various treatments in Peach Blossom. The shoot length varied from 0.13 to 0.63 cm. However, significant difference was observed among the various treatments in Tropic Seas. The highest shoot length was recorded in the treatment SPG4 (1.27 cm). The treatments SPG1 (0.17 cm), SPS1 (0.20 cm) and SPG2 (0.20 cm) produced shortest shoots.

4.1.2.6 Amino acid

Varietal difference was noticed when the basal medium was supplemented with varying levels of amino acids, glycine and arginine. In Peach Blossom, all the treatments involving different concentrations of glycine produced callus (Plate 13) (Table 20). Shoots were produced in treatments devoid of glycine (16.00). SAR1 and SAR2 produced shoots. Roots with prominent root hairs were observed in SAR1. Higher concentration of arginine in the treatment SAR3 produced callus. For the variety Tropic Seas also, the different concentrations of glycine produced callus (Plate 14). Shoots were observed in treatments without glycine (Table 20). All the treatments involving arginine produced shoots. In SAR3 roots were also produced (Plate 15).

4.1.2.7 Coconut water

The effect of coconut water on shoot proliferation varied significantly in both the varieties (Table 21). All the treatments produced callus in the variety Peach Blossom. However, in the variety Tropic Seas all the treatments produced shoots (Plate 16). Significant difference was not noticed among the different treatments with respect to the number of shoots. The length of the

Table 20 Effect of amino acids on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
SGL0	100.00	16.00	0.43	100.00	16.67	0.33
SGL1	100.00	@	-	100.00	@	-
SGL2	100.00	@	-	100.00	@	-
SGL3	100.00	@	-	100.00	@	-
SAR1	100.00	17.33	0.13	100.00	13.67	0.30
SAR2	100.00	14.00	0.13	100.00	16.00	0.26
SAR3	100.00	@	-	100.00	14.67	0.33

@ Callusing

◈Treatment combinations are given in Table 7.

The data represents mean value of three replications

Table 21 Effect of coconut water on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
SCW1	100.00	@	-	100.00	20.57	1.51
SCW2	100.00	@	-	100.00	21.57	0.50
SCW3	100.00	@	-	100.00	22.29	0.66
Control	100.00	21.86	0.16	100.00	22.28	0.19
F	-	-	-	-	NS	9.54*
CD (0.05)	-	-	-	-	-	0.52

@ Callusing

◈Treatment combinations are given in Table 8.

The data represents mean value of seven replications

* Significant at 5 % level

Plate 13. Callus formation in cv. Peach Blossom on MS medium supplemented with amino acid, glycine 100.00 mgl^{-1}

Plate 14. Callus formation in cv. Tropic Seas on MS medium supplemented with amino acid, glycine 100.00 mgl^{-1}

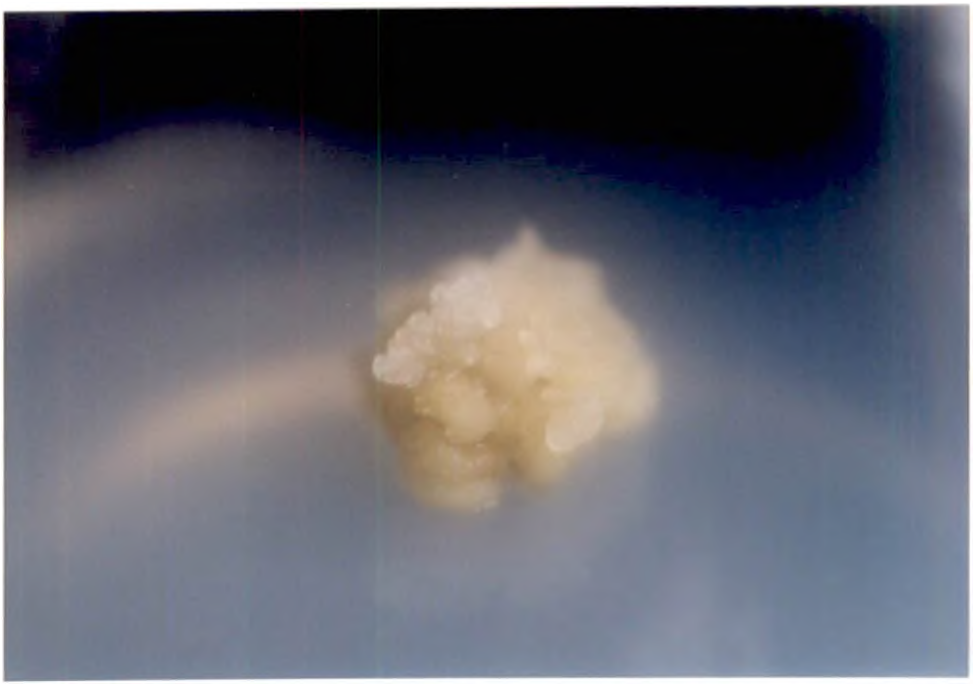
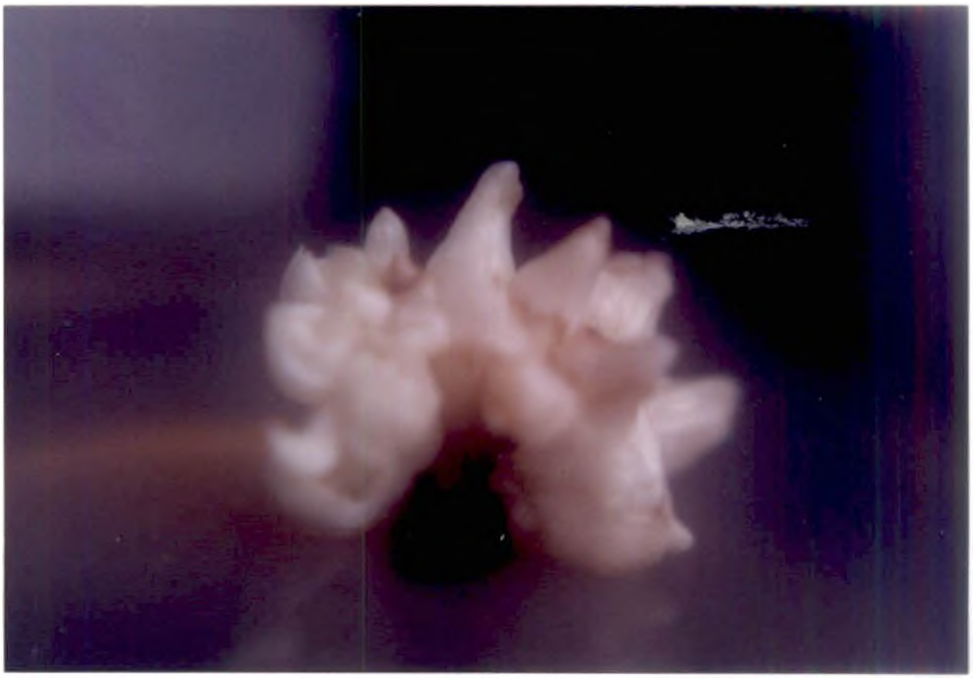


Plate 15. Induction of roots in cv. Tropic Seas in multiple shoot proliferation medium supplemented with amino acid arginine 100.00 mgl^{-1}

Plate 16. Multiple shoot proliferation in cv. Tropic Seas on MS medium supplemented with coconut water 200.00 ml l^{-1}


17



shoots varied significantly, the longest (1.51 cm) being observed in treatment SCW1 supplemented with 50.00 ml l^{-1} coconut water. The shortest shoot length was recorded in control (0.19 cm), which was on par with SCW2 (treatment with 100.00 ml l^{-1} CW) and SCW3 (treatment with 200.00 ml l^{-1} CW).

4.1.2.8 Activated charcoal

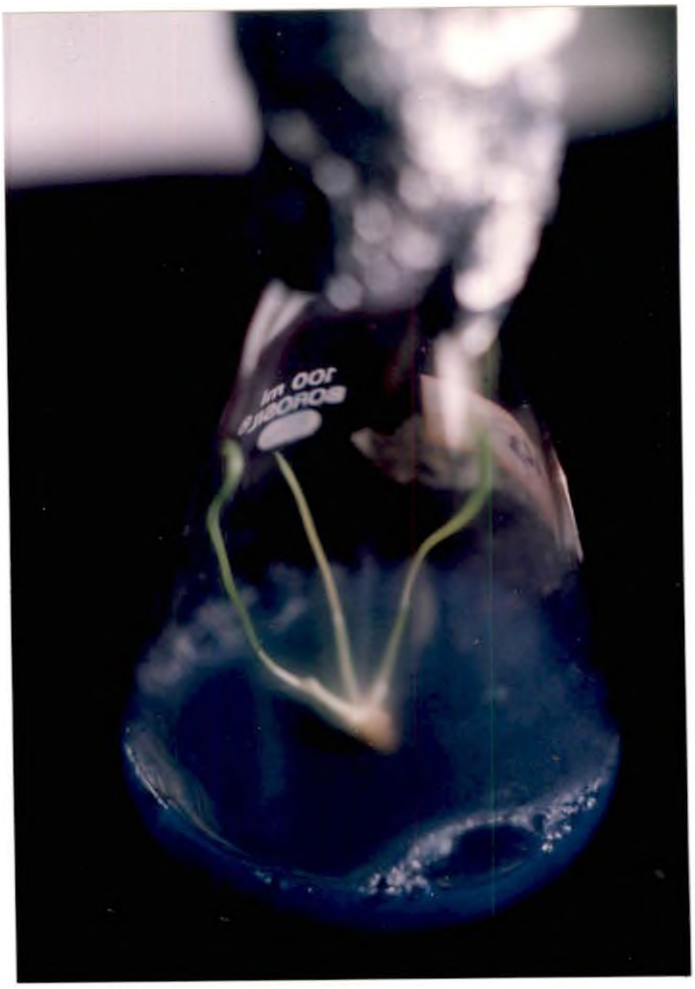
Comparing the three different levels of activated charcoal tried, no significant difference was observed, with regard to the number of shoots in both the varieties. However, significant difference was noticed between the treatments and control regarding the shoot number and shoot length in both the varieties.

In Peach Blossom, the highest shoot number was obtained in control (21.86). Among the three treatments tried SAC1 produced 3.00 shoots, followed by SAC2 (2.14) and SAC3 (1.14) (Table 22). In Tropic Seas also, the control recorded highest rate of shoot proliferation (22.28). Out of the three treatments, SAC1 produced 2.00 shoots (Plate 18), followed by SAC2 and SAC3 with average shoot numbers of 1.86 and 1.00, respectively.

There was no significant difference among the three treatments tried in the cv. Peach Blossom with regard to the length of longest shoots produced. The highest shoot length was noticed in SAC3 (16.93 cm) (Plate 17). The control produced shortest shoot length of 0.16 cm. In Tropic Seas, significant difference was noticed among the three treatments and also between the treatments and control with respect to shoot length. Among the three

Plate 17. **Rapid shoot elongation in cv. Peach Blossom on MS medium supplemented with activated charcoal 2.00 mg l^{-1}**

Plate 18. **Rapid shoot elongation in cv. Tropic Seas on MS medium supplemented with activated charcoal 0.50 mg l^{-1}**



treatments SAC2 produced longest shoots (15.00 cm), which was on par with SAC3 (14.03 cm). The control recorded shoot length of 0.19 cm.

4.1.2.9 Solidifying agent

No significant difference was noticed among the different concentrations of agar on the number of shoots produced in both the varieties (Table 23).

In Peach Blossom significant difference was noticed among the treatments with regard to the lengths of shoots. The treatments SAG1, SAG2 and SAG3 produced shoots of length 0.53 cm, 0.53 cm and 0.51, respectively, which were on par. In Tropic Seas, there was no significant difference among the treatments with regard to the shoot length.

4.1.2.10 Culture conditions

Light had significant influence on the rate of shoot proliferation (Table 24). Callus initiation was found in cultures under dark. In Peach Blossom, cultures under light condition produced on an average 24.46 shoots, while that under dark condition produced only 11.77 shoots. In Tropic Seas, shoot number recorded under light condition was 25.92 and that under dark condition was 11.85.

4.1.3 *In vitro* rooting

4.1.3.1 Plant growth substances

The response to different growth substances tried for rooting varied with the varieties. The different levels of auxins (IBA, NAA and IAA) tried, influenced root production significantly. The treatments with various levels of 2,4-D (0.10, 0.20, 0.40 and 0.80 mg l^{-1}) did not produce roots.

Table 22 Effect of activated charcoal on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
SAC1	100.00	3.00	14.96	100.00	2.00	10.43
SAC2	71.42	2.14	13.54	71.42	1.86	15.00
SAC3	85.71	1.14	16.93	42.85	1.00	14.03
Control	100.00	21.86	0.16	100.00	22.28	0.19
F	-	NS	NS	-	NS	5.11*
CD (0.05)	-	-	-	-	-	3.10

◈Treatment combinations are given in Table 9.

The data represents mean value of seven replications

* Significant at 5 % level

Table 23 Effect of solidifying agent on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
SAG1	100.00	19.29	0.53	100.00	21.70	0.30
SAG2	100.00	22.14	0.53	100.00	25.42	0.29
SAG3	100.00	22.43	0.51	100.00	21.71	0.30
Control	100.00	21.86	0.16	100.00	22.28	0.19
F	-	NS	11.92*	-	NS	NS
CD (0.05)	-	-	0.16	-	-	-

◈Treatment combinations are given in Table 10.

The data represents mean value of seven replications

* Significant at 5 % level

Out of the different plant growth substances tried, the treatment R3 (IBA 2.00 mg l^{-1}) showed earliest rooting (7.00 days) in Peach Blossom (Plate 19). Treatments R7, R2, R1, R6 and R9 were found to be on par (Table 25a). Control recorded 24.67 days for rooting. In the variety Tropic Seas also significant difference was observed among the various treatments with respect to the number of days taken for rooting. Earliest days (7.00) for root initiation were recorded in R9 (IAA 2.00 mg l^{-1}) (Plate 20). This treatment was found to be on par with R7, R8, R2, R1 and R6.

With regard to the highest number of roots produced in the cv. Peach Blossom, the treatment R5 was superior. This treatment produced 24.00 roots and was on par with R3, which produced 21.33 roots. The lowest number of roots was obtained in treatments R8, R6 and control (1.33). In the variety Tropic Seas, the highest number (17.33) of roots was recorded in R2 (IBA 1.00 mg l^{-1}). Treatments R5, R4 and R1 were found to be on par with R2 (Table 25a). The lowest number of roots was recorded in control (Plate 21), R6 and R8 (1.33).

Significant difference was noticed with respect to the length of the roots also, in both the varieties. The highest root length in Peach Blossom was recorded in R3 (5.00) followed by R2 (4.43 cm), R9 (4.10 cm) (Plate 21) and R5 (3.27 cm). Lowest root length was noticed in control (0.53 cm) and R8 (0.80 cm). In Tropic Seas, the treatment R4 produced longest root (4.23 cm). This was on par with R7 (4.17 cm). The shortest root length was observed in control, R8 and R6.

Table 24 Effect of light on multiple shoot proliferation of gladiolus varieties

Culture period : 6 weeks

Treatment	Peach Blossom			Tropic Seas		
	Survival (%)	Shoots per culture	Length of longest shoot (cm)	Survival (%)	Shoots per culture	Length of longest shoot (cm)
Light	100.00	24.46	0.14	100.00	25.92	0.12
Dark	76.20	11.77	0.15	100.00	11.85	0.11
F	-	97.58*	NS	-	126.37*	NS
CD (0.05)	-	2.65	-	-	2.59	-

The data represents mean value of thirteen replications

* Significant at 5 % level

Table 25a Effect of plant growth substances on *in vitro* rooting of gladiolus varieties

Culture period : 4 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Days for root initiation	No. of roots	Length of longest root (cm)	Days for root initiation	No. of roots	Length of longest root (cm)
R1	10.33	1.67	2.43	11.00	10.33	1.67
R2	10.00	7.00	4.43	9.00	17.33	2.27
R3	7.00	21.33	5.00	18.00	5.67	2.67
R4	18.67	11.33	2.03	19.67	13.00	4.23
R5	14.33	24.00	3.27	17.67	15.67	1.50
R6	11.00	1.33	2.60	12.00	1.33	0.80
R7	8.67	5.67	2.50	8.33	5.67	4.17
R8	13.67	1.33	0.80	8.67	1.33	0.67
R9	12.67	2.00	4.10	7.00	6.33	2.00
Control	24.67	1.33	0.53	28.33	1.33	0.59
F	2.57**	5.31**	NS	6.97*	4.39*	6.42*
CD (0.05)	6.40	11.23	-	5.38	8.27	1.50

◈Treatment combinations are given in Table 11

The data represents mean value of three replications

*Significant at 5 % level

** Significant at 1 % level

Plate 19. ***In vitro*** **rooting in cv. Peach Blossom in MS medium supplemented with IBA 2.00 mg l^{-1}**

Plate 20. ***In vitro*** **rooting in cv. Tropic Seas in MS medium supplemented with IAA 2.00 mg l^{-1}**

Table 25b Effect of plant growth substances on the nature of roots produced in gladiolus varieties

Treatment	Peach Blossom	Tropic Seas
R1	Thin, long, light brown	Medium thick, cream, branched
R2	Thin, cream	Thick, stout, cream
R3	Thin, light brown, branched, root hair present	Thick, stout, cream
R4	Thick, short, dark brown	Thick, dark brown
R5	Medium thick, cream, root hair present	Thick, dark brown
R6	Very thin, black	Thin, black
R7	Very thin, black	Very thin, black
R8	Thin, black	Very thin, black
R9	Thin, black	Thin, long, dark brown
Control	Very thin, long, light brown	Very thin, long, light brown.

Very thin - < 0.5 mm Thin - 0.5 – 1.0 mm
Medium thick - 1.0 – 1.5 mm Thick - > 1.5 mm

4.1.3.2 Carbon sources

On comparing the effect of different levels of carbon sources, significant difference was noticed with respect to the number of days taken for root initiation (Table 26) in both the varieties. In Peach Blossom, least number of days (8.75) for root initiation was noticed in the treatment RS2 (sucrose 30.00 g l^{-1}). This treatment was on par with RG3 and RS3. The utmost days for root initiation (18.20) were recorded for the treatment RG1, followed by RS1 and RG2. The least number of days for root initiation (9.25) in Tropic Seas were recorded for the treatment RS3 (9.25), which was on par with RS2 (9.75) and RS1 (11.50). The utmost number of days for rooting was observed in RG1 (19.50).

The effect of carbon sources on the number of roots produced was not significant in both the varieties. In Peach Blossom, highest number of roots (25.75) was produced in the treatment RG3 and lowest number (9.50) in RG2. In Tropic Seas, the treatment RS2 produced highest number of roots (26.75) and RS1 produced lowest number of roots (15.00).

The length of the roots differed significantly among the different treatments of carbon sources tried in Peach Blossom (Table 26). The longest root (3.63 cm) was observed in the treatment RS2. The shortest root (0.83 cm) was measured in the treatment RS1. In Tropic Seas, no significant difference was noticed among the various treatments, with respect to the length of roots.

Table 26 Effect of carbon sources on *in vitro* rooting of gladiolus varieties

Culture period : 4 weeks

◈Treatment	Peach Blossom			Tropic Seas		
	Days for root initiation	No. of roots	Length of longest root (cm)	Days for root initiation	No. of roots	Length of longest root (cm)
RS1	15.50	24.50	0.83	11.50	15.00	1.20
RS2	8.75	22.25	3.63	9.75	26.75	1.50
RS3	11.00	15.50	1.58	9.25	16.50	1.98
RG1	18.20	17.75	0.88	19.50	24.50	0.80
RG2	15.00	9.50	1.23	14.00	22.75	1.25
RG3	11.50	25.75	1.18	13.50	24.00	1.50
F	4.11**	NS	13.78**	9.34*	NS	NS
CD (0.05)	5.04	-	0.87	3.65	-	-

◈Treatment combinations are given in Table 12.

The data represents mean value of four replications

* Significant at 5 % level

** Significant at 1 % level

***In vitro* corm formation**

In cv. Peach Blossom *in vitro* corm formation was noticed in some cultures, nine weeks after culture in rooting medium supplemented with IBA 2.00 mg l^{-1} (Plate 22).

4.1.4 *Ex vitro* rooting

The plantlets subjected to *ex vitro* rooting survived only for three days. The plantlets exhibited symptoms of rotting and died.

4.1.5 Planting out and acclimatization

The *in vitro* rooted plantlets were planted out in sterilized media in plastic pots (Plate 23) and kept inside humidified polythene tent to maintain humidity (Plate 24).

Among the three different media tried, sand : soil (2 : 1) recorded 100.00 per cent survival rate in both the varieties after 15 days. In the media sand, the survival rate was 40.00 per cent and 22.22 per cent in Peach Blossom and Tropic Seas, respectively. The survival rate was poor in soilrite (Table 27).

Table 27 Survival rate of plantlets 15 days after planting out

Media	Peach Blossom (%)	Tropic Seas (%)
Sand	40.00	22.22
Soilrite	14.29	0.00
Sand : soil	100.00	100.00

Plate 21. *In vitro* rooting in cv. Tropic Seas

1. Rooting in MS medium without plant growth substances
2. Rooting in MS medium supplemented with IAA 2.00 mg l^{-1}
3. Rooting in MS medium supplemented with IBA 2.00 mg l^{-1}

Plate 22. *In vitro* corm formation in cv. Peach Blossom 9 weeks after culture in MS medium supplemented with IBA 2.00 mg l^{-1}

Plate 23. Hardening of *in vitro* raised plantlets of gladiolus

Plate 24. Plantlets kept inside polythene tent

Discussion

5. DISCUSSION

Gladiolus is an important bulbous ornamental crop valued for its attractive spikes. This is a recently introduced cut flower crop in Kerala, which can be successfully cultivated by planting during November (Suneetha, 1994). It is commercially propagated through corms and cormels. But the rate of multiplication is extremely slow as the daughter corms or cormels are few in number and due to their small size, they require three to four seasons to develop into an ideal size to produce a marketable spike, as the spike size depends on the corm size. A considerable time is therefore required to get sufficient quantity of planting material of a newly evolved cultivar or hybrid. The dormancy of corms and cormels lasting for three to four months is another serious problem, which limits the commercial production of planting material. Diseases in field conditions and in storage are also factors affecting the multiplication rate. *In vitro* propagation methods can act as a viable alternative to overcome these problems. It enables large-scale production of disease free plantlets of desired cultivars within short period of time.

Micropropagation of gladiolus is achieved through enhanced release of axillary buds, somatic embryogenesis and somatic organogenesis. *In vitro* propagation through enhanced release of axillary buds was found effective for the rapid clonal propagation of gladiolus (Hussey, 1977; Ziv, 1979; Hussey, 1982; Dickens *et al.*, 1986; Dantu and Bhojwani, 1987; Lilien-Kipnis and Kochba, 1987; Ziv, 1990; Zakutskaya and Murin, 1990; Rao *et al.*, 1991; Rumynin *et al.*, 1990; Nagaraju and Parthasarathy, 1995; Pathania *et al.*, 2001).

Varietal difference in response to *in vitro* culture is obvious in gladiolus cultivars. Two varieties *viz.*, Peach Blossom and Tropic Seas found suitable for the southern region of Kerala, was selected for the present study. The outcome of the investigations is discussed in this chapter.

The culture establishment medium is useful for conditioning of the explant and for stimulating its initial growth. In order to standardize a suitable hormone combination for better culture establishment, studies were carried out using BA, kinetin, NAA and IAA at various concentrations. BA 4.00 mg l^{-1} along with NAA 0.50 mg l^{-1} was best for the initial culture establishment of both the varieties, Peach Blossom and Tropic Seas. Kinetin and its combination with auxin could induce early bud break (Table 14), but compared to BA and its combinations with auxin, the efficiency was less with regard to the shoot number and further shoot growth. A similar response was reported by Hussain (1995) also. Treatments involving auxin alone did not induce early bud break and inhibited shoot growth. This was in conformation with the findings of Hussey (1976a) and Hussain (1995). MS medium supplemented with BAP 3.00 mg l^{-1} followed by BAP 3.00 mg l^{-1} + NAA 0.20 mg l^{-1} were excellent for the initial establishment of the cultures of cv. American Beauty (Misra and Singh, 1999). BAP 2.00 – 4.00 mg l^{-1} along with GA3 was found optimal for the culture establishment of cvs. Eurovision and Wine and Roses (Pathania *et al.*, 2001).

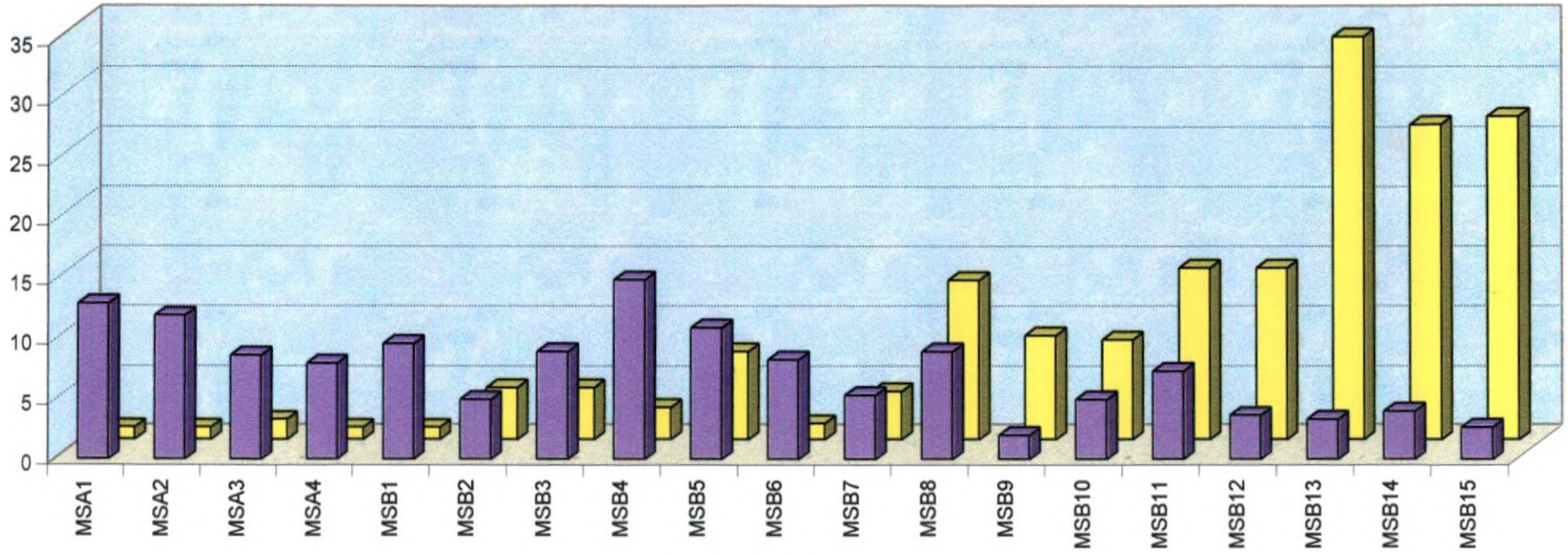
Growth and morphogenesis *in vitro* are regulated by the interaction and balance between the growth regulators supplied in the medium and the growth substances produced endogenously by cultured cells (Krikorian, 1982). A balance between auxin and cytokinin is required for shoot proliferation

(Skoog and Miller, 1957). In the present study, the frequency of multiple axillary bud production was high at higher levels of BA (4.00 mg l^{-1}) in combination with auxin in both the varieties (Fig. 1 - 4). But the lengths of individual shoots were less with high rate of proliferation. According to Hussey (1976a) higher levels of BAP promoted secondary axillary bud production leading to crowding and non-distichous arrangement. The increasing level of BAP was required to promote a steady rate of axillary branching in any cultivar. Beura and Singh (1998) also observed a higher rate of bud proliferation in MS medium supplemented with high concentration of BAP (4.00 mg l^{-1}). Increased shoot regeneration rate with increase in kinetin and BAP (1.00 – 4.00 mg l^{-1}) was obtained in cv. Friendship by Anandhi and Sekhar (2000).

In contrast, BAP at low concentration of 0.75 mg l^{-1} gave highest number of shoots and axillary buds in cv. Cream White (Nagaraju and Parthasarathy, 1995). Hussain (1995) obtained very high rate of multiple axillary bud production in MS medium supplemented with BAP 1.00 mg l^{-1} + NAA 0.50 mg l^{-1} or BAP 2.00 mg l^{-1} + NAA 0.50 mg l^{-1}.

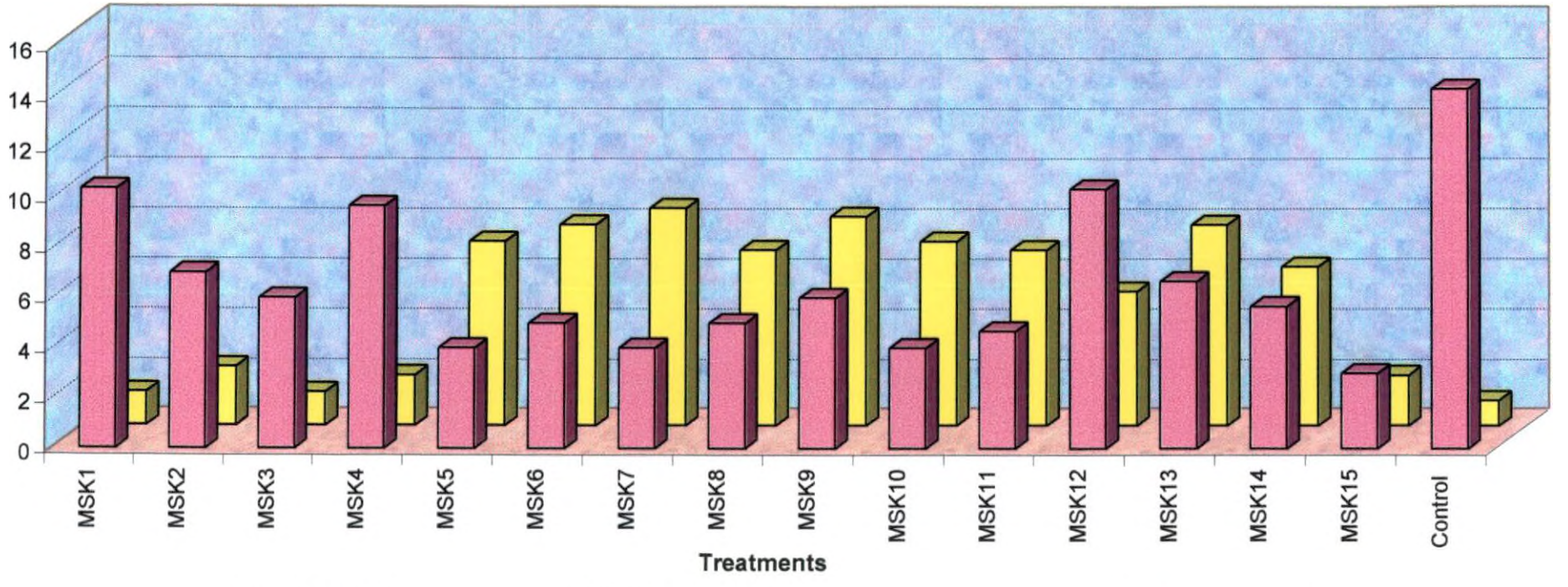
The effect of BAP on different gladiolus varieties such as Bellarina, Blue Moon, Cream White, Friendship, Her Majesty and Top Brass was found to be non-uniform (Nagaraju *et al.*, 2000). The genotypes varied in their response to BAP with respect to a set of characters including shoot number, shoot length, shoot weight, bud number and bud length. Such variations may be due to differences in the endogenous level of auxins and cytokinins among the different varieties.

Fig. 1 Effect of plant growth substances (BA and auxins) on multiple shoot proliferation of gladiolus cv. Peach Blossom



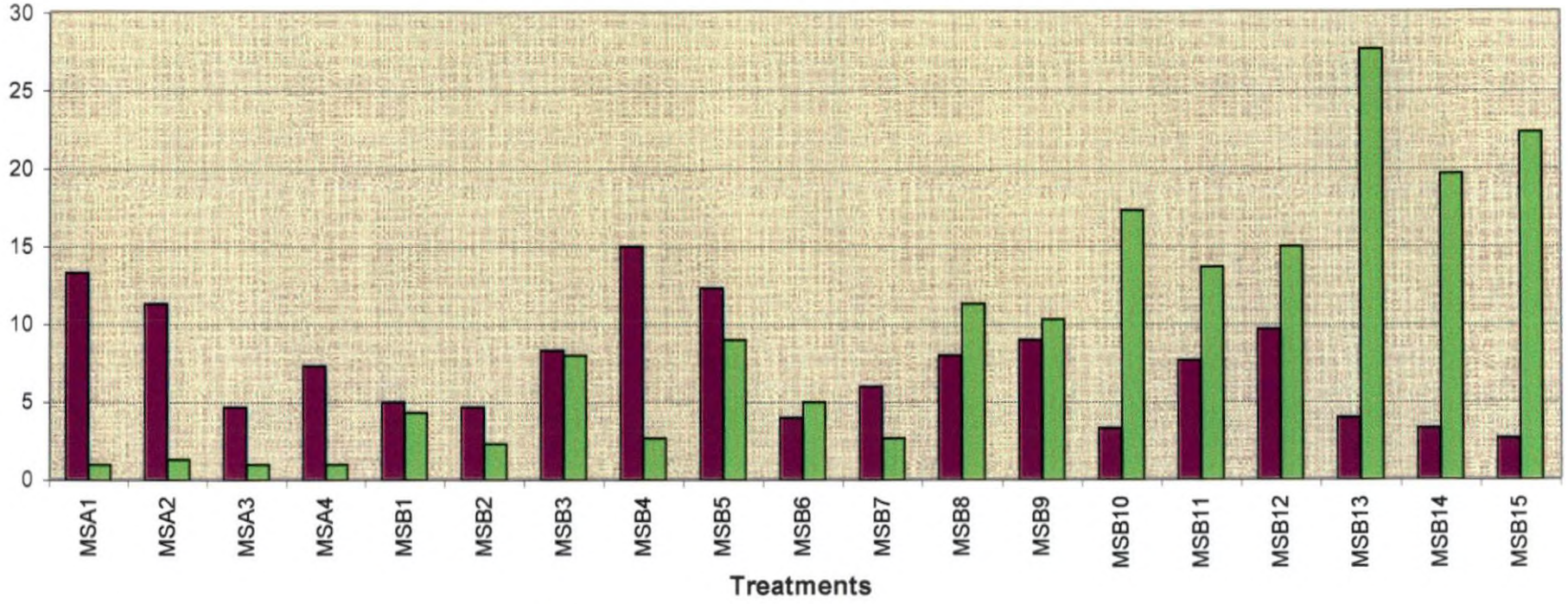
MSA1 - BA 0.00 + NAA 0.10
MSA2 - BA 0.00 + NAA 0.50
MSA3 - BA 0.00 + IAA 1.00
MSA4 - BA 0.00 + IAA 2.00
MSB1 - BA 1.00
MSB2 - BA 1.00 + NAA 0.10
MSB3 - BA 1.00 + NAA 0.50
MSB4 - BA 1.00 + IAA 1.00
MSB5 - BA 1.00 + IAA 2.00
MSB6 - BA 2.00
MSB7 - BA 2.00 + NAA 0.10
MSB8 - BA 2.00 + NAA 0.50
MSB9 - BA 2.00 + IAA 1.00
MSB10 - BA 2.00 + IAA 2.00
MSB11 - BA 4.00
MSB12 - BA 4.00 + NAA 0.10
MSB13 - BA 4.00 + NAA 0.50
MSB14 - BA 4.00 + IAA 1.00
MSB15 - BA 4.00 + IAA 2.00

Fig. 2 Effect of plant growth substances (kinetin and auxins) on multiple shoot proliferation of gladiolus cv. Peach Blossom



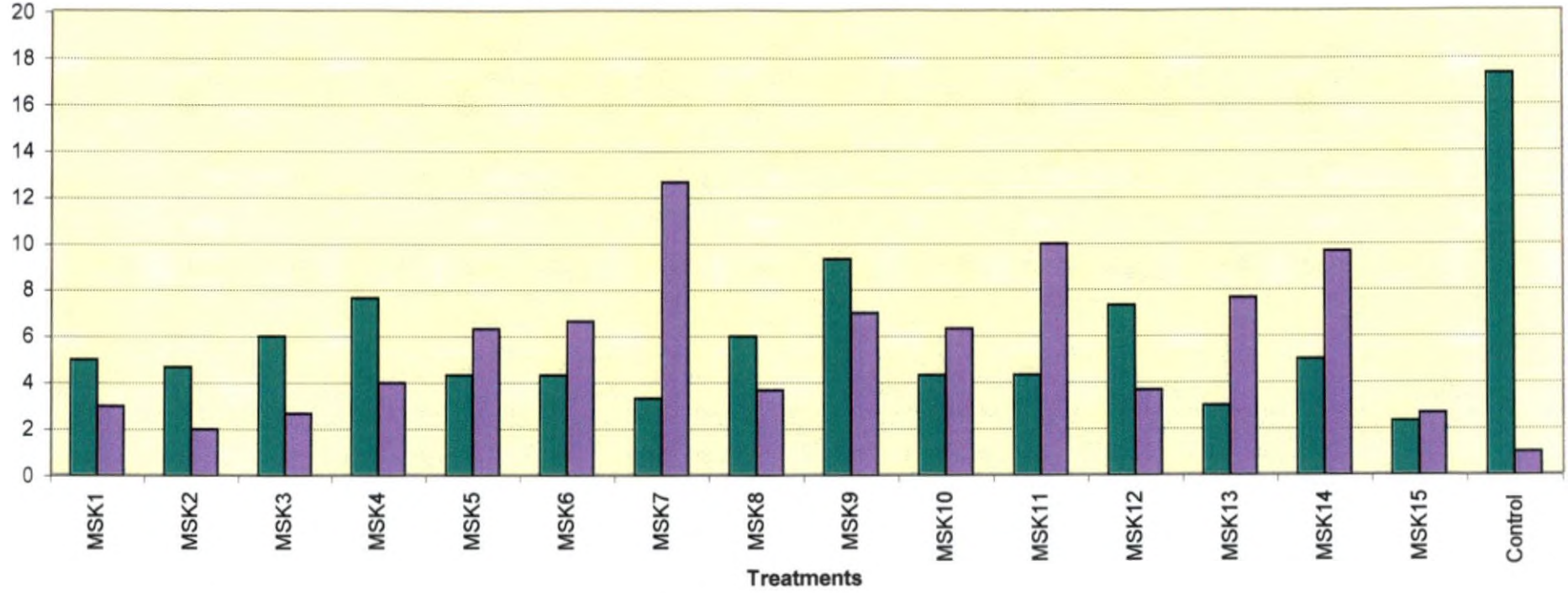
MSK1 - Kinetin 1.00
MSK2 - Kinetin 1.00 + NAA 0.10
MSK3 - Kinetin 1.00 + NAA 0.50
MSK4 - Kinetin 1.00 + IAA 1.00
MSK5 - Kinetin 1.00 + IAA 2.00
MSK6 - Kinetin 2.00
MSK7 - Kinetin 2.00 + NAA 0.10
MSK8 - Kinetin 2.00 + NAA 0.50
MSK9 - Kinetin 2.00 + IAA 1.00
MSK10 - Kinetin 2.00 + IAA 2.00
MSK11 - Kinetin 4.00
MSK12 - Kinetin 4.00 + NAA 0.10
MSK13 - Kinetin 4.00 + NAA 0.50
MSK14 - Kinetin 4.00 + IAA 1.00
MSK15 - Kinetin 4.00 + IAA 2.00

Fig. 3 Effect of plant growth substances (BA and auxins) on multiple shoot proliferation of gladiolus cv. Tropic Seas



MSA1 - BA 0.00 + NAA 0.10
MSA2 - BA 0.00 + NAA 0.50
MSA3 - BA 0.00 + IAA 1.00
MSA4 - BA 0.00 + IAA 2.00
MSB1 - BA 1.00
MSB2 - BA 1.00 + NAA 0.10
MSB3 - BA 1.00 + NAA 0.50
MSB4 - BA 1.00 + IAA 1.00
MSB5 - BA 1.00 + IAA 2.00
MSB6 - BA 2.00
MSB7 - BA 2.00 + NAA 0.10
MSB8 - BA 2.00 + NAA 0.50
MSB9 - BA 2.00 + IAA 1.00
MSB10 - BA 2.00 + IAA 2.00
MSB11 - BA 4.00
MSB12 - BA 4.00 + NAA 0.10
MSB13 - BA 4.00 + NAA 0.50
MSB14 - BA 4.00 + IAA 1.00
MSB15 - BA 4.00 + IAA 2.00

Fig. 4 Effect of plant growth substances (kinetin and auxins) on multiple shoot proliferation of gladiolus cv. Tropic Seas



MSK1 - Kinetin 1.00
MSK2 - Kinetin 1.00 + NAA 0.10
MSK3 - Kinetin 1.00 + NAA 0.50
MSK4 - Kinetin 1.00 + IAA 1.00
MSK5 - Kinetin 1.00 + IAA 2.00
MSK6 - Kinetin 2.00
MSK7 - Kinetin 2.00 + NAA 0.10
MSK8 - Kinetin 2.00 + NAA 0.50
MSK9 - Kinetin 2.00 + IAA 1.00
MSK10 - Kinetin 2.00 + IAA 2.00
MSK11 - Kinetin 4.00
MSK12 - Kinetin 4.00 + NAA 0.10
MSK13 - Kinetin 4.00 + NAA 0.50
MSK14 - Kinetin 4.00 + IAA 1.00
MSK15 - Kinetin 4.00 + IAA 2.00

The basal medium requirement is highly tissue dependant and varies from one variety to another. In the present investigation, MS medium was superior to SH and B5 media with respect to shoot proliferation in both the varieties (Table 16). This is in agreement with the observations of Hussain (1995), who reported that MS and modified MS medium were good for proliferation of multiple axillary bud aggregates. MS medium was utilized for micropropagation of gladiolus by many workers (Kim and Lee, 1993; Jager *et al.*, 1998; Nagaraju *et al.*, 1998; Misra and Singh, 1999; Anandhi and Sekhar, 2000; Pathania *et al.*, 2001).

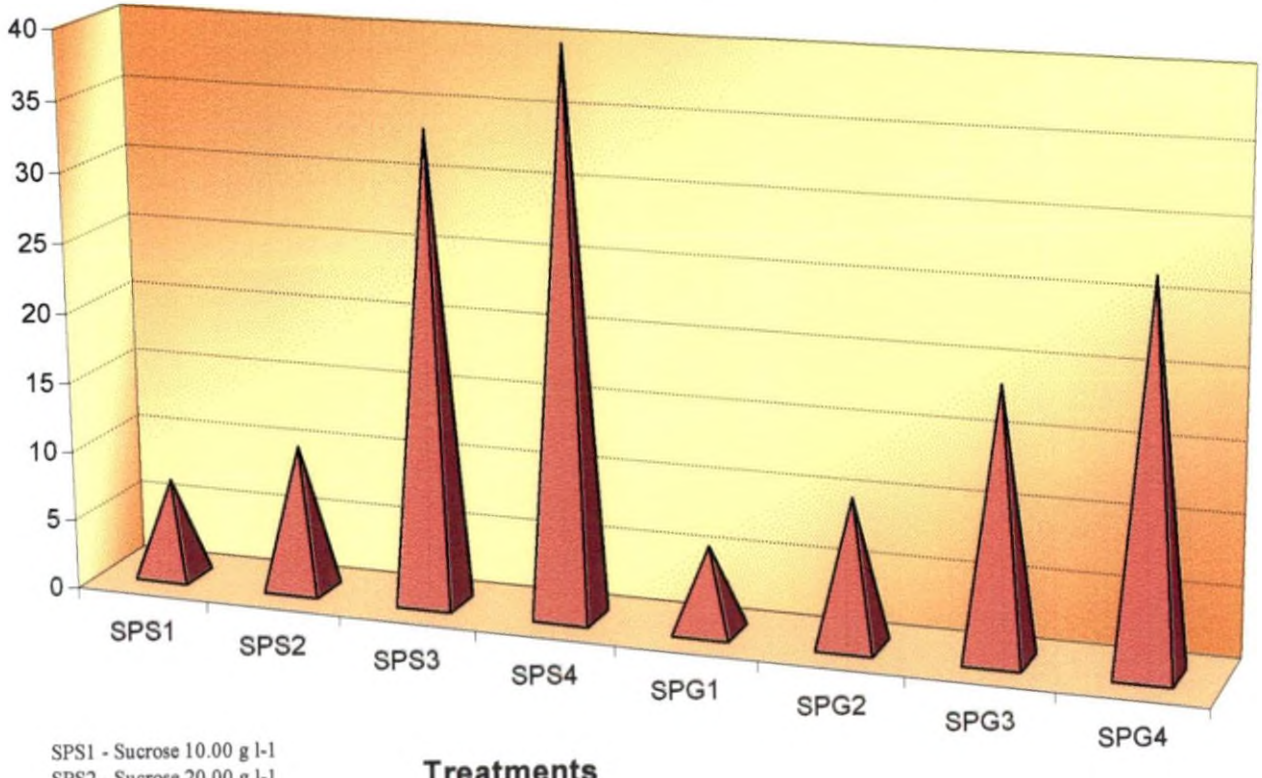
Murashige and Skoog medium at full strength was used for the micropropagation of many gladiolus varieties (Hussey, 1977; Ziv, 1979; Bajaj *et al.*, 1983; Dantu and Bhojwani, 1987; Ziv, 1990; Kumar *et al.*, 1999; Nagaraju *et al.*, 2000; Babu and Chawla, 2000). In the present study, the highest shoot proliferation rate was obtained in full MS. However, length of shoots was highest in half MS than in full MS (Table 17). Similar effect was reported by Hussain (1995).

Solid culture was better than liquid culture in both the varieties tried for the present study with regard to shoot proliferation. But the lengths of shoots were highest in liquid media (Table 18). *In vitro* propagation of gladiolus in agar cultures was reported by many workers (Hussey, 1977; Ziv, 1979; Bajaj *et al.*, 1983; Logan and Zettler, 1985; Lilien-Kipnis and Kochba, 1987). Aseptic bud explants of cv. Eurovision subcultured from agar to liquid medium increased in size and proliferation (Ziv, 1989). The promotive effect of liquid MS medium on elongation of shoot cultures was reported by Dantu and Bhojwani (1995).

Sucrose is the most utilizable carbon form and also energy source and an osmoregulatory factor (George and Sherrington, 1984). In the present study, a comparison of the two carbon sources showed that sucrose was superior to glucose for multiple shoot proliferation (Fig. 5 - 6). Sucrose 40.00 g l^{-1} produced highest number of shoots followed by sucrose 30.00 g l^{-1} in both the varieties (Table 19). Also at higher concentrations, the buds produced were healthy and vigorous. Higher sucrose concentrations of six or nine percent were found best for shoot production at 15^0C in *Gladiolus tristis* by De Bruyn and Ferreira (1992). The general superiority of sucrose over glucose for the culture of organized plant tissues may be on account of the more effective translocation of sucrose to the apical meristems (Butcher and Street, 1964).

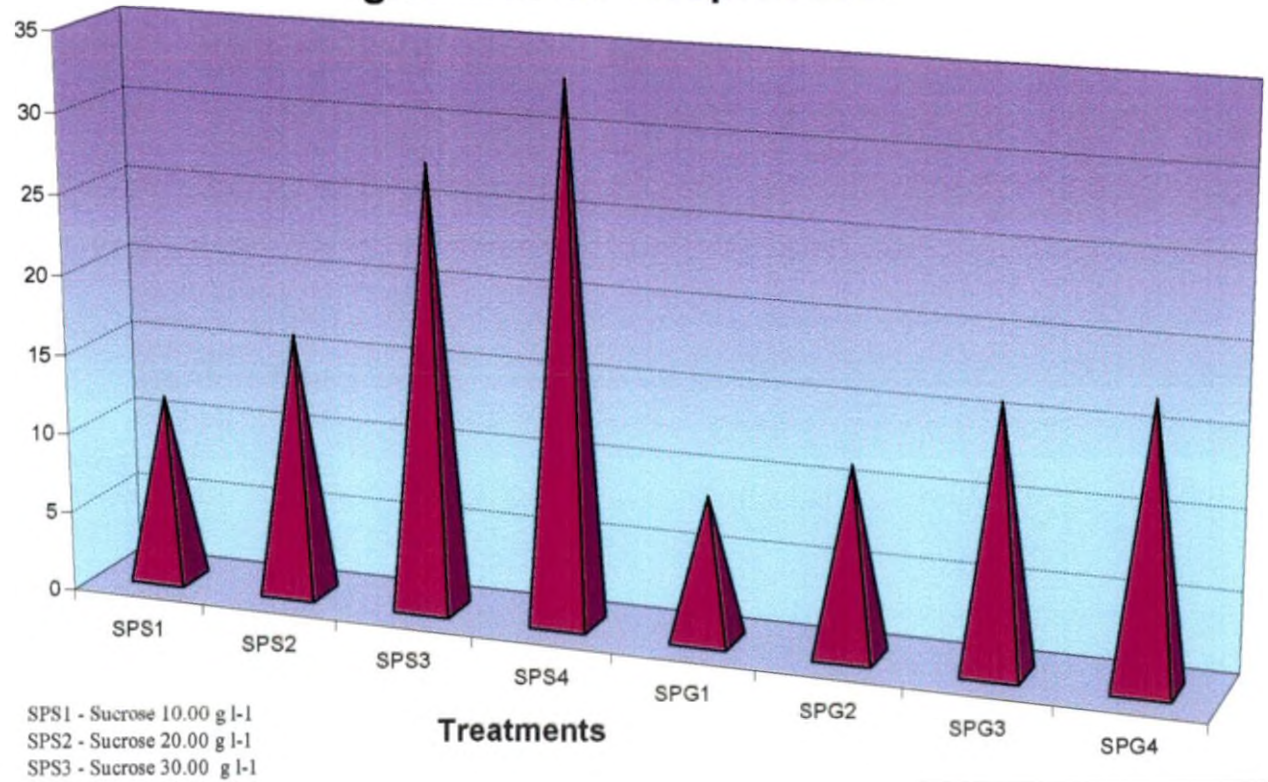
The addition of amino acids to media is important for stimulating cell growth. Certain specific amino acids added to the medium inhibit growth. The present investigation showed that the response to amino acids was cultivar dependant (Table 20). All the treatments involving glycine produced callus in both the varieties. Arginine at 25.00 and 50.00 mg l^{-1} produced multiple shoots and at 100.00 mg l^{-1} produced callus in cv. Peach Blossom. In the cv. Tropic Seas, all the treatments involving arginine produced shoots (Table 20). The addition of arginine strongly suppressed cytokinin-induced bud formation in Wishbone flower, *Torenia fournieri* (Tanimoto and Harada, 1983). In orchid variety, Sonia-17, the addition of amino acid, glutamine produced no favourable effect on shoot proliferation rate (Kuriakose, 1997).

Fig. 5 Effect of carbon sources on multiple shoot proliferation of gladiolus cv. Peach Blossom



SPS1 - Sucrose 10.00 g l-1
SPS2 - Sucrose 20.00 g l-1
SPS3 - Sucrose 30.00 g l-1
SPS4 - Sucrose 40.00 g l-1
SPG1 - Glucose 10.00 g l-1
SPG2 - Glucose 20.00 g l-1
SPG3 - Glucose 30.00 g l-1
SPG4 - Glucose 40.00 g l-1

Fig. 6 Effect of carbon sources on multiple shoot proliferation of gladiolus cv. Tropic Seas



SPS1 - Sucrose 10.00 g l-1
SPS2 - Sucrose 20.00 g l-1
SPS3 - Sucrose 30.00 g l-1
SPS4 - Sucrose 40.00 g l-1
SPG1 - Glucose 10.00 g l-1
SPG2 - Glucose 20.00 g l-1
SPG3 - Glucose 30.00 g l-1
SPG4 - Glucose 40.00 g l-1

Coconut water contains cytokinin like substances (Shantz and Steward, 1952; Letham, 1974 and Van Standen and Drewes, 1975). In the present study, coconut water at different concentrations induced callus in the cv. Peach Blossom. However, in the cv. Tropic Seas shoots were produced, but the shoot number recorded was not statistically significant (Table 21). CW proved to be inhibitory to growth in some instances (George and Sherrington, 1984). In *Anthurium andreanum* cv. Dragon's Tongue, CW inhibited shoot proliferation (Thomas, 1996). Hussain (1995) reported that gladiolus cultures in CW supplemented media took more days for shoot elongation and produced lesser number of shoots, thus conforming the present results.

Activated charcoal adsorbs the toxic substances and residual cytokinin from the medium (Fridborg *et al.*, 1978). The present studies on the effect of activated charcoal showed rapid shoot elongation, which was followed by rooting. Shoot proliferation was absent in all the cultures treated with AC (Table 22). This response was similar to the cultures in MS basal media lacking plant growth substances (Plate 25). So the rapid shoot elongation and subsequent rooting may be attributed to the adsorption of phytohormones on AC. However, Hussain (1995) obtained a reduction in shoot length also, in addition to reduction in shoot number in gladiolus.

The optimum agar concentration creates an osmotic potential favourable for the uptake of nutrients. The different concentrations of agar tried in the present study failed to show any significant difference in shoot proliferation (Table 23). Agar 0.80 per cent, was beneficial in many cultivars (Steinitz *et al.*, 1991; Jager *et al.*, 1998; Nagaraju *et al.*, 2000; Anandhi and Sekhar, 2000).

Plate 25. Rapid shoot elongation and rooting from bud aggregates of cv. Peach Blossom in MS medium devoid of plant growth substances (left) and MS medium supplemented with BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1} and activated charcoal 0.50 mg l^{-1} (right)

Light had significant influence on shoot proliferation. Under dark condition callus was produced (Table 24). This may be due to an increased content of auxin co-factors in the etiolated tissues, which increase the tissues' specificity to exogenously applied auxin (Herman and Hess, 1963). The bud aggregates proliferated under dark was pale and chlorotic. These buds lack chlorophyll, which is required for photosynthesis, and in turn shoot regeneration. This may be the reason for reduced rate of shoot proliferation of cultures under dark condition.

Auxins frequently used for inducing roots are NAA, IBA and IAA (George and Sherrington, 1984). The rooting response to each of the hormones with respect to earliness in root induction, highest number of roots produced and longest root recorded was significantly different in both the varieties. There was also considerable variation in rooting response depending on the type and concentration of auxins used (Table 25a). In the present investigation, MS medium supplemented with IBA 2.00 mg l^{-1} induced earliest rooting (7.00 days) in cv. Peach Blossom, however the same delayed root induction upto 18.00 days in cv. Tropic Seas. In the latter, IAA 2.00 mg l^{-1} induced earliest rooting (7.00 days). Highest root number (24.00) was obtained in cv. Peach Blossom in the treatment having NAA 1.00 mg l^{-1}, however in cv. Tropic Seas highest root number (17.33) was recorded in the treatment having IBA 2.00 mg l^{-1}. The longest root in Peach Blossom and Tropic Seas were recorded in treatments having IBA 2.00 mg l^{-1} and NAA 0.50 mg l^{-1}, respectively. Such varietal difference in response to different types and concentrations of auxins were noticed in many varieties. In the cv.

American Beauty, NAA at a lower concentration of 0.50 mg l^{-1} induced earliest rooting as well as longest root (Misra and Singh, 1999). However, in cv. Friendship NAA at higher concentration (4.00 mg l^{-1}) was reported good for rhizogenesis by Anandhi and Sekhar (2000). In the cv. Friendship, Hussain *et al.* (1994) got extensive root system in *in vitro* raised shoots cultured in MS medium supplemented with IBA 2.00 mg l^{-1}.

High sugar concentration was beneficial for morphogenesis in gladiolus (Steinitz *et al.*, 1991). In the present investigation, glucose at 40.00 g l^{-1} recorded the highest root number (25.75) in the cv. Peach Blossom. However, sucrose 30.00g l^{-1} induced earliest rooting (8.75 days) and longest root (3.63 cm) (Table 26). In the cv. Tropic Seas higher sucrose concentration (40.00 g l^{-1}) produced earliest root initiation (9.25 days) and longest root (1.98 cm). But root number was highest (26.75) at sucrose 30.00 g l^{-1}. Collins and Dixon (1992) reported that a higher concentration of 40.00 g l^{-1} sucrose was ideal for improved rooting of *in vitro* shoots of Australian terrestrial orchid. Higher sucrose concentrations (8.00, 10.00 and 12.00 per cent) increased the number of roots in gladiolus cvs. Her Majesty and Aldebaran (Kumar *et al.*, 1999). The beneficial effect of high sucrose concentration might be because the optimum level of sucrose for root formation depends on a balance between the sucrose and total nitrogen in the medium.

Debergh and Maene (1981) emphasized that the *in vitro* rooting of propagated shoot is expensive. Labour involved in handling individual shoots can cause this stage of micropropagation to amount for 30-75 per cent of total cost of plants propagated through tissue culture. Also there is difficulty of inducing a root system that will not be fully effective when planted out.

Unrooted shoots of certain plants root more readily in *ex vitro*, if exposed to auxin before being planted into a rooting substrate (George and Sherrington, 1984).

However, in the present study the unrooted shoots of gladiolus subjected to treatments with IBA (50.00 and 100.00 mg l^{-1}) overnight, failed to produce roots and the shoots rotted after three days. Similar results were obtained in orchids by Kuriakose (1997).

The difficulties associated with the survival and growth of tissue cultured plants after transplanting are attributed to poor control of water loss from the plants and their need to switch from heterotrophic to photo autotrophic nutrition (Conner and Thomas, 1982).

According to Lilien-Kipnis and Kochba (1987) serious acclimatization problems were encountered with rising temperature in late spring, owing to impeded root development *ex vitro* at higher temperature. Rumynin *et al.* (1990) raised gladiolus plantlets in a substrate covered with a sand layer at 20-25^{0}C and 60-80 per cent relative humidity under continuous illumination (3000 – 5000 lux). The survival rate ranged from 21.00 per cent in cv. King Arthur to 86.00 per cent in cv. Bright Eyes.

In the present study, the best survival rate of *in vitro* rooted plantlets was obtained for the media sand : soil. A survival rate of 100.00 per cent was obtained in sand : soil, 15 days after planting out in both the varieties. The survival rate of plantlets was found to be poor in sand and soilrite (Table 27) The poor survival rate in soilrite might be due to excess water retention of the media.

Ex vitro establishment studies need to be further standardized. For micropropagation of gladiolus to be made commercially viable, further studies involving different potting media, containers, growing conditions (temperature, light and humidity) and use of plant hormones, which can induce hardiness in the plantlets, should be taken up.

Plate 26. **Different stages of *in vitro* clonal propagation of gladiolus *via* enhanced release of axillary buds**

1. **Intact cormels**

2, 3 and 4. **Stages of shoot proliferation**

5. ***In vitro* rooting**

Summary

6. SUMMARY

Attempts were made in the Department of Pomology and Floriculture, College of Agriculture, Vellayani during 2000-2001 to evolve protocol for the *in vitro* clonal propagation of two gladiolus varieties, namely Peach Blossom and Tropic Seas.

The effect of different basal media, mode of culture, plant growth substances, sucrose, glucose, activated charcoal, agar, amino acids and coconut water on culture establishment, multiple shoot proliferation and *in vitro* rooting were studied. The *in vitro* response as influenced by culture condition (light and darkness) was also tried. *Ex vitro* rooting as well as *ex vitro* establishment were conducted.

The salient findings of the above studies are summarized below :

1. The survival rates of Peach Blossom and Tropic Seas in culture establishment media were 79.41 per cent and 80.39 per cent respectively.

2. Among the plant growth substances, kinetin 2.00 mgl^{-1} + NAA 0.10 mgl^{-1} induced earliest bud initiation in both the varieties. However, further survival and shoot growth were better in treatments involving BA (2.00 and 4.00 mgl^{-1}) and its combination with auxins. BA 4.00 mgl^{-1}+ NAA 0.50 mgl^{-1} resulted in the production of highest number of shoots in both the cultivars.

3. Among the different plant growth substances tried for multiple shoot proliferation, highest shoot proliferation was obtained with BA 4.00 mgl^{-1} + NAA 0.50 mgl^{-1}, in both the varieties. Hence this combination was used for standardization of other media components. However, for further shoot multiplication low concentration of BA (1.00 and 2.00 mg l^{-1}) along with NAA (0.10 and 0.50 mg l^{-1}) was ideal.

4. Out of the three basal media tried for shoot proliferation, MS media was best with respect to highest shoot proliferation in both the varieties.

5. Full strength MS medium produced highest shoot number and ½ MS medium produced longest shoots, in both the varieties.

6. Solid medium was superior to liquid medium with respect to highest shoot number in both the varieties. But the liquid media recorded longest shoot in both the cultivars.

7. Sucrose 40.00 gl^{-1} in the shoot proliferation medium recorded highest proliferation of healthy and vigorous shoots in both the varieties.

8. Addition of activated charcoal had no beneficial effect on shoot proliferation in both the varieties.

9. There was no significant effect of different concentrations of agar on shoot proliferation.

10. Varietal difference was noticed when amino acids, glycine and arginine, were added in the basal media. In Peach Blossom, all the treatments involving glycine and higher concentration of arginine produced callus. In Tropic Seas all the treatments involving glycine

produced callus. There was no beneficial effect of amino acids on shoot proliferation.

11. The response to addition of coconut water to shoot proliferation medium varied differently in both the varieties. In Peach Blossom, coconut water at different concentration induced callus, however in Tropic Seas the same produced multiple shoots.

12. Light was necessary for shoot proliferation in both the varieties.

13. Among the various plant growth substances tried for rooting, IBA 2.00 mg 1^{-1} and IAA 2.00 mg 1^{-1} induced earliest rooting in Peach Blossom and Tropic Seas, respectively. Sucrose 30.00 g 1^{-1} and 40.00 g1^{-1} induced early rooting and longest root in Peach Blossom and Tropic Seas, respectively.

14. *Ex vitro* rooting tried was not successful in both the varieties.

15. *In vitro* rooted plantlets planted out in sand : soil (2 : 1) recorded 100.00 per cent survival rate in both the varieties after 15 days.

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*Original not seen

Appendices

APPENDIX – I

Composition of MS, SH and B5 basal media

Nutrient	Quantity (mg l^{-1})		
	MS	SH	B5
Macronutrients			
$NH_4 NO_3$	1650.00	-	-
$(NH_4)_2 SO_4$	-	-	134.00
$NH_4 H_2PO_4$	-	300.00	-
KNO_3	1900.00	2500.00	2500.00
KH_2PO_4	170.00	-	-
$MgSO_4, 7H_2O$	370.00	400.00	250.00
$NaH_2PO_4. H_2O$	-	-	150.00
$CaCl_2. 2 H_2O$	440.00	200.00	150.00
Micronutrients			
H_3BO_3	6.20	5.00	3.00
$MnSO_4. 4H_2O$	22.30	13.20	10.00
$ZnSO_4. 7H_2O$	8.60	1.00	2.00
KI	0.83	1.00	0.75
$Na_2 MoO_4. 2H_2O$	0.25	0.10	0.25
$CuSO_4. 5H_2O$	0.025	0.20	0.025
$CoCl_2. 6H_2O$	0.025	0.10	0.025
$FeSO_4. 7H_2O$	27.85	15.00	27.85
$Na_2EDTA. 2H_2O$	37.25	20.00	37.25

APPENDIX – I Contd...

Nutrient	Quantity (mg l^{-1})		
	MS	SH	B5
Vitamins			
Thiamine, HCl	0.10	5.00	10.00
Pyridoxine, HCl	0.50	0.50	1.00
Nicotinic acid	0.50	5.00	1.00
Amino acid			
Glycine	2.00	-	-
Others			
Inositol	100.00	1000.00	100.00
Sucrose *	30.00	30.00	20.00
Agar *	8.00	8.00	8.00

* g l^{-1}

APPENDIX – II

Abstract of analysis of variance for the effect of different treatments

Sl. No.	Character	Treatment mean squares		Error mean squares	
		Peach Blossom	Tropic Seas	Peach Blossom	Tropic Seas
I.	**Culture establishment**				
1.	**Effect of plant growth substances**				
	i) Days for bud initiation	172.08	160.27	15.18	21.16
	ii) Number of shoots	0.63	0.12	0.23	9.91
II.	**Multiple shoot proliferation**				
1.	**Effect of plant growth substances**				
	i) Days for bud initiation	30.81	30.28	10.46	9.16
	ii) Number of shoots	183.46	129.40	18.30	10.30
	iii) Length of longest shoot	96.22	98.95	7.81	6.43
2.	**Effect of basal media**				
	i) Number of shoots	170.33	99.48	14.34	21.11
	ii) Length of longest shoot	3.25	2.68	0.14	0.16
3.	**Effect of MS basal media**				
	i) Number of shoots	293.78	144.85	27.98	36.75
	ii) Length of longest shoot	2.12	1.44	0.22	0.09
4.	**Effect of mode of culture**				
	i) Number of shoots	808.65	216.35	15.17	25.78
	ii) Length of longest shoot	1.21	2.11	0.09	0.14
5.	**Effect of carbon sources**				
	i) Number of shoots	507.08	215.04	7.25	7.21
	ii) Length of longest shoot	0.07	0.45	0.10	0.11

APPENDIX – II Contd...

Sl. No.	Character	Treatment mean squares		Error mean squares	
		Peach Blossom	Tropic Seas	Peach Blossom	Tropic Seas
6.	**Effect of activated charcoal**				
	i) Number of shoots	6.05	2.05	3.27	4.39
	ii) Length of longest shoot	20.24	40.43	10.49	7.91
7.	**Effect of solidifying agent**				
	i) Number of shoots	14.67	0.51	21.20	11.38
	ii) Length of longest shoot	0.24	0.02	0.02	0.02
8.	**Effect of coconut water**				
	i) Number of shoots	-	5.19	-	156.98
	ii) Length of longest shoot	-	2.09	-	0.22
9.	**Effect of light**				
	i) Number of shoots	1047.12	1288.04	10.73	10.19
	ii) Length of longest shoot	0.002	0.002	0.001	0.002
III.	***In vitro* rooting**				
1.	**Effect of plant growth substances**				
	i) Days for root initiation	36.26	69.45	14.13	9.97
	ii) Number of roots	230.57	103.68	43.33	23.60
	iii) Length of longest root	5.19	5.01	5.34	0.78
2.	**Effect of carbon sources**				
	i) Days for root initiation	47.24	56.27	11.49	6.03
	ii) Number of roots	152.14	89.27	93.29	63.97
	iii) Length of longest root	4.67	0.62	0.34	0.29

APPENDIX III

CHARACTERISTICS OF THE VARIETIES PEACH BLOSSOM AND TROPIC SEAS

Sl. No.	Characters	Peach Blossom	Tropic Seas
1	Flower colour	Pinkish Orange	Light Violet
2	Days to 100% emergence of spikes	92.10	99.40
3	Length of spikes (cm)	65.20	68.42
4	Number of florets per spike	11.80	12.80
5	Vase life of flowers	11.95	14.75
6	Weight of corms (g)	46.50	50.25
7	Weight of cormels (g)	3.47	3.87
8	Number of cormels per plant	7.80	8.60

Source : KAU,1996

IN VITRO CLONAL PROPAGATION OF TWO PROMISING GLADIOLUS (*Gladiolus grandiflorus* L.) VARIETIES

BY

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ABSTRACT OF THE THESIS
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ABSTRACT

Studies were conducted to evolve protocol for the *in vitro* clonal propagation of *Gladiolus grandiflorus* L. varieties during 2000-2001 in the Department of Pomology and Floriculture, College of Agriculture, Vellayani.

Two varieties Peach Blossom and Tropic Seas were selected for the study. Cormels were used as explant. The effects of culture medium (basal medium, strength of MS basal medium, mode of culture, plant growth substances, carbon sources, activated charcoal, solidifying agent, amino acids and coconut water) and culture conditions on *in vitro* shoot proliferation *via* enhanced release of axillary buds were studied.

MS medium supplemented with kinetin 2.00 mg l^{-1} and NAA 0.10 mgl^{-1} induced earliest bud initiation in both the cultivars, in the initial culture establishment medium.

Highest shoot proliferation in both the varieties was obtained in full strength solid MS medium supplemented with BA 4.00 mg l^{-1}, NAA 0.50 mg l^{-1} and sucrose 40.00 g l^{-1} under light.

In vitro rooting in cultivar Peach Blossom was best obtained in MS medium supplemented with IBA 2.00 mg l^{-1} and sucrose 30.00 g l^{-1}. In the cultivar Tropic Seas, *in vitro* rooting was best in MS medium supplemented with IAA 2.00 mg l^{-1} and sucrose 40.00 g l^{-1}.

The different levels of agar tried had no significant effect on multiple shoot proliferation. Similarly activated charcoal, coconut water and amino acids (glycine and arginine) had no beneficial effect on multiple shoot proliferation.

Ex vitro rooting studies were not successful.

Planting out of *in vitro* rooted plantlets in sand : soil (2 : 1) media recorded a survival rate of 100.00 per cent, after 15 days.