EX VITRO ESTABLISHMENT OF GLADIOLUS (*Gladiolus grandiflorus* L.)

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DECLARATION

I hereby declare that this thesis entitled "***Ex vitro* establishment of Gladiolus (*Gladiolus grandiflorus* L.)**" 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.

Vellayani,
19.10.2004

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CERTIFICATE

Certified that this thesis entitled "***Ex vitro* establishment of Gladiolus (*Gladiolus grandiflorus* L.)**" is a record of research work done independently by Ms. Sheena, A. (2002-12-02) 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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LIST OF ABBREVIATIONS

AMF	–	Arbuscular mycorrhizal fungi
BA	–	Benzyl adenine
cm	–	Centimetre
cm/s	–	Centimetre per second
CD	–	Critical difference
CGR	–	Crop growth rate
cm^2	–	Square centimetre
CRD	–	Completely Randomised design
DAP	–	Days after planting
°C	–	Degree Celsius
Fig.	–	Figure
FYM	–	Farmyard manure
g	–	Gram
HCl	–	Hydrochloric acid
IBA	–	3-Indole butyric acid
KOH	–	Potassium hydroxide
LAI	–	Leaf area index
MS	–	Murashige and Skoog
mg	–	Milligram
mg/l	–	Milligram per litre
µg	–	Microgram
NAA	–	α - Naphthalene acetic acid
p.s.i	–	Pounds per square inch
%	–	Per cent
Rs.	–	Rupees
RGR	–	Relative growth rate
v/v	–	Volume by volume

CONTENTS

INTRODUCTION

1. INTRODUCTION

Gladiolus is an important cut flower crop belonging to the family Iridaceae. It is popular in many parts of the world due to the beauty and vase life of the inflorescence. The elegant spikes are widely used in flower arrangement, bouquet making and other floral decorations. It is also valued for use in herbaceous borders, beddings, rockeries and for pots. In India, gladiolus is the major bulbous ornamental cultivated in an area of 1200 ha (Mishra and Pathania, 2002).

Gladiolus can be successfully cultivated in Kerala conditions (Suneetha, 1994). It is traditionally propagated by corms and cormels. But under the climatic conditions of our State, the multiplication of corms and cormels is at a very slow pace and it cannot satisfy the demand for planting materials for large-scale cultivation. Dormancy of the cormels coupled with diseases in storage as well as under field condition creates problems in multiplication of planting material. Tissue culture technique, which enables large-scale production of disease free plants, will help in overcoming scarcity of planting material of gladiolus. The technique is based on *in vitro* culture of various explants capable of regeneration on suitable media as reported by several workers (Ziv *et al.*, 1970; Hussey, 1977; Bajaj *et al.*, 1983; Logan and Zettler, 1985; Hussain, 1995; Misra and Singh, 1999; Pathania *et al.*, 2001).

The technique for micropropagation has been standardized. However, problems have been encountered in *ex vitro* establishment of plantlets. The physiological and anatomical characteristics of micropropagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field. Normal development of micropropagated plantlets during acclimatization and hardening stage is mandatory to ensure a high per cent of survival after

transplanting to *ex vitro* conditions. Hardening and *ex vitro* establishment of plantlets are the most difficult stages in the micropropagation of gladiolus (Razdan, 2003). Micropropagation on a large scale can be successful only when plantlets after transfer from culture to soil show high survival rates. Standardizing the techniques for *ex vitro* establishment of tissue culture plantlets of gladiolus will streamline the supply of elite planting material in sufficient number for large-scale cultivation in the state.

The present study was undertaken with the objective of standardizing the *ex vitro* establishment techniques with different treatments involving potting media, growth retardant and mycorrhizae for easy establishment so as to reduce the mortality rate of tissue culture plantlets of gladiolus.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Micropropagation has become a main tool for large-scale multiplication of horticultural crops nowadays. But the survival of plantlets on field transfer is problematic in many crops. Considerable efforts have been directed to optimize the conditions for *in vitro* stages of micropropagation, but the process of acclimatization and *ex vitro* establishment of micropropagated plants has not merited the attention it deserves. In a crop like gladiolus where corm dormancy is a major hindrance in propagation, multiplication by tissue culture methods assume great significance. But hardening and *ex vitro* establishment of plantlets need further refinement.

This review encompasses the research work on various aspects of *ex vitro* establishment of tissue cultured plantlets with reference to potting media, mycorrhizae and growth retardant.

2.1 *IN VITRO* CULTURE OF GLADIOLUS

Hussey (1975) reported that MS medium was ideal for the *in vitro* propagation of members of the family Iridaceae, Liliaceae and Amaryllidaceae, if supplemented with growth factors. MS medium as the ideal basal medium for gladiolus was reported by several workers (Ziv, 1979; Rao *et al.*, 1991; Hussain, 1995; Pathania *et al.*, 2001).

Dehusked intact cormels were identified as successful explant in gladiolus (Nagaraju and Parthasarathy, 1995). Cormel shoot tip was also identified as an excellent explant in gladiolus (Misra and Singh, 1999).

MS medium containing IBA 4.00 mg l^{-1} was best for rooting of separated shoots of gladiolus (Arora and Grewal, 1990). BA 4.00 mg l^{-1} + NAA 0.50 mg l^{-1} resulted in the production of highest number of shoots. IBA 2.00 mg l^{-1} and IAA 2.00 mg l^{-1} induced earliest rooting in cv. Peach Blossom and Tropic Seas respectively (Priyakumari, 2001).

2.2 CHARACTERISTICS OF *IN VITRO* GROWN PLANTS INFLUENCING *EX VITRO* ESTABLISHMENT

Grout and Aston (1978) reported that the transition zone between shoot and root was abnormal in micropropagated cauliflower plants. A continuous vascular connection between the shoot and root was critical for efficient water flow for reducing the mortality during stress conditions. Debergh and Maene (1981) found that *in vitro* produced roots die soon after transfer and new *in vitro* adapted roots are quickly produced to sustain the plant to the non-sterile environment.

Leaves existing in culture made only small or negative photosynthetic contribution following transplanting and the first new leaves formed after transplanting had an intermediate photosynthetic capacity. Only new leaves initiated following transfer to soil had full photosynthetic competence and successful acclimatization was dependent on such leaves (Donnelly and Vidaver, 1984; Grout and Millam, 1985). Smith *et al.* (1986) observed that the *in vitro* plantlets are having diminished stature and reduced cell size during the period of acclimatization. He also opined that high rate of water loss incurred by *in vitro* shoots impose severe limitation in acclimatization. Fabbri *et al.* (1986) reported that persistent leaves of tissue cultured strawberry plantlets formed during acclimatization exhibit anatomical characteristics intermediate between that of *in vitro* formed leaves and that of green house grown plants. The stomata are unable to close and as cuticular wax in the leaf surface is minimal, stomata are unable to control water loss. Improper development of vascular connection between shoots and roots may cause poor establishment of the plantlets (Langford and Wainwright, 1987). Short *et al.* (1987) reported that the plantlets cultured at 80 per cent relative humidity have increased wax deposition on their leaves and they showed better *ex vitro* establishment because of reduced water loss.

Reuther (1988) observed that the roots formed *in vitro* had no root hairs and physiologically, the leaves of plantlets grown *in vitro* are incapable of significant photosynthesis. Lee *et al.* (1988) reported that the leaves of plantlets developed *in vitro* are smaller and thinner than those developed *in vivo*. Roots produced in agar are hairless, easily damaged on transfer and have limited ability to function in composts (Wainwright, 1988). The capability of *in vitro* stomata to adapt to the new environmental conditions by modifying guard cells during acclimatization enlighten the role of stomata in the death of micropropagated *Prunus cerasus* plants after transfer to external environment (Marin *et al.*, 1988). Blanke and Belcher (1989), showed that small humidity gradient between the intercellular leaf space and the saturated atmosphere was responsible for the poor development of morphological stature of leaves and lack of stomatal functioning in tissue cultured apple, which in turn resulted in high mortality of plantlets after transfer to the *ex vitro* conditions. Ziv and Ariel (1992) reported that the failure of stomata in vitreous leaves to close, resulted from structural changes in the cell wall and it caused problems in survival and quality of micropropagated plants. During the initial stages of acclimatization of micropropagated plantlets, *in vitro* leaves are the only source to cover metabolic demands and to sustain the plant's adaptation and regrowth. However, the way these leaves act can differ depending on plant species and *in vitro* conditions (Huylenbroeck *et al.*, 1998).

With the formation of new leaves, the photosynthetic activity of the persistent leaves decreased and their function was taken over by the newly formed ones (Borkowska, 2001). Stomata of *in vitro* developed leaves closed slowly and the number of stomata of newly developed leaves decreased during acclimatization. *In vitro* propagated roots generally lose their hairs during acclimatization but they develop new fully functional roots from them (Vegvari, 2003). Water loss from leaves of *in vitro* cultured plantlets was much higher than that of acclimatized plants or seedlings. Leaves of *in vitro* grown plants showed open stomata and

collapsed guard cells, while acclimatized leaves presented closed stomata. Stomatal density, stomatal aperture and guard cell protuberance decreased during the acclimatization period (Romano and Martins-Loucao, 2003).

2.3 ACCLIMATIZATION AND ITS IMPORTANCE IN *EX VITRO* ESTABLISHMENT

The survival and growth of *in vitro* propagated plants after removal from culture is a main problem in micropropagation. The acclimatization period between transplanting *in vitro* regenerated plantlets and their full establishment under greenhouse conditions is complex and often results in low rates of plantlet survival (Wardle *et al.*, 1983). If survival is achieved, growth rates are generally poor leading to delay in attainment of completely acclimatized plants (Lakso *et al.*, 1986). During *ex vitro* acclimatization, plantlets are transferred from an *in vitro* controlled environment to an external uncontrolled environment, where they have to establish normal photosynthetic activity and water relations (Desjardins *et al.*, 1987). According to Lilien-Kipnis and Kochba (1987) serious acclimatization problems were encountered in gladiolus owing to impeded root development *ex vitro* at higher temperature.

Acclimatization prior to transplantation of micropropagated plants is essential as it is the process which hardens the plants to survive after transfer to field. Different processes of acclimatization are necessary for different plant genera (Agarwal and Dutta, 1990). In the greenhouse and in the field, the irradiance is much higher and humidity much lower than in culture vessels. Even if the water potential of the substrate is higher than the water potential of the media with sucrose, the plantlets may quickly wilt as water loss from their leaves is not restricted. In addition water supply can be limiting because of low hydraulic conductivity of roots and root-stem connections (Fila *et al.*, 1998). During *in vitro* culture, plantlets grown in relatively air tight vessels with higher humidity and lower irradiance cause problems in *ex vitro* establishment (Pospisilova *et al.*, 1999).

The control of transpiration during early stage after transplanting plays a key role in the acclimatization process and photoautotrophic conditions could be a solution to solve the problems associated with transplantation stress (Jeong-Hoon *et al.*, 2000).

The benefit of any micropropagation system can, however, only be fully realized by the successful transfer of plantlets from tissue culture vessel to the ambient conditions found *ex vitro*. Most species grown *in vitro* require an acclimatization process in order to ensure that sufficient number of plants survive when transferred to soil (Hazarika, 2003). Two major strategies involved in the successful acclimatization are encouraging micropropagated plants to become autotrophic and reducing water stress by changing the culture environment (Rohr *et al.*, 2003).

The transplanting losses during acclimatization were higher for plantlets derived from *in vitro* rooted microcuttings. Acclimatization in fog favoured the survival rate and reduced transplanting losses (Hatzilazarou, 2003). During acclimatization, physiological and structural changes allow micropropagated plants to adapt to the new environmental conditions, mainly to low relative humidity and high light intensity. As a result, plants become autotrophic and develop as normal plants. However acclimatization is not easy in many species obtaining low survival percentage (Marin, 2003). Since both the anatomy and morphology of leaves and the physiology of the *in vitro* derived plantlets are different from those of the normal plants, it is important to consider these differences while acclimatizing them. Hence, hardening plays a vital role in the survival and establishment of the *in vitro* derived plantlets to the *in vivo* condition (Indumathi *et al.*, 2003).

2.4 FACTORS AFFECTING *EX VITRO* ESTABLISHMENT

2.4.1 Potting Media

Rao *et al.* (1991) cultured cormel shoot tips of gladiolus cv. Vinks Glory and successfully transferred the plantlets to pots containing FYM,

sand and soil (2:1:1 v/v). Ajithkumar (1993) observed that plastic pot grown anthurium plants and soil grown plants recorded maximum number of leaves during *ex vitro* establishment. Soilrite grown anthurium plants gave maximum leaf area during third and fourth week after transplanting.

Micropropagated rose plantlets planted in sand and soilrite potting mixtures recorded 62.5 per cent survival three weeks after root induction. But there was no survival after four weeks (Wilson *et al.*, 1997). *In vitro* rooted plantlets of *Gladiolus carneus* were successfully hardened off in a sand : soil (2 :1) mix in a mist house for four weeks and then transferred to greenhouse (Jager *et al.*, 1998). A sterilized potting mixture containing FYM + sand + loam (2 : 1 : 1 v/v) resulted in 60 per cent survival of *in vitro* propagated plantlets of gladiolus cv. American Beauty after transplantation (Misra and Singh, 1999).

Different substrates with different nutrient status, aeration and water holding capacity have different influence on growth of mycorrhizal fungi and plant growth (You-Shan *et al.*, 2001). *In vitro* rooted plantlets of dracaena were transferred to glass jars containing a medium of peat + soilrite (1 : 1 v/v) and more than 95 per cent survival was achieved (Singh *et al.*, 2001). Hundred per cent survival of micropropagated carnation was reported in soil rite (peat + perlite 1 : 1) due to the optimum conditions of aeration, water holding capacity and nutrients present in the medium (Jagannatha *et al.*, 2001). *In vitro* rooted *Acorus calamus* plantlets transferred to pots registered 80-90 per cent survival and were transferred to the field after one month (Anu *et al.*, 2001). Asiatic hybrid lily plantlets were transferred to pots containing a potting mixture of leaf mould : sand (3:1) and after hardening bulblets of 0.5 to 1.0 cm diameter were planted in the field (Misra and Datta, 2001). Micro corms of gladiolus formed *in vitro* gave higher survival rate than the rooted plantlets when transferred to soil (Pathania *et al.*, 2001). Priyakumari (2001) reported 100 per cent survival of gladiolus cultivar Tropic Seas and Peach Blossom plantlets after 15 days of planting out in a 1:1 sand :soil mixture.

Salvi (2002) reported 95 per cent survival of micropropagated turmeric plantlets planted in paper cups filled with sterilized soil. Pot mixture significantly influenced the rate of survival of micropropagated *Mussaenda phillipica* plantlets. Maximum survival of 83.3 per cent was obtained in pots with sterilized mixture of sand, FYM and loam soil (1:1:1) and minimum survival of 33.3 per cent in sand (Mishra and Tiwari, 2002). *In vitro* grown pineapple plantlets were successfully transferred to cups with soil or soilrite and after six weeks transplanted to field. Field grown plants showed 90 per cent survival (Soneji *et al.*, 2002).

In vitro grown micro shoots of Chrysanthemum were successfully transferred to pots containing 1:1 mixture of sand and soil (Meenakumari and Varghese, 2003). The success rate of transplantation of gerbera plantlets was 95 per cent when sand : FYM : red soil (1:1:1) was used as potting media (Aswath *et al.*, 2003). Singh and Dubey (2003) obtained eighty three per cent survival when micropropagated rose shoots with 2-3 roots were transferred to a 1:1 medium of soil and leaf mould. Potting media in different combination were better than soil alone. Different combinations of potting media produced more growth and vigour of *Scindaspus aureus* plantlets (Ahmad and Qasim, 2003). Indumathi *et al.* (2003) reported that for *Dendrobium* hybrid Sonia-17, the medium consisting of charcoal + brick + cocopeat (1:1:1) produced maximum plant height, leaf size and root length.

Non-hyperhydrated plantlets of *Globularia alypum* L. were acclimatized with 100 per cent success on peat + perlite (1:1 v/v); increasing the perlite ratio in the medium decreased the ability of acclimatization (Bertsouklis *et al.*, 2003). In a 1:1 peat - perlite medium, 37.5 per cent of micropropagated citrus plantlets formed lateral roots. On soil : manure : peat (1:1:1) mixture 12.5 per cent of the plantlets died and those that survived formed a poor root system. The plants grown on sand, peat, sand - perlite (1:1) and peat - perlite (1:1), developed a good root

system (Plastira and Karetsos, 2003). Chan *et al.* (2003) obtained 93 per cent survival for *in vitro* rooted *Musa sapientum* plantlets after being acclimatized in intermittent mist house for 10-14 days.

Good vessel ventilation combined with artificial substrate perlite was beneficial for acclimatization of *in vitro* rooted *Phillyrea* plantlets (Lucchesini and Mensuali-Sodi, 2004). *Aloe vera* plantlets planted in plastic pots containing soil + FYM (1:1) were kept in a greenhouse for 10 days and survived when shifted to a shade house (Aggarwal and Barna, 2004). *In vitro* rooted shoots of gerbera were transferred to pots of 10 cm diameter containing FYM : sand (1:1) and covered with jars to maintain high relative humidity. After 14 weeks, jars were removed and plants were kept in glass house and gradually exposed to normal conditions (Kumar *et al.*, 2004).

2.4.2 Arbuscular Mycorrhizal Fungi (AMF)

The micropropagation technique does not take into consideration the existence of mutualistic symbiosis of mycorrhiza and other associative plant growth promoting fungi. The media used are devoid of symbiotic propagules and plantlets obtained from these systems are not associated with any friendly fungi and bacteria. However, early inoculation of these plants with appropriate symbiotic organisms like AMF improves plant survival and performance (Sahay and Varma, 2000).

2.4.2.1 Effect of AMF on Survival of Micropropagated Plants

Cent per cent of micropropagated *Leucaena leucocephala* plantlets survived in AMF inoculated soil where as only 20 per cent of plantlets survived without inoculation (Puthur *et al.*, 1988). Inoculation of potting medium with *Glomus etunicatum* and *Glomus fasciculatum* favoured 100.00 and 80.00 per cent *ex vitro* establishment of micropropagated jack plantlets. In the treated plants, plant height, fresh weight, dry weight, number of leaves and total leaf area were significantly increased (Ramesh, 1990).

Micropropagated plantlets of Avocado exhibited a very slow rate of growth during acclimatization phase. Inoculation of plantlets with vesicular arbuscular mycorrhizal fungus *Glomus fasciculatum* improved the formation of a well developed root system and helped the plantlets to tolerate environmental stress at transplanting (Vidal *et al.*, 1992). Various strains of mycorrhizal fungi caused varied survival rate in micropropagated gerbera, nephrolepis and syngonium (Wang *et al.*, 1993). Schultz *et al.* (1998) reported that survival rate of micropropagated oil palm clones during acclimatization was increased from 70 per cent to 90-95 per cent when vesicular arbuscular fungi was inoculated. Sato *et al.*(1999) inoculated heliconia and pot gerbera plants with AMF. Although colonization was high, heliconia did not benefit from inoculation. However gerbera benefited from inoculation regarding survival, fresh and dry weights. Micropropagated banana plantlets inoculated with mycorrhizal fungi colonized well in the roots and increased the growth of plants (Severn-Ellis, 1999; Trindade *et al.*, 2003; Lins *et al.*, 2003).

2.4.2.2 Effect of AMF on Biometric Characters

Introduction of the mycorrhizal fungus at the time plantlets were transferred from *in vitro* condition to *ex vitro* conditions improved shoot and root growth, enhanced shoot : root ratio and increased concentration of N, P and K in micropropagated avocado plants (Vidal *et al.*, 1992). AMF inoculated plantlets of jack recorded significantly higher plant height, shoot and root fresh weight. *Glomus mosseae* was most effective in stimulating plant growth (Sivaprasad *et al.*, 1995).

Leaf area was found to be higher in *Glomus fasciculatum* and *Glomus mosseae* associated plants in mulberry due to high P uptake (Setua *et al.*, 1999). Micropropagated banana plantlets inoculated with AMF had greater height, leaf area and fresh weight of shoots and roots, as well as higher rates of photosynthesis and transpiration (Yano-Melo *et al.*, 1999). Hernandez-Sebastia *et al.* (1999) reported that stomatal conductance of

strawberry plantlets was affected by inoculation of *Glomus intraradices*. Root colonization by *Glomus intraradices* modified water status, control of water losses and osmotic relations of leaves and roots of micropropagated strawberry plantlets under *in vitro* conditions. Micropropagated banana plantlets raised in soil inoculated with *Glomus mosseae* and amended with 50 per cent phosphorus fertilizer showed an increase in plant height, number of leaves, stem girth, root volume and total biomass (Shashikara *et al.*, 1999).

Estrada-Luna (2000) reported that micropropagated guava plantlets inoculated with mycorrhiza had greater shoot growth rates, leaf production rates, shoot length, leaf area and dry matter production. During the acclimatization period, Chile ancho pepper plantlets inoculated with AMF showed decreased stomatal conductance and increased stomatal resistance (Estrada-Luna *et al.*, 2001).

Better water transpiration efficiency by increased number and size of stomata was reported in leaves of *Simarouba glauca* due to inoculation of *Glomus mosseae* by Sailo *et al.* (2002). Micropropagated banana plants colonized by AMF had larger shoot and root dry weight and P content than non-mycorrhizal plants (Declerck *et al.*, 2002). Varshney *et al.* (2002) reported that micropropagated onion bulblets inoculated with AMF showed better shoot weight, shoot length, number of leaves and leaf area than uninoculated plants.

Shoot growth depression and low root colonization was observed during acclimatization of micropropagated persimon plantlets when inoculated with *Glomus mosseae*. Shoot and root growth enhanced when *Glomus intraradices* was inoculated (Marin *et al.*, 2003). Significant increase in the overall growth including shoot, root and leaf parameters was observed in micropropagated banana plantlets inoculated with AMF. Prolific mycelium of AMF with its small diameter several folds smaller as compared to the root hairs of the host, was able to penetrate into those areas that are otherwise not accessible by the root hairs of the host. The plant

nutrient uptake thereby increased leading to improved growth (Thaker and Jasrai, 2003).

2.4.3 Triazoles

2.4.3.1 Triazoles on Survival of Micropropagated Plants

Davis *et al.* (1986) reported that triazole treatment while transplanting to greenhouse cause better survival of ornamental plants. Triazoles are reported to protect plants from injury due to various stresses, including low and high temperature, drought and air pollutants (Fletcher *et al.*, 1986). Smith *et al.* (1990) reported that growth retardants reduced damage due to wilting while *ex vitro* establishment. Ziv (1991) reported that addition of paclobutrazol to the medium resulted in the formation of cormels and 100 per cent survival in gladiolus following transfer to green house. Addition of paclobutrazol to the rooting medium enhanced dessication tolerance of micropropagated chrysanthemum and rose (Robertz *et al.*, 1992).

Hazarika *et al.* (2002a) reported that paclobutrazol, a triazole compound in the rooting medium of citrus reduced plant growth and led to reduction in wilting when plantlets were transferred to greenhouse. Addition of paclobutrazol 1 mg l^{-1} in the growth medium also increased survival of citrus plantlets. Triadimefon, a triazole fungicide, upon application either as a foliar spray or pre-sowing soaking treatment increased several morphological and yield parameters in both dicots and monocots (Devetha *et al.*, 2003).

2.4.3.2 Effect of Triazoles on Biometric Characters

Novello *et al.* (1992) reported that micropropagated grape vine plantlets grown in vessels with reduced relative humidity and with paclobutrazol showed a rapid initial reduction in leaf conductance followed by a more gradual reduction. Bishnoi *et al.* (1994) reported that growth retardants decreased the stomatal conductance of plants.

In vitro application of triadimefon (2 mg l^{-1}) obviated the need of hardening micropropagated banana plantlets under a polythene tent. Triadimefon treated plants were turgid and healthy compared with control plants when transferred to 1:1 mix of peat and sand (Murali and Duncan, 1995). Triadimefon increased tillers $plant^{-1}$, green leaves $plant^{-1}$ and leaf area $plant^{-1}$ though individual leaf size was slightly reduced in wheat (Sairam *et al.*, 1995).

In micropropagated sugarcane, higher root length was obtained by treatment with 2 µg of triadimefon. Root dry weight was greatest with 20 microgram paclobutrazol and 2 µg triadimefon. Leaf area index was highest after treatment with 15 mM calcium chloride followed by 2 mM triadimefon (Dhaliwal *et al.*, 1997). Triadimefon 10 mg/l increased shoot diameter, thickness of collenchyma, phloem and xylem thickness and overall diameter of vascular bundles of China aster (Nagarajaiah *et al.*, 1997).

Sujatha *et al.* (1999) reported that triadimefon treatment ameliorated the deleterious effects of sodium chloride salinity stress on bhindi seedlings. Triadimefon 5 and 10 mg l^{-1} and paclobutrazol 5 and 20 mg l^{-1} increased net photosynthetic rate of *Panicum miliaceum*. Transpiration rate and stomatal resistance showed definite correlation with the increase in net photosynthetic rate (Bisht *et al.*, 2000).

Samasya (2000) reported that CGR, NAR and RGR of micropropagated orchid plantlets treated with 5 mg l^{-1} of triazole performed better than non-treated plants. However, towards the later stages these plants were on par with that of normal plants.

Triadimefon enhanced length of root, shoot, number of leaves, total leaf area, leaf area index, fresh and dry mass of leaves and shoot, relative growth rate and net productivity of greengram (Rajendiran and Ramanujan, 2003). Paclobutrazol inhibited the stem elongation and primary leaf

expansion of bean seedlings. The stomatal density increased on both surfaces and water loss was reduced due to reduced leaf area (Tari, 2003).

2.4.3.3 Effect of Triazole on AMF

Sugarcane plants treated with triadimefon and *Glomus clarum* showed greater plant weight than plants treated with triadimefon only (Reis *et al.*, 1994).

The share of ectomycorrhizas with *Hebeloma crustuliniforme* in the root systems of scots pines treated with triadimefon was three times greater than in control plants. Average growth of mycorrhiza inoculated and non-inoculated plants where triadimefon applied were not much different (Trzcinska, 2001). Application of triadimefon did not affect the colonization of beneficial fungus such as AMF (Devetha *et al.*, 2003).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Investigations were carried out at the Department of Pomology and Floriculture, College of Agriculture, Vellayani with the objective of standardizing *ex vitro* establishment techniques in gladiolus during the year 2002-2004. The materials and methods tried for the *ex vitro* establishment study are described in this chapter.

3.1 MATERIALS USED

The gladiolus variety chosen for the experiment was Vinks Glory. *In vitro* rooted plantlets of Vinks Glory comprised the experimental material. These plantlets were produced based on the following protocol. Cormels were collected from field grown gladiolus plants. Cormels were dehusked and immersed in 1000 times diluted labolene solution for 30 minutes, washed thoroughly in running tap water for five minutes and then in distilled water. These cormels were surface sterilized with 0.08 per cent mercuric chloride for ten minutes with intermittent shaking inside a laminar air flow chamber. The solution was drained and cormels were washed four to five times with sterile distilled water. Cormels were inoculated in MS medium (Murashige and Skoog, 1962) with BA 2.00 mg/l and NAA 0.50 mg/l and multiple shoot initiation was obtained (Plate 1). After 2-3 subcultures, individual shoots measuring 2.50-3.50 cm length excised from the shoot proliferating cultures were subjected to *in vitro* rooting in IBA 2.00 mg/l (Plate 2). Composition of MS media is given in Appendix I.

3.2 METHODS

3.2.1 Design of the Experiment

The experiment was laid out in CRD with six replications.

Plate 1. Cormel of gladiolus showing multiple shoot initiation in the medium supplemented with BA 2.00 mg l^{-1} + NAA 0.50 mg l^{-1}

Plate 2. Plantlets of gladiolus subjected to *in vitro* rooting in the medium supplemented with IBA 2.00 mg l^{-1}

3.2.2 Treatments

3.2.2.1 Potting Media

Five different potting media were prepared using different proportions of sand, soilrite, soil, coirpith and leaf mould. The composition of potting media are furnished in the following table.

Table 1 Composition of potting media

Treatments	Potting media	Composition
A_1	Sand	
A_2	Soilrite	
A_3	Sand : soil	1:1
A_4	Sand : Soil: coirpith	1:1:1
A_5	Sand : Soil : Leaf mould	1:1:1

The potting media were sterilized by autoclaving them at 15 p.s.i for 45 minutes.

3.2.2.2 Growth Retardant

B_0 - control

B_1 - 4 mg/l

B_2 - 8 mg/l

4 mg/l and 8 mg/l solutions of a triazole compound, Triadimefon were prepared and applied to the potting media.

3.2.2.3 Mycorrhizal Fungi

C_1-*Glomus fasciculatum*

C_2-*Glomus monosporum*

The *in vitro* grown plantlets were planted in sterile potting media inoculated with a mixture of 5 g AMF infected root bits of guinea grass.

3.2.2.4 Height of Potting Media

D_1- 4 cm

D_2 - 6 cm

Height of the potting media inside the container was maintained at 4 cm and 6 cm levels.

3.2.3 Treatment Combinations

Treatment combinations are given in Table 2.

3.2.4 *Ex vitro* Establishment

The culture vessels with *in vitro* rooted plantlets were opened and plantlets were taken out using sterilized forceps. The agar adhering to the roots were completely removed by thorough washing. For this first the plantlets were kept under running tap water and then washed in distilled water. During all these processes, care was taken for not damaging the roots. The plantlets were subjected to a fungicide treatment in 0.1 per cent Indofil (Dithane M- 45) by dipping in it for a period of 10 minutes. These plantlets were planted in disposable cups filled with sterilized potting media and subjected to different treatments (Plate 3 and 4). The plants were kept inside a humidity chamber covered with polythene sheets of 350 gauge thickness for a period of 30 days (Plate 5).

3.2.5 Aftercare of Plantlets

After 15 days of planting out, the plantlets were irrigated twice a week with 0.1 MS solution. Humidity inside the chamber was adjusted by lifting the polythene sheet and plantlets were gradually exposed to sunlight. After the observation period, some of the plants were transferred to pots and placed outside (Plate 6).

Table 2 Treatment combinations

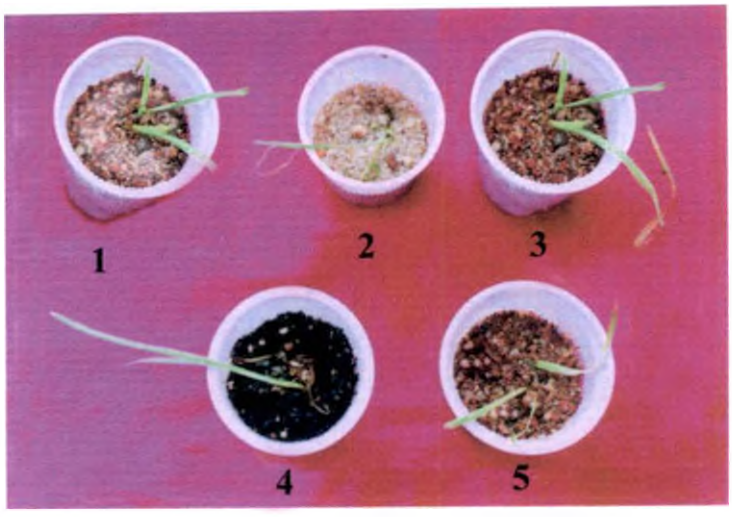
Treatments	Combinations
$A_1B_0C_1D_1$	Sand; Triazole 0; *Glomus fasciculatum*; Height 4 cm
$A_1B_0C_1D_2$	Sand; Triazole 0; *Glomus fasciculatum*; Height 6 cm
$A_1B_0C_2D_1$	Sand; Triazole 0; *Glomus monosporum* ; Height 4 cm
$A_1B_0C_2D_2$	Sand; Triazole 0; *Glomus monosporum*; Height 6 cm
$A_1B_1C_1D_1$	Sand; Triazole 4 mg/l; *Glomus fasciculatum*; Height 4 cm
$A_1B_1C_1D_2$	Sand; Triazole 4 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_1B_1C_2D_1$	Sand; Triazole 4mg/l; *Glomus monosporum*; Height 4 cm
$A_1B_1C_2D_2$	Sand; Triazole 4 mg/l; *Glomus monosporum*; Height 6 cm
$A_1B_2C_1D_1$	Sand; Triazole 8 mg/l; *Glomus fasciculatum*; Height 4 cm
$A_1B_2C_1D_2$	Sand; Triazole 8 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_1B_2C_2D_1$	Sand; Triazole 8 mg/l; *Glomus monosporum*; Height 4 cm
$A_1B_2C_2D_2$	Sand; Triazole 8 mg/l; *Glomus monosporum*; Height 6 cm
$A_2B_0C_1D_1$	Soilrite; Triazole 0; *Glomus fasciculatum*; Height 4 cm
$A_2B_0C_1D_2$	Soilrite; Triazole 0; *Glomus fasciculatum*; Height 6 cm
$A_2B_0C_2D_1$	Soilrite; Triazole 0; *Glomus monosporum* ; Height 4 cm
$A_2B_0C_2D_2$	Soilrite; Triazole 0; *Glomus monosporum*; Height 6 cm
$A_2B_1C_1D_1$	Soilrite; Triazole 4 mg/l; *Glomus fasciculatum* ; Height 4 cm
$A_2B_1C_1D_2$	Soilrite; Triazole 4 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_2B_1C_2D_1$	Soilrite; Triazole 4 mg/l; *Glomus monosporum*; Height 4 cm
$A_2B_1C_2D_2$	Soilrite; Triazole 4 mg/l; *Glomus monosporum*; Height 6 cm
$A_2B_2C_1D_1$	Soilrite; Triazole 8 mg/l; *Glomus fasciculatum*; Height 4 cm
$A_2B_2C_1D_2$	Soilrite; Triazole 8 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_2B_2C_2D_1$	Soilrite; Triazole 8 mg/l; *Glomus monosporum*; Height 4 cm
$A_2B_2C_2D_2$	Soilrite; Triazole 8 mg/l; *Glomus monosporum*; Height 6 cm
$A_3B_0C_1D_1$	Sand: Soil; Triazole 0; *Glomus fasciculatum*; Height 4 cm
$A_3B_0C_1D_2$	Sand: Soil; Triazole 0; *Glomus fasciculatum*; Height 6 cm

Table 2 Continued

Treatments	Combinations
$A_3B_0C_2D_1$	Sand: Soil; Triazole 0; *Glomus monosporum* ; Height 4 cm
$A_3B_0C_2D_2$	Sand: Soil; Triazole 0; *Glomus monosporum*; Height 6 cm
$A_3B_1C_1D_1$	Sand: Soil; Triazole 4 mg/l; *Glomus fasciculatum* ; Height 4 cm
$A_3B_1C_1D_2$	Sand: Soil; Triazole 4 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_3B_1C_2D_1$	Sand: Soil; Triazole 4mg/l; *Glomus monosporum*; Height 4 cm
$A_3B_1C_2D_2$	Sand: Soil; Triazole 4 mg/l; *Glomus monosporum*; Height 6 cm
$A_3B_2C_1D_1$	Sand: Soil; Triazole 8 mg/l; *Glomus fasciculatum*; Height 4 cm
$A_3B_2C_1D_2$	Sand: Soil; Triazole 8 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_3B_2C_2D_1$	Sand: Soil; Triazole 8 mg/l; *Glomus monosporum*; Height 4 cm
$A_3B_2C_2D_2$	Sand: Soil; Triazole 8 mg/l; *Glomus monosporum*; Height 6 cm
$A_4B_0C_1D_1$	Sand: Soil: coir pith; Triazole 0; *Glomus fasciculatum*; Height 4 cm
$A_4B_0C_1D_2$	Sand: Soil: coir pith; Triazole 0; *Glomus fasciculatum*; Height 6 cm
$A_4B_0C_2D_1$	Sand: Soil: coir pith; Triazole 0; *Glomus monosporum* ; Height 4 cm
$A_4B_0C_2D_2$	Sand: Soil: coir pith; Triazole 0; *Glomus monosporum*; Height 6 cm
$A_4B_1C_1D_1$	Sand: Soil: coir pith; Triazole 4 mg/l; *Glomus fasciculatum*; Height 4 cm
$A_4B_1C_1D_2$	Sand: Soil: coir pith; Triazole 4 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_4B_1C_2D_1$	Sand: Soil: coir pith; Triazole 4mg/l; *Glomus monosporum*; Height 4 cm
$A_4B_1C_2D_2$	Sand: Soil: coir pith; Triazole 4 mg/l; *Glomus monosporum*; Height 6 cm
$A_4B_2C_1D_1$	Sand: Soil: coir pith; Triazole 8 mg/l; *Glomus fasciculatum*; Height 4 cm
$A_4B_2C_1D_2$	Sand: Soil; Triazole 8 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_4B_2C_2D_1$	Sand: Soil: coir pith; Triazole 8 mg/l; *Glomus monosporum*; Height 4 cm

Table 2 Continued

Treatments	Combinations
$A_4B_2C_2D_2$	Sand: Soil: coir pith; Triazole 8 mg/l; *Glomus monosporum*; Height 6 cm
$A_5B_0C_1D_1$	Sand: Soil: Leaf mould; Triazole 0; *Glomus fasciculatum*; Height 4 cm
$A_5B_0C_1D_2$	Sand: Soil: Leaf mould; Triazole 0; *Glomus fasciculatum*; Height 6 cm
$A_5B_0C_2D_1$	Sand: Soil: Leaf mould; Triazole 0; *Glomus monosporum*; Height 4 cm
$A_5B_0C_2D_2$	Sand: Soil: Leaf mould; Triazole 0; *Glomus monosporum* Height 6 cm
$A_5B_1C_1D_1$	Sand: Soil: Leaf mould; Triazole 4 mg/l; *Glomus fasciculatum*; Height 4 cm
$A_5B_1C_1D_2$	Sand: Soil: Leaf mould; Triazole 4 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_5B_1C_2D_1$	Sand: Soil: Leaf mould; Triazole 4mg/l; *Glomus monosporum*; Height 4 cm
$A_5B_1C_2D_2$	Sand: Soil: Leaf mould; Triazole 4 mg/l; *Glomus monosporum*; Height 6 cm
$A_5B_2C_1D_1$	Sand: Soil: Leaf mould; Triazole 8 mg/l; *Glomus fasciculatum*; Height 4 cm
$A_5B_2C_1D_2$	Sand: Soil: Leaf mould; Triazole 8 mg/l; *Glomus fasciculatum*; Height 6 cm
$A_5B_2C_2D_1$	Sand: Soil: Leaf mould; Triazole 8 mg/l; *Glomus monosporum*; Height 4 cm
$A_5B_2C_2D_2$	Sand: Soil: Leaf mould; Triazole 8 mg/l; *Glomus monosporum*; Height 6 cm



1. Sand : soil
2. Sand
3. Sand : soil : coirpith
4. Soilrie
5. Sand : soil : leafmould

Plate 3. Gladiolus plantlets transferred to different potting media

Plate 4. Plantlets subjected to various treatments kept for acclimatization

Plate 5. Humidity chamber used for acclimatization of plantlets

Plate 6. Gladiolus plants established in pots, 90 days after planting

3.3 OBSERVATIONS

3.3.1 Survival of the plantlets

Survival of the plantlets were observed at fortnightly interval and survived plants were observed for further study.

3.3.2 Mycorrhizal Colonization

Mycorrhizal colonization pattern in the roots was studied according to the method described by Phillips and Hayman (1970). FAA solution was prepared (formaldehyde: Acetic acid: Ethanol @ 5:5:90) and gladiolus roots were cut into pieces of 1cm length and immersed in this solution and kept overnight. After draining this solution, ten per cent KOH solution was poured into the root bits, autoclaved for ten minutes for softening the roots so as to make them vulnerable to staining. After draining KOH solution, roots were treated with one per cent HCl for ten minutes for neutralizing the effect of KOH.

Trypan blue dye was prepared in 0.05 per cent lactophenol (lactic acid-20ml, phenol-20ml, glycerol-40ml, distilled water-40ml) for staining mycorrhiza. The roots were treated with the dye for one to five minutes. The dye was drained and the root bits were observed under microscope for mycelia, vesicles and arbuscules. At least 50 bits were examined for each treatment for better precision (Plates 7 and 8).

$$\text{AMF colonization (\%)} = \frac{\text{Number of root bits positive for AMF colonization}}{\text{Total number of root bits observed}} \times 100$$

3.3.3 Biometric Observations

All the biometric observations were recorded for a period of one month.

Plate 7. Roots of gladiolus showing mycelia of AMF, 45 days after planting

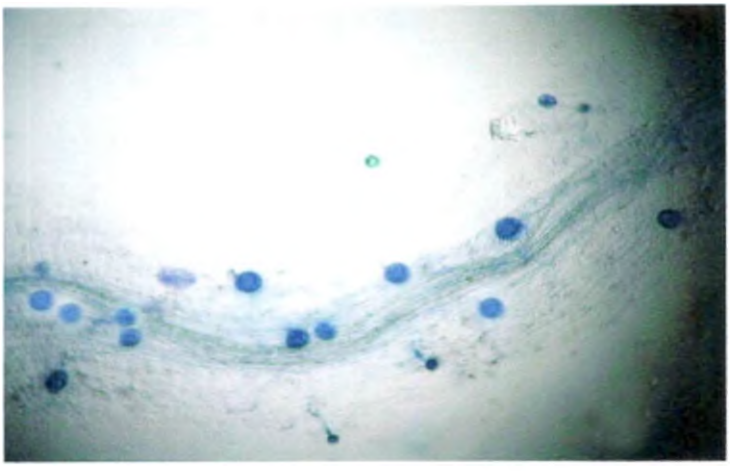


Plate 8. Roots of gladiolus showing vescicles of AMF, 45 days after planting

3.3.3.1 Height of the Plant

This was measured from the collar region to the tip and the mean length was expressed in cm. The observations were made at 15days interval.

3.3.3.2 Number of Leaves

The total number of fully opened leaves developed per plantlet was counted and the mean value was expressed at 15 days interval.

3.3.3.3 Number of Roots

The total number of roots per plantlet was counted and the mean value was expressed at 15 days interval.

3.3.3.4 Fresh Weight and Dry Weight

Fresh weight of individual plants were recorded at 15 days interval. These plants were dried at 70 °C for 48 hours and the dry weight was recorded and expressed as g plant^{-1}.

3.3.3.5 Phyllochron

Number of leaves produced were noted at weekly interval to find out phyllochron. Time taken for emergence of first and second *ex vitro* produced leaves were expressed in days.

3.3.4 Physiological Observations

3.3.4.1 Stomatal Conductance

Stomatal resistance of individual plants was recorded using a Porometer (Delta T devices-Cambridge-UK) at fortnightly interval during 10 to 12 am.

$$\text{Stomatal conductance} = \frac{1}{\text{Stomatal resistance}}$$

Stomatal conductance was expressed in cm/s.

3.3.4.2 Leaf Area Index

Leaf area index was calculated by employing the formula of Rajeevan *et al.* (1992)

Leaf area = length of leaf × breadth of leaf × 0.635 +12.9

$$\text{Leaf area index} = \frac{\text{Leaf area per plant}}{\text{Area occupied per plant}}$$

3.3.4.3 Crop Growth Rate (CGR)

The CGR was worked out by using the formula of Watson (1971) and expressed in mg cm^{-2} day^{-1}

$$CGR = \frac{W_2 - W_1}{p\,(t_2 - t_1)}$$

where W_1 and W_2 –Whole plant dry weight at t_1 and t_2 respectively.

t_1 and t_2- time in days

p - ground area on which W_1 and W_2 was estimated.

3.3.4.4 Relative Growth Rate (RGR)

The RGR was determined by utilizing the following formula (Williams, 1946) and expressed in mg g^{-1} day^{-1}.

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

W_1 and W_2 - plant dry weight at t_1 and t_2 respectively

t_1 and t_2 - time in days.

3.3.5 Economics of Acclimatization

Economics of acclimatization was worked out. In computing the cost involved, the prevailing market rates during 2003-2004 was considered.

3.3.6 Statistical Analysis

The data were statistically analysed as per the procedure outlined by Panse and Sukhatme (1995). Data regarding fresh weight, dry weight, CGR and RGR were analysed as per CRD. All other observations were analysed as per factorial CRD.

RESULTS

4. RESULTS

The results of the trials conducted on the *ex vitro* establishment of tissue cultured plantlets of gladiolus variety Vinks Glory is presented in this chapter. All the main effects and two factor interactions effects have been presented in this chapter. The mean values and three factor interactions are included in Appendices II – VI.

4.1 SURVIVAL OF THE PLANTLETS

Survival of the plantlets were observed at fortnightly interval for a month. The data regarding survival rate as influenced by potting media, triazole, mycorrhizae and height of potting media is given in Table 3.

Among the different potting media tried for *ex vitro* establishment, soilrite recorded highest survival per cent of 86.11 followed by sand with a survival of 77.77 per cent. The lowest survival per cent was obtained in sand : soil : coir pith (58.33) and sand : soil : leaf mould (59.72) at 15 DAP. One month after planting, soilrite recorded 80.55 per cent survival followed by sand (59.72 per cent). The lowest survival was recorded by sand : soil : leaf mould (41.66 per cent).

Application of triazole at a concentration of 4 mg/l showed 73.33 and 54.16 per cent survival 15 and 30 DAP respectively. Survival of plantlets recorded in 8 mg/l triazole application was 70.83 and 55.83 per cent at 15 and 30 DAP respectively. Compared to treated plants, untreated plants showed a lower survival per cent one month after planting.

Among the two mycorrhizae tried, *Glomus fasciculatum* inoculated plants recorded 66.11 per cent and 58.33 per cent survival 15 and 30 DAP respectively. Survival of *Glomus monosporum* inoculated plants were 64.44 and 52.77 per cent.

Table 3 Survival rate of plantlets as influenced by potting media, triazole, mycorrhizae and height of the potting media in the container

Treatment	Survival rate (%)	
	15 DAP	30 DAP
1. Potting media		
Sand (A_1)	77.77	59.72
Soilrite (A_2)	86.11	80.55
Sand : Soil (A_3)	72.22	52.77
Sand : Soil : Coirpith (A_4)	58.33	44.44
Sand : Soil : Leaf mould (A_5)	59.72	41.66
2. Triazole		
Control (B_0)	63.33	47.50
4 mg/l (B_1)	73.33	54.16
8 mg/l (B_2)	70.83	55.83
3. Mycorrhizae		
Glomus fasciculatum (C_1)	66.11	58.33
Glomus monosporum (C_2)	64.44	52.77
4. Height of potting media		
4 cm (D_1)	73.33	68.33
6 cm (D_2)	61.66	51.11

15 DAP, 73.33 per cent of plantlets survived in the potting media with a height of 4 cm. After 30 days it was 68.33 per cent. When height of potting media was 6 cm, 61.66 per cent and 51.11 per cent of plantlets survived 15 and 30 DAP respectively.

The data regarding survival rate of plantlets subjected to various treatment combinations are included in Appendix VII.

4.2 MYCORRHIZAL COLONIZATION

The data recorded on mycorrhizal colonization is presented in Table 4.

Glomus fasciculatum recorded 100 per cent colonization in treatments $A_1B_1D_1$ and $A_2B_1D_1$ and no colonization in the treatment $A_5B_0D_2$. Highest colonization recorded by *Glomus monosporum* inoculated plantlets was 94.10 per cent in the treatment $A_2B_1D_1$ while treatment $A_3B_2D_2$ recorded no colonization.

4.3 BIOMETRIC OBSERVATIONS

4.3.1 Leaf Number

4.3.1.1 Main Effects of the Factors on Leaf Number

The data is presented in Table 5.

The effect of potting media was not significantly different regarding leaf number during the observation period. Among the media, soil rite recorded the highest value for leaf number and sand : soil : leaf mould recorded the lowest leaf number both 15 and 30 days after planting.

Application of triazole compound did not show significant effect on leaf number. Control plants showed increase in leaf number compared to that of triazole treated plants. Highest value was recorded for treatments without triazole (4.70 and 5.60 respectively for 15 and 30 DAP).

Glomus fasciculatum and *Glomus monosporum* inoculation did not show significant difference in leaf number. But *Glomus monosporum*

Table 4 Mycorrhizal colonization percentage of gladiolus plants inoculated with *Glomus fasciculatum* and *Glomus monosporum*

Treatments	Colonization percentage	
	Glomus fasciculatum (C_1)	*Glomus monosporum* (C_2)
$A_1B_0D_1$	91.66	73.30
$A_1B_0D_2$	90.00	71.40
$A_1B_1D_1$	100.00	75.00
$A_1B_1D_2$	87.50	72.70
$A_1B_2D_1$	94.40	73.30
$A_1B_2D_2$	78.50	60.00
$A_2B_0D_1$	85.00	75.00
$A_2B_0D_2$	91.60	86.60
$A_2B_1D_1$	100.00	94.10
$A_2B_1D_2$	75.00	60.00
$A_2B_2D_1$	80.00	70.50
$A_2B_2D_2$	86.50	37.50
$A_3B_0D_1$	93.30	72.20
$A_3B_0D_2$	90.00	46.15
$A_3B_1D_1$	93.30	50.00
$A_3B_1D_2$	80.00	70.00
$A_3B_2D_1$	94.10	66.60
$A_3B_2D_2$	63.60	0.00
$A_4B_0D_1$	80.00	41.60
$A_4B_0D_2$	76.10	37.50
$A_4B_1D_1$	26.60	56.25
$A_4B_1D_2$	50.00	69.20
$A_4B_2D_1$	70.00	54.50
$A_4B_2D_2$	75.00	61.10
$A_5B_0D_1$	70.50	26.60
$A_5B_0D_2$	0.00	32.00
$A_5B_1D_1$	53.00	42.80
$A_5B_1D_2$	75.00	52.30
$A_5B_2D_1$	61.10	62.50
$A_5B_2D_2$	45.00	38.80

Table 5 Main effects of potting media, triazole, mycorrhizae and height of potting media on leaf number and plant height

Treatment	Leaf number		Plant height (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP
1. Potting media				
A_1	5.00	5.67	13.15	16.42
A_2	5.04	5.96	13.12	16.54
A_3	4.54	5.67	13.58	17.26
A_4	4.79	5.33	12.98	14.37
A_5	4.08	4.75	10.86	12.40
SE	0.25	0.29	0.30	0.67
CD (0.05)	NS	NS	0.85	1.91
2. Triazole				
B_0	4.88	5.68	12.58	15.81
B_1	4.70	5.60	12.54	14.87
B_2	4.50	5.37	12.49	14.86
SE	0.20	0.23	0.23	0.28
CD (0.05)	NS	NS	NS	0.79
3. Mycorrhizae				
C_1	4.68	5.63	13.05	15.94
C_2	4.70	5.32	12.03	14.41
SE	0.16	0.19	0.19	0.43
CD (0.05)	NS	NS	0.54	1.21
4. Height of potting media				
D_1	4.68	5.45	12.30	14.90
D_2	4.70	5.50	12.77	15.46
SE	0.16	0.19	0.19	0.43
CD (0.05)	NS	NS	NS	NS

inoculated plantlets recorded highest leaf number (4.70) 15 DAP. At the same time *Glomus fasciculatum* inoculated plants recorded highest leaf number (5.63) one month after planting (Plate 9).

Height of the potting media in the container had no significant effect on the leaf number. Highest value for leaf number was recorded when the height of potting media was 6 cm both 15 and 30 DAP.

4.3.1.2 Interaction Effects of the Factors on Leaf Number

There was no significant difference between the treatments regarding number of leaves due to the interaction between potting media and triazole (Table 6).

The interaction between potting media and mycorrhizae did not significantly affect the leaf number during the acclimatization period (Table 7). Type of potting media and height of potting media in the container did not show significant interaction effect on leaf number (Table 8).

Leaf number was found to be affected by interaction effect of triazole and mycorrhizae. Fifteen days after planting, highest leaf number of 5.25 was obtained for the treatment combination of B_1C_2 and lowest leaf number of 3.95 was obtained for B_2C_2. But during the later stages there was no significant interaction between triazole and mycorrhizae on leaf number (Table 9).

The interaction between triazole and height of potting media in the container did not significantly affect the leaf number (Table 10) during the observation period. The interaction between mycorrhizae and height of potting media did not affect the leaf number (Table 11).

4.3.2 Height of the Plant

4.3.2.1 Main Effects of the Factors on Height of the Plant

The data is presented in Table 5.

Table 6 Interaction effect of potting media and triazole on leaf number and plant height

Treatments	Leaf number		Plant height (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP
A_1B_0	4.88	5.38	13.64	17.34
A_1B_1	5.38	6.13	12.54	15.66
A_1B_2	4.75	5.50	13.29	16.28
A_2B_0	4.75	5.75	13.76	16.86
A_2B_1	5.25	6.13	12.34	15.45
A_2B_2	5.13	6.00	12.35	16.81
A_3B_0	4.75	5.88	13.30	17.35
A_3B_1	4.75	5.88	12.10	17.17
A_3B_2	4.13	5.25	11.66	16.95
A_4B_0	4.50	5.63	13.01	14.48
A_4B_1	4.50	4.88	12.58	13.91
A_4B_2	5.38	5.50	11.86	14.73
A_5B_0	4.63	5.75	14.36	14.54
A_5B_1	4.50	5.00	11.76	13.15
A_5B_2	3.13	3.50	8.95	9.51
SE	0.44	0.51	0.52	1.17
CD (0.05)	NS	NS	1.48	NS

Table 7 Interaction effect of potting media and mycorrhizae on leaf number and plant height

Treatments	Leaf number		Plant height (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP
A_1C_1	4.67	5.50	13.53	16.85
A_1C_2	5.33	5.83	12.78	16.00
A_2C_1	4.92	5.83	13.83	17.42
A_2C_2	5.17	6.08	11.74	15.20
A_3C_1	4.67	5.83	12.49	15.88
A_3C_2	4.42	5.50	13.33	16.89
A_4C_1	4.83	5.92	12.90	15.43
A_4C_2	4.75	4.75	13.07	13.32
A_5C_1	4.33	5.08	12.50	14.13
A_5C_2	3.83	4.42	9.22	10.67
SE	0.36	0.41	1.21	0.95
CD (0.05)	NS	NS	0.43	NS

Table 8 Interaction effect of type of potting media and height of potting media in the container on leaf number and plant height

Treatments	Leaf number		Plant height (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP
A_1D_1	4.83	5.50	12.94	16.53
A_1D_2	5.17	5.83	13.37	16.52
A_2D_1	5.25	6.17	11.78	15.66
A_2D_2	4.83	5.75	12.45	15.43
A_3D_1	4.33	5.58	12.97	16.26
A_3D_2	4.75	5.75	14.19	18.06
A_4D_1	4.67	4.83	12.78	13.32
A_4D_2	4.92	5.83	13.18	15.43
A_5D_1	4.33	5.17	11.05	12.95
A_5D_2	3.83	5.33	10.67	11.85
SE	0.36	0.41	0.43	0.95
CD (0.05)	NS	NS	NS	NS

Different potting media significantly affected the plant height both 15 and 30 days after planting. During the first fortnight, sand : soil(1:1) recorded maximum plant height of 13.58 cm. This was followed by sand (13.15 cm) and soil rite (13.12 cm). During the second fortnight also sand : soil recorded highest value of 17.26 cm followed by soil rite (16.54 cm) . The lowest value during both period was recorded by sand : soil : leaf mould media. The values were 10.86 cm and 12.40 cm respectively after the first and second fortnight of planting.

Triazole did not show any effect on plant height 15 DAP. But plant height was shown to be affected significantly by triazole treatment after 30 days of planting. Highest plant height of 15.81 cm was observed for untreated plants and lowest value of 14.86 cm for the treatment with high concentration of triazole (8 mg/l) which was closely followed by treatment with 4 mg/l concentration (14.87) one month after planting (Plate 10).

Mycorrhizal inoculation affected the plant height significantly. *Glomus fasciculatum* inoculated plants recorded maximum plant height during the observation period. The values were 13.05 cm and 15.94 cm respectively for 15 and 30 DAP.

Height of the potting media in the container did not show any significant effect on plant height.

4.3.2.2 Interaction Effects of the Factors on Height of the Plant

Plant height was significantly affected by the interaction between potting media and triazole 15 DAP (Table 6). The highest value for plant height was recorded for A_5B_0 (14.36 cm) which was on par with A_1B_0, A_2B_0 and A_3B_0. The values were 13.64, 13.76 and 13.30 cm respectively. The lowest plant height of 8.95 cm was recorded for A_5B_2 15 DAP.

The highest plant height of 17.35 cm was recorded for the interaction A_3B_0 in the second fortnight. It was followed by A_1B_0 (17.34 cm),

1. *Glomus monosporum*
2. *Glomus fasciculatum*

Plate 9. Plants inoculated with AMF, 30 days after planting

1. **Untreated plant**
2. **Treated plant**

Plate 10. Effect of triazole on the plants, 30 days after planting

Table 9 Interaction effect of triazole and mycorrhizae on leaf number and plant height

Treatments	Leaf number		Plant height (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP
B_0C_1	4.50	5.55	14.21	15.91
B_0C_2	4.90	5.80	12.54	16.51
B_1C_1	4.50	5.65	12.50	15.42
B_1C_2	5.25	5.55	12.66	14.33
B_2C_1	5.05	5.70	12.54	15.72
B_2C_2	3.95	4.60	10.88	13.20
SE	0.28	0.32	0.33	0.74
CD (0.05)	0.79	NS	0.93	NS

Table 10 Interaction effect of triazole and height of potting media in the container on leaf number and plant height

Treatments	Leaf number		Plant height (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP
B_0D_1	4.65	5.70	13.16	16.38
B_0D_2	4.75	5.65	13.03	15.49
B_1D_1	4.90	5.60	11.70	14.12
B_1D_2	4.85	5.60	13.46	15.63
B_2D_1	4.50	5.05	12.06	14.22
B_2D_2	4.50	5.25	11.84	15.24
SE	0.28	0.32	0.33	0.74
CD (0.05)	NS	NS	NS	NS

Table 11 Interaction effect of mycorrhizae and height of potting media in the container on leaf number and plant height

Treatments	Leaf number		Plant height (cm)	
	15 DAP	30 DAP	15 DAP	30 DAP
C_1D_1	4.60	5.53	12.83	15.57
C_1D_2	4.77	5.73	13.27	16.32
C_2D_1	4.77	5.37	11.78	14.24
C_2D_2	4.63	5.27	12.27	14.59
SE	0.23	0.26	0.27	0.60
CD (0.05)	NS	NS	NS	NS

A_3B_1 (17.17 cm) and A_3B_2 (16.95 cm). The lowest value was recorded for A_5B_2 (9.51 cm).

Interaction effect of potting media and mycorrhizae significantly influenced the plant height 15 DAP. The interaction A_2C_1 gave the maximum plant height of 13.83 cm followed by A_1C_1 (13.53 cm). Lowest value was recorded for the interaction A_5C_2 (9.22 cm). After 30 days of planting, the interaction effect was not significant on the treatments (Table 7).

Plant height was not affected by the interaction between type of potting media and height of potting media in the container (Table 8). Interaction of triazole and mycorrhizae significantly affected the plant height 15 DAP (Table 9). But the interaction was not significant one month after planting. During the first fortnight, highest value was obtained for the interaction B_0C_1 (14.21 cm) and lowest value for B_2C_2 (10.88 cm). During the second fortnight highest plant height was obtained for the interaction B_0C_2 (16.51 cm) and lowest for B_2C_2 (13.20 cm).

The interaction between triazole and height of potting media in the container did not significantly influence the plant height during the observation period (Table 10). There was no significant difference in the plant height due to interaction of mycorrhizae and height of potting media in the container (Table 11).

4.3.3 Number of Roots

4.3.3.1 Main Effects of the Factors on Number of Roots

The data is presented in Table 12.

Different potting media significantly affected the number of roots during the first fortnight. Sand recorded the highest number of 6.17 and sand : soil : leaf mould recorded the lowest number of 4.46. One month after planting, sand : soil recorded the highest value of 9.29 and the rest of the treatments were not significantly different.

Table 12 Main effects of potting media, triazole, mycorrhizae and height of potting media on root number and phyllochron

Treatment	Root number		Phyllochron (days)	
	15 DAP	30 DAP	First leaf	Second leaf
1. Potting media				
A_1	6.17	8.92	10.04	13.58
A_2	4.92	8.83	9.38	13.67
A_3	4.92	9.29	10.79	14.25
A_4	5.63	8.38	10.50	14.21
A_5	4.46	8.21	9.54	14.17
SE	0.32	0.32	0.42	0.32
CD (0.05)	0.89	NS	NS	NS
2. Triazole				
B_0	5.05	8.23	9.35	13.30
B_1	5.53	9.18	10.35	14.15
B_2	5.68	9.38	10.45	14.48
SE	0.24	0.25	0.33	0.25
CD (0.05)	NS	0.71	0.92	0.70
3. Mycorrhizae				
C_1	5.57	9.22	10.28	14.38
C_2	5.27	8.63	9.82	13.57
SE	0.20	0.25	0.27	0.20
CD (0.05)	NS	0.52	NS	0.57
4. Height of potting media				
D_1	5.57	9.07	10.25	14.38
D_2	5.27	8.78	9.85	13.57
SE	0.20	0.21	0.27	0.20
CD (0.05)	NS	NS	NS	NS

Triazole treatment did not significantly influence root number 15 DAP. But 30 DAP, root number was affected significantly by triazole treatment. Triazole at 8 mg/l recorded highest root number (9.38) while control plants recorded the lowest value (8.23).

Mycorrhizal treatment did not show significant effect on root number 15 DAP. *Glomus fasciculatum* and *Glomus monosporum* significantly affected root number during the second fortnight. Plants inoculated with *Glomus fasciculatum* recorded higher number (9.22) than that of *Glomus monosporum* (8.63).

Height of potting media in the container did not affect the root number significantly.

4.3.3.2 Interaction Effects of the Factors on Number of Roots

There was no significant interaction between the potting media and triazole with respect to root number of the plantlets (Table 13) during the observation period.

The interaction effect of potting media and mycorrhizae (Table 14) did not have significant influence on root number both 15 and 30 DAP.

Interaction between type of potting media and height of potting media in the container did not influence the number of roots (Table 15).

Triazole and mycorrhizae significantly affected number of roots. The interaction B_2C_1 gave the highest number of roots (6.75) and B_0C_2 gave the lowest number (4.90) 15 DAP. B_1C_1 recorded highest root number (9.65) and B_0C_1 recorded lowest number of 7.80 one month after planting (Table 16).

Triazole application and height of potting media in the container did not affect the root number during the observation period (Table 17). The interaction between mycorrhizae and height of potting media in the container also did not influence the number of roots (Table 18) both 15 and 30 DAP.

Table 13 Interaction effect of potting media and triazole on root number and phyllochron

Treatments	Root number		Phyllochron (days)	
	15 DAP	30 DAP	First leaf	Second leaf
A_1B_0	5.88	9.00	9.50	11.25
A_1B_1	6.63	8.75	10.25	13.38
A_1B_2	6.00	9.00	10.38	14.13
A_2B_0	4.63	8.88	9.38	13.10
A_2B_1	5.00	8.75	9.75	14.28
A_2B_2	5.13	8.88	10.38	13.50
A_3B_0	4.50	9.13	9.63	14.50
A_3B_1	4.63	9.50	10.13	13.88
A_3B_2	5.63	9.25	11.00	14.38
A_4B_0	5.00	9.88	9.50	13.13
A_4B_1	5.88	10.13	11.13	14.50
A_4B_2	6.00	8.13	10.38	13.38
A_5B_0	5.25	10.00	10.50	13.10
A_5B_1	5.00	8.75	11.25	14.08
A_5B_2	5.63	5.88	11.63	15.00
SE	0.55	0.98	0.73	0.55
CD (0.05)	NS	NS	1.06	1.56

Table 14 Interaction effect of potting media and mycorrhizae on root number and phyllochron

Treatments	Root number		Phyllochron (days)	
	15 DAP	30 DAP	First leaf	Second leaf
A_1C_1	6.33	9.17	9.83	13.75
A_1C_2	6.00	8.67	10.25	13.42
A_2C_1	5.42	8.92	9.33	13.50
A_2C_2	4.42	8.75	9.75	13.83
A_3C_1	4.92	9.08	10.58	14.17
A_3C_2	4.92	9.50	10.42	14.33
A_4C_1	5.67	9.75	11.00	14.83
A_4C_2	5.58	9.00	10.58	13.58
A_5C_1	5.50	9.17	10.67	15.67
A_5C_2	5.42	7.25	10.01	13.67
SE	1.26	0.46	1.68	1.27
CD (0.05)	NS	NS	NS	0.45

Table 15 Interaction effect of type of potting media and height of potting media in the container on root number and phyllochron

Treatments	Root number		Phyllochron (days)	
	15 DAP	30 DAP	First leaf	Second leaf
A_1D_1	6.00	9.08	10.17	14.25
A_1D_2	6.33	8.75	9.92	12.92
A_2D_1	5.25	8.67	9.50	13.92
A_2D_2	4.58	9.00	9.58	13.43
A_3D_1	5.17	9.42	10.67	14.42
A_3D_2	4.67	9.17	10.33	14.08
A_4D_1	6.25	9.25	10.67	13.75
A_4D_2	5.00	9.50	10.92	14.67
A_5D_1	5.17	8.90	10.25	15.58
A_5D_2	5.75	7.50	8.50	12.75
SE	0.45	0.46	0.59	1.20
CD (0.05)	NS	NS	NS	NS

4.3.4 Phyllochron

4.3.4.1 Main Effects of the Factors on Pyllochron

The data regarding phyllochron of the plantlets is presented in Table 12.

Effect of potting media on phyllochron was not significant. The days taken for emergence of first leaf ranged from 9.38 to 10.79 days and for second leaf, it ranged from 13.58 to 14.25 days.

Triazole treatment significantly affected the leaf production interval. Triazole at a concentration of 8 mg/l took 10.45 days for the emergence of first leaf and 14.48 days for the second leaf. At the same time untreated plants took only 9.35 days to emerge the first leaf and 13.30 days for the second leaf. Emergence of first leaf was not affected by inoculation of mycorrhizae. But the second leaf production was significantly affected by mycorrhizal inoculation. *Glomus monosporum* inoculated plants showed shorter leaf production interval (13.57 days) compared to those inoculated with *Glomus fasciculatum* (14.38 days).

Height of potting media in the container did not show significant influence on phyllochron.

4.3.4.2 Interaction Effects of the Factors on Phyllochron

Leaf production was significantly affected by interaction of potting media and triazole (Table 13). The interaction A_5B_2 took highest leaf production interval of 11.63 days for the emergence of first leaf followed by A_5B_1 (11.25 days). Shortest leaf production interval for first leaf was obtained in the interaction A_2B_0 (9.38 days).

The interaction A_5B_2 took highest interval (15.00 days) for emergence of second leaf. The lowest phyllochron for second leaf was obtained for A_1B_0 (11.25 days) followed by A_2B_0 and A_5B_0 with a value of 13.10 days.

Interaction effect of potting media and mycorrhizae was significant for emergence of second leaf (Table 14). But it was not significant for the production of first leaf. The interaction A_5C_1 recorded highest duration (15.67 days) to produce second leaf whereas the interaction A_1C_2 recorded the shortest duration (13.42 days).

Interaction effect of type of potting media and height of potting media in the container did not influence the phyllochron (Table 15). The interaction between triazole and mycorrhizae had no effect on emergence of first leaf but it exerted significant effect on emergence of second leaf (Table 16). Longest period for first leaf production was noticed for B_2C_1 (14.45 days) followed by B_1C_2 (14.20 days). Shortest duration for second leaf emergence was obtained for B_0C_1 (12.15 days).

Interaction of triazole and height of potting media in the container did not show influence on phyllochron (Table 17). The interaction between mycorrhizae and height of potting media in the container did not significantly affect the phyllochron (Table 18).

4.3.5 Fresh Weight

The data on fresh weight is given in Table 19.

There was significant effect on the fresh weight of the plants due to the treatments both 15 and 30 DAP.

Highest fresh weight was recorded for the treatment $A_1B_1C_2$ followed by $A_1B_1C_1$ and $A_4B_2C_2$ 15 DAP. The values were 1.302, 1.254 and 1.218 g plant^{-1} respectively. The lowest value for fresh weight was recorded for the treatment $A_2B_0C_2$ and the value was 0.928 g plant^{-1}.

After one month of planting, the highest value for fresh weight was observed in the treatment $A_1B_1C_2$ (1.697 g plant^{-1}). It was on par with $A_1B_1C_1$ (1.568 g plant^{-1}), $A_2B_1C_1$ (1.565 g plant^{-1}) and $A_4B_2C_2$ (1.621 g plant^{-1}). The treatment $A_2B_0C_2$ recorded the lowest fresh weight of 1.150 g plant^{-1} followed by $A_4B_0C_2$ (1.157 g plant^{-1}) thirty days after planting.

Table 16 Interaction effect of triazole and mycorrhizae on root number and phyllochron

Treatments	Root number		Phyllochron (days)	
	15 DAP	30 DAP	First leaf	Second leaf
B_0C_1	5.20	7.80	9.95	12.75
B_0C_2	4.90	8.70	8.75	13.18
B_1C_1	5.75	9.65	10.60	14.10
B_1C_2	5.30	9.20	10.10	14.20
B_2C_1	6.75	8.65	10.60	14.45
B_2C_2	5.60	9.35	10.30	14.00
SE	0.35	0.36	0.46	0.35
CD (0.05)	0.54	0.38	NS	0.98

Table 17 Interaction effect of triazole and height of potting media in the container on root number and phyllochron

Treatments	Root number		Phyllochron (days)	
	15 DAP	30 DAP	First leaf	Second leaf
B_0D_1	5.10	9.25	10.55	14.65
B_0D_2	5.00	9.50	10.35	14.30
B_1D_1	5.75	9.70	10.85	14.50
B_1D_2	5.30	8.65	9.85	13.80
B_2D_1	5.85	8.25	9.35	14.00
B_2D_2	5.50	8.20	9.35	12.60
SE	0.35	0.36	0.46	0.35
CD (0.05)	NS	NS	NS	NS

Table 18 Interaction effect of mycorrhizae and height of potting media in the container on root number and phyllochron

Treatments	Root number		Phyllochron (days)	
	15 DAP	30 DAP	First leaf	Second leaf
C_1D_1	5.73	9.13	10.27	14.77
C_1D_2	5.40	9.30	10.30	14.00
C_2D_1	5.40	9.00	10.23	14.00
C_2D_2	5.13	8.27	9.40	13.13
SE	0.28	0.29	0.38	0.28
CD (0.05)	NS	NS	NS	NS

4.3.6 Dry Weight

The data relating to dry weight of the plants is presented in Table 19.

The treatments significantly affected the dry weight of the plants. Highest value for dry weight after the first fortnight was obtained in the treatment $A_1B_1C_2$ (0.508 g plant^{-1}). It was on par with the treatments $A_1B_0C_2$ (0.409 g plant^{-1}), $A_2B_0C_2$ (0.379 g plant^{-1}), $A_3B_2C_1$ (0.419 g plant^{-1}) and $A_5B_2C_1$ (0.382 g plant^{-1}). The lowest value for dry weight 15 DAP was recorded by the treatment $A_2B_0C_1$ (0.251 g plant^{-1}).

One month after planting the highest value for dry weight was obtained by the treatments $A_2B_1C_2$ and $A_2B_2C_1$ with a value of 0.918 g plant^{-1}. The lowest value for dry weight was 0.621 g plant^{-1} recorded for the treatment $A_1B_0C_2$ which was on par with 0.651 g plant^{-1} recorded for the treatment $A_4B_0C_2$.

4.4 PHYSIOLOGICAL OBSERVATIONS

4.4.1 Stomatal Conductance

4.4.1.1 Main Effects of the Factors on Stomatal Conductance

The data is presented in Table 20.

Potting media significantly affected the stomatal conductance values. Highest value was obtained for sand : soil (1.01 cm/s) 15 DAP. Lowest value was obtained for sand (0.59 cm/s). After 30 days of planting, sand : soil : leaf mould recorded highest value of 0.26 cm/s and lowest value was obtained for soilrite (0.13 cm/s).

Triazole application influenced the stomatal conductance significantly. Lowest value was recorded by plants treated with 8 mg/l triazole. The values were 0.62 cm/s and 0.10 cm/s respectively for 15 and 30 DAP. Highest values were obtained for untreated plants. The values were 0.79 cm/s and 0.20 cm/s respectively for 15 and 30 DAP.

Table 19 Fresh weight and dry weight as influenced by potting media, triazole and mycorrhizae

Treatment	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})	
	15 DAP	30 DAP	15 DAP	30 DAP
$A_1B_0C_1$	1.100	1.546	0.302	0.854
$A_1B_0C_2$	1.093	1.496	0.409	0.621
$A_1B_1C_1$	1.254	1.568	0.313	0.809
$A_1B_1C_2$	1.302	1.697	0.508	0.895
$A_1B_2C_1$	1.065	1.401	0.272	0.852
$A_1B_2C_2$	1.122	1.412	0.317	0.849
$A_2B_0C_1$	1.201	1.523	0.251	0.746
$A_2B_0C_2$	0.928	1.150	0.379	0.833
$A_2B_1C_1$	1.195	1.565	0.273	0.889
$A_2B_1C_2$	1.148	1.250	0.277	0.918
$A_2B_2C_1$	1.101	1.473	0.361	0.918
$A_2B_2C_2$	1.130	1.442	0.313	0.866
$A_3B_0C_1$	1.039	1.332	0.393	0.792
$A_3B_0C_2$	0.995	1.281	0.337	0.767
$A_3B_1C_1$	1.134	1.461	0.361	0.916
$A_3B_1C_2$	0.998	1.296	0.336	0.780
$A_3B_2C_1$	1.199	1.436	0.419	0.859
$A_3B_2C_2$	1.121	1.442	0.289	0.834
$A_4B_0C_1$	1.133	1.362	0.324	0.814
$A_4B_0C_2$	1.067	1.157	0.316	0.651
$A_4B_1C_1$	1.078	1.331	0.362	0.806
$A_4B_1C_2$	1.105	1.521	0.331	0.712
$A_4B_2C_1$	1.068	1.307	0.314	0.845
$A_4B_2C_2$	1.218	1.621	0.290	0.902
$A_5B_0C_1$	1.073	1.345	0.280	0.762
$A_5B_0C_2$	1.068	1.417	0.305	0.849
$A_5B_1C_1$	0.998	1.212	0.331	0.859
$A_5B_1C_2$	1.009	1.177	0.363	0.861
$A_5B_2C_1$	1.112	1.437	0.382	0.838
$A_5B_2C_2$	1.012	1.356	0.258	0.759
SE	0.084	0.168	0.051	0.072
CD (0.05)	0.243	0.485	0.146	0.208

Table 20 Main effects of potting media, triazole, mycorrhizae and height of potting media on stomatal conductance and leaf area index

Treatment	Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	
1. Potting media			
A_1	0.59	0.15	1.03
A_2	0.61	0.13	1.13
A_3	1.01	0.22	1.01
A_4	0.64	0.19	0.97
A_5	0.69	0.26	0.84
SE	0.02	0.01	0.02
CD (0.05)	0.07	0.03	0.05
2. Triazole			
B_0	0.79	0.20	1.08
B_1	0.73	0.15	1.00
B_2	0.62	0.10	0.94
SE	0.02	0.01	0.01
CD (0.05)	0.05	0.02	0.04
3. Mycorrhizae			
C_1	0.68	0.15	1.00
C_2	0.75	0.19	0.99
SE	0.01	0.01	0.01
CD (0.05)	0.11	0.02	NS
4. Height of potting media			
D_1	0.73	0.15	1.01
D_2	0.70	0.19	0.98
SE	0.01	0.01	0.01
CD (0.05)	NS	NS	NS

Mycorrhizal inoculation significantly affected the stomatal conductance. *Glomus monosporum* recorded higher values both 15 and 30 DAP. The values were 0.75 and 0.19 cm/s respectively.

Height of potting media in the container did not influence the stomatal conductance.

4.4.1.2 Interaction Effects of the Factors on Stomatal Conductance

Interaction of potting media and triazole significantly affected the stomatal conductance. 15 DAP, highest value was recorded for the interaction A_3B_2 (1.48 cm/s) followed by A_3B_1 (1.07 cm/s). Lowest values was obtained for A_5B_0 (0.16 cm/s) followed by A_1B_0 (0.37 cm/s). One month after planting, the value was highest for A_4B_0 (0.29 cm/s) followed by A_3B_0 (0.27 cm/s). The value was lowest for A_1B_2 (0.10 cm/s) which was on par with A_1B_1 (10.11 cm/s) and A_2B_1 (0.12 cm/s) (Table 21).

Stomatal conductance was significantly affected by interaction between potting media and mycorrhizae (Table 22). The value was highest for the interaction A_3C_2 (1.13 cm/s) during the first fortnight. The lowest value was recorded by A_2C_1 (0.45 cm/s). One month after planting, the interaction A_3C_2 recorded highest value (0.23 cm/s) and this was closely followed by A_4C_2 (0.22 cm/s) and A_1C_1 (0.21 cm/s). Lowest value was given by A_2C_2 (0.06 cm/s).

Type of potting media and height of potting media in the container significantly affected the stomatal conductance. Value was highest for the interaction A_2D_2 (1.10 cm/s) and lowest for A_5D_1 (0.49 cm/s) after 15 days of planting. Highest value of 0.24 cm/s was obtained for the interaction A_2D_2 and lowest value of 0.10 cm/s for A_1D_1 30 DAP (Table 23). Interaction of triazole and mycorrhizae significantly affected the stomatal conductance values 15 DAP (Table 24). The highest value was obtained for the interaction B_0C_2 (0.83 cm/s) and the lowest value was recorded for B_2C_1 (0.56 cm/s). But the effect was not significant after 30

Table 21 Interaction effect of potting media and triazole on stomatal conductance and leaf area index

Treatments	Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	30 DAP
A_1B_0	0.37	0.24	1.00
A_1B_1	0.82	0.11	0.99
A_1B_2	1.06	0.10	1.09
A_2B_0	0.56	0.14	1.24
A_2B_1	0.57	0.12	1.10
A_2B_2	0.70	0.13	1.05
A_3B_0	0.48	0.27	1.02
A_3B_1	1.07	0.17	1.07
A_3B_2	0.68	0.21	0.93
A_4B_0	1.48	0.29	0.95
A_4B_1	0.53	0.15	0.95
A_4B_2	0.61	0.14	0.64
A_5B_0	0.94	0.21	1.00
A_5B_1	0.68	0.16	0.88
A_5B_2	0.16	0.20	1.00
SE	0.04	0.02	0.03
CD (0.05)	0.11	0.06	0.08

Table 22 Interaction effect of potting media and mycorrhizae on stomatal conductance and leaf area index

Treatments	Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	30 DAP
A_1C_1	0.82	0.21	1.04
A_1C_2	0.67	0.09	1.02
A_2C_1	0.45	0.20	1.08
A_2C_2	0.77	0.06	1.17
A_3C_1	0.88	0.20	1.03
A_3C_2	1.13	0.23	0.99
A_4C_1	0.57	0.17	0.97
A_4C_2	0.64	0.22	0.97
A_5C_1	0.65	0.17	0.90
A_5C_2	0.54	0.15	0.78
SE	0.09	0.05	0.07
CD (0.05)	0.03	0.02	0.02

Table 23 Interaction effect of type of potting media and height of potting media in the container on stomatal conductance and leaf area index

Treatments	Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	30 DAP
A_1D_1	0.80	0.10	1.11
A_1D_2	0.70	0.20	0.95
A_2D_1	0.62	0.12	1.07
A_2D_2	1.10	0.24	1.19
A_3D_1	0.92	0.20	1.02
A_3D_2	0.60	0.23	1.00
A_4D_1	0.60	0.18	0.94
A_4D_2	0.61	0.21	0.99
A_5D_1	0.49	0.16	0.90
A_5D_2	0.69	0.16	0.78
SE	0.03	0.02	0.02
CD (0.05)	0.09	0.05	NS

days of planting. Interaction of triazole and height of potting media on the stomatal conductance was not significant (Table 25).

Interaction between mycorrhizae and height of potting media in the container significantly influenced the stomatal conductance (Table 26). The interaction C_2D_1 gave highest value of 0.87 cm/s and C_1D_1 gave lowest value of 0.58 cm/s after a fortnight of planting. After one month of planting the interaction C_1D_2 gave highest value (0.23 cm/s) and C_2D_2 gave lowest value (0.14 cm/s).

4.4.2 Leaf Area Index (LAI)

4.4.2.1 Main Effects of the Factors on Leaf Area Index

The data is presented in Table 20.

Leaf area index was significantly affected by different potting media. Soilrite recorded the maximum leaf area index of 1.13 one month after planting. This was closely followed by sand (1.03) and sand : soil (1.01). Leaf area index was lowest for sand : soil : leaf mould and the value was 0.84.

Triazole application significantly influenced LAI of the plants. Untreated plants recorded highest LAI of 1.08 and triazole treated plants recorded lowest leaf area index. Triazole at a concentration of 8 mg/l recorded a leaf area index of 0.94.

Mycorrhizal inoculation did not affect the leaf area index significantly. *Glomus fasciculatum* inoculated plants gave higher leaf area index of 1.00 compared to *Glomus monosporum* inoculated plants which recorded LAI of 0.99.

Height of potting media did not show influence on leaf area index of the plants.

Table 24 Interaction effect of triazole and mycorrhizae on stomatal conductance and leaf area index

Treatments	Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	30 DAP
B_0C_1	0.75	0.22	1.03
B_0C_2	0.83	0.18	1.00
B_1C_1	0.75	0.19	0.97
B_1C_2	0.72	0.12	1.05
B_2C_1	0.56	0.16	0.98
B_2C_2	0.71	0.15	0.90
SE	0.03	0.01	0.02
CD (0.05)	0.09	NS	0.07

Table 25 Interaction effect of triazole and height of potting media in the container on stomatal conductance and leaf area index

Treatments	Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	30 DAP
B_0D_1	0.73	0.17	1.07
B_0D_2	0.85	0.23	1.02
B_1D_1	0.70	0.15	0.99
B_1D_2	0.76	0.16	1.00
B_2D_1	0.74	0.13	0.96
B_2D_2	0.49	0.18	0.92
SE	0.03	0.01	0.02
CD (0.05)	NS	NS	NS

Table 26 Interaction effect of mycorrhizae and height of potting media in the container on stomatal conductance and leaf area index

Treatments	Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	30 DAP
C_1D_1	0.58	0.15	1.01
C_1D_2	0.77	0.23	0.99
C_2D_1	0.87	0.15	1.01
C_2D_2	0.63	0.14	0.98
SE	0.02	0.01	0.01
CD (0.05)	0.06	0.03	NS

4.4.2.2 Interaction Effect of the Factors on Leaf Area Index

The interaction between potting media and triazole significantly affected the LAI of the plants (Table 21). The highest leaf area index was recorded by the interaction A_2B_0 (1.24) and lowest by A_4B_2 (0.64).

The interaction between potting media and mycorrhizae influenced the LAI significantly (Table 22). Highest value for LAI was obtained for A_2C_2 (1.17), followed by A_2C_1 (1.08) and A_1C_1 (1.04). The value was lowest for A_5C_2 (0.78).

Type of potting media and height of potting media in the container did not affect the LAI significantly (Table 23).

LAI was significantly affected by the interaction effect of triazole and mycorrhizae (Table 24). The interaction B_1C_2 recorded maximum LAI of 1.05 followed by B_0C_1 (1.03) and B_0C_2 (1.00). The lowest value for LAI was obtained for B_2C_2 (0.90).

Interaction effect of triazole and height of potting media in the container did not significantly affect the LAI (Table 25).

Interaction of mycorrhizae and height of potting media in the container did not show significant effect on the leaf area index (Table 26).

4.4.3 Crop Growth Rate

The data relating to CGR is presented in Table 27.

Significant difference was noticed among the treatments with respect to the CGR values. Highest CGR was observed in the treatment $A_2B_1C_1$ followed by $A_2B_1C_2$. The treatments recorded values of 1.330 mg cm^{-2} day^{-1} and 1.312 mg cm^{-2} day^{-1}respectively. The lowest value for CGR was obtained for the treatment $A_1B_0C_2$ (0.391 mg cm^{-2} day^{-1}).

4.4.4 Relative Growth Rate

The data pertaining to RGR is given in Table 27.

Table 27 CGR and RGR as influenced by potting media, triazole and mycorrhizae one month after planting

Treatment	CGR (mg cm^{-2} day^{-1})	RGR (mg g^{-1} day^{-1})
$A_1B_0C_1$	1.021	0.069
$A_1B_0C_2$	0.391	0.027
$A_1B_1C_1$	0.919	0.063
$A_1B_1C_2$	0.716	0.041
$A_1B_2C_1$	1.069	0.076
$A_1B_2C_2$	0.923	0.061
$A_2B_0C_1$	0.916	0.072
$A_2B_0C_2$	0.840	0.052
$A_2B_1C_1$	1.330	0.086
$A_2B_1C_2$	1.312	0.089
$A_2B_2C_1$	1.031	0.061
$A_2B_2C_2$	1.029	0.067
$A_3B_0C_1$	0.740	0.047
$A_3B_0C_2$	0.792	0.055
$A_3B_1C_1$	1.034	0.062
$A_3B_1C_2$	0.822	0.056
$A_3B_2C_1$	0.815	0.047
$A_3B_2C_2$	1.011	0.071
$A_4B_0C_1$	0.924	0.043
$A_4B_0C_2$	0.913	0.052
$A_4B_1C_1$	0.828	0.055
$A_4B_1C_2$	1.076	0.067
$A_4B_2C_1$	0.983	0.064
$A_4B_2C_2$	1.133	0.075
$A_5B_0C_1$	0.798	0.065
$A_5B_0C_2$	0.860	0.062
$A_5B_1C_1$	0.969	0.058
$A_5B_1C_2$	0.919	0.064
$A_5B_2C_1$	0.844	0.052
$A_5B_2C_2$	0.923	0.071
SE	0.112	0.008
CD (0.05)	0.323	0.025

RGR values were affected by the treatments significantly. The treatment $A_2B_1C_2$ recorded the highest value for RGR followed by $A_2B_1C_1$. The treatments recorded 0.089 and 0.086 mg g^{-1} day^{-1} respectively. The lowest value was observed for the treatment $A_1B_0C_2$ and the value was 0.027 mg g^{-1} day^{-1}.

4.5 ECONOMICS OF ACCLIMATIZATION

Economics of acclimatization was worked out by considering the cost of production of *in vitro* plantlets (Appendix VIII) and the expenses involved in their *ex vitro* establishment. By combining these two aspects the cost of an individual plantlet was worked out for each treatment. The data is presented in Table 28.

Cost of acclimatization was found to be highest for the treatments $A_2B_2C_1D_2$ and $A_2B_2C_2D_2$ which recorded a value of Rs. 1.84 per plantlet. The lowest cost of acclimatization was recorded in the treatments $A_3B_0C_1D_1$ and $A_3B_0C_2D_1$ with a value of Rs. 1.09 per plantlet. Cost of production of *in vitro* plantlet was estimated to be Rs.3.41 per plantlet. The total cost of a plant after acclimatization was found to be ranged from Rs. 4.50 to 5.25.

Table 28 Economics of acclimatization and total cost of individual plants after acclimatization

Treatments	Sand (A_1)		Soilrite (A_2)		Sand : Soil (A_3)		Sand : Soil : Coirpith (A_4)		Sand : Soil : Leaf mould (A_5)	
	Cost of acclimatization (Rs.)	Total cost per plantlet (Rs.)	Cost of acclimatization (Rs.)	Total cost per plantlet (Rs.)	Cost of acclimatization (Rs.)	Total cost per plantlet (Rs.)	Cost of acclimatization (Rs.)	Total cost per plantlet (Rs.)	Cost of acclimatization (Rs.)	Total cost per plantlet (Rs.)
$B_0C_1D_1$	1.11	4.52	1.60	5.01	1.09	4.50	1.16	4.57	1.10	4.51
$B_0C_1D_2$	1.15	4.56	1.80	5.21	1.12	4.53	1.21	4.62	1.13	4.54
$B_0C_2D_1$	1.11	4.52	1.60	5.01	1.09	4.50	1.16	4.57	1.10	4.51
$B_0C_2D_2$	1.15	4.56	1.80	5.21	1.12	4.53	1.21	4.62	1.13	4.54
$B_1C_1D_1$	1.13	4.54	1.62	5.03	1.11	4.52	1.18	4.60	1.12	4.53
$B_1C_1D_2$	1.17	4.58	1.82	5.23	1.14	4.56	1.23	4.65	1.16	4.57
$B_1C_2D_1$	1.13	4.54	1.62	5.03	1.11	4.52	1.18	4.60	1.12	4.53
$B_1C_2D_2$	1.17	4.58	1.82	5.23	1.14	4.56	1.23	4.65	1.16	4.57
$B_2C_1D_1$	1.16	4.57	1.64	5.05	1.14	4.54	1.21	4.62	1.15	4.56
$B_2C_1D_2$	1.19	4.60	1.84	5.25	1.17	4.58	1.26	4.67	1.18	4.60
$B_2C_2D_1$	1.16	4.57	1.64	5.05	1.14	4.54	1.21	4.62	1.15	4.56
$B_2C_2D_2$	1.19	4.60	1.84	5.25	1.17	4.58	1.26	4.67	1.18	4.60

DISCUSSION

5. DISCUSSION

Efficient commercial micropropagation depends on rapid and extensive proliferation along with the use of large scale cultures for the multiplication phase. But normal plant development during *ex vitro* establishment stage is very much essential to ensure a high per cent of survival after field planting (Preece and Sutter, 1991). Plantlets that have grown *in vitro* have been continuously exposed to a unique microenvironment that has been selected to provide minimal stress and optimum conditions for plant multiplication. These conditions contribute a culture induced phenotype that cannot survive the environmental conditions when directly placed in greenhouse or field (Hazarika, 2003). Transfer of *in vitro* plantlets to *ex vitro* conditions is the most critical stage in the widespread use of micropropagation. To promote *ex vitro* survival and physiological competence, especially to protect the plants from various stresses and to encourage autotrophy, a transitional environment is needed during the acclimatization phase. Manipulating the acclimatization conditions prior to or upon transplanting usually reduced losses (Huyelenbroeck and Debergh, 1986; Preece and Sutter, 1991; Desjardins, 1995) however at additional cost to the producers.

The present study was undertaken with the objective to standardise the *ex vitro* establishment techniques in gladiolus with reference to potting media, growth retardant and mycorrhizae. The results obtained are discussed in this chapter.

Survival per cent of plantlets with respect to different potting media were studied at fortnightly intervals. Among the different potting media tried, soilrite was found to be the best potting media regarding plant survival. Soilrite recorded 86.11 per cent survival after the first fortnight and 80.55 per cent survival after the second fortnight of planting. This might be due to the optimum conditions of aeration, water holding

capacity and nutrients present in soilrite. Similar results were observed in micropropagated carnation by Jagannatha *et al.* (2001). Media with two or more components showed relatively low survival. Sand : soil : leaf mould (1 : 1 : 1) recorded a survival of 41.66 per cent and sand : soil : coirpith (1 : 1 :1) recorded 44.44 per cent after one month of planting (Fig. 1). This is in agreement with Bilderbach *et al.* (1982) who obtained higher plant growth in a media composed of single component as compared to that of blended media.

Soilrite recorded highest number of leaves (5.96) one month after planting (Table 5). Similar results were obtained in micropropagated anthurium during the *ex vitro* establishment stage by Ajithkumar (1993).

Height of the plant was highest in sand : soil (17.26 cm) followed by soilrite (16.54 cm) and sand (16.42 cm) after one month of planting (Table 5). As in the case of height of the plant, sand : soil recorded the highest number of roots(9.29) one month after planting. Number of days taken for emergence of first leaf was shortest for soilrite (9.38 days) and for second leaf it was shortest for sand (13.58 days) closely followed by soilrite (13.67 days). At the same time, sand : soil (1 : 1) took 10.74 and 14.25 days for emergence of first and second leaf respectively (Fig. 2). This indicates that number of leaves is basically a genetic factor which can be modified by physical conditions.

Stomatal conductance was high in sand : soil after the first and second fortnight. The values were 1.01 and 0.22 cm/s respectively. As stomatal conductance is a measure of the rate of passage of either water vapour or carbon dioxide through the stomata, this plays a vital role in acclimatization of micropropagated plantlets. In the present study stomatal conductance was found to be higher in the first fortnight and it gradually decreased by the end of second fortnight. This might be due to the fact that the leaves of *in vitro* grown plants showed open stomata and collapsed guard cells, while acclimatized leaves presented closed stomata as well as

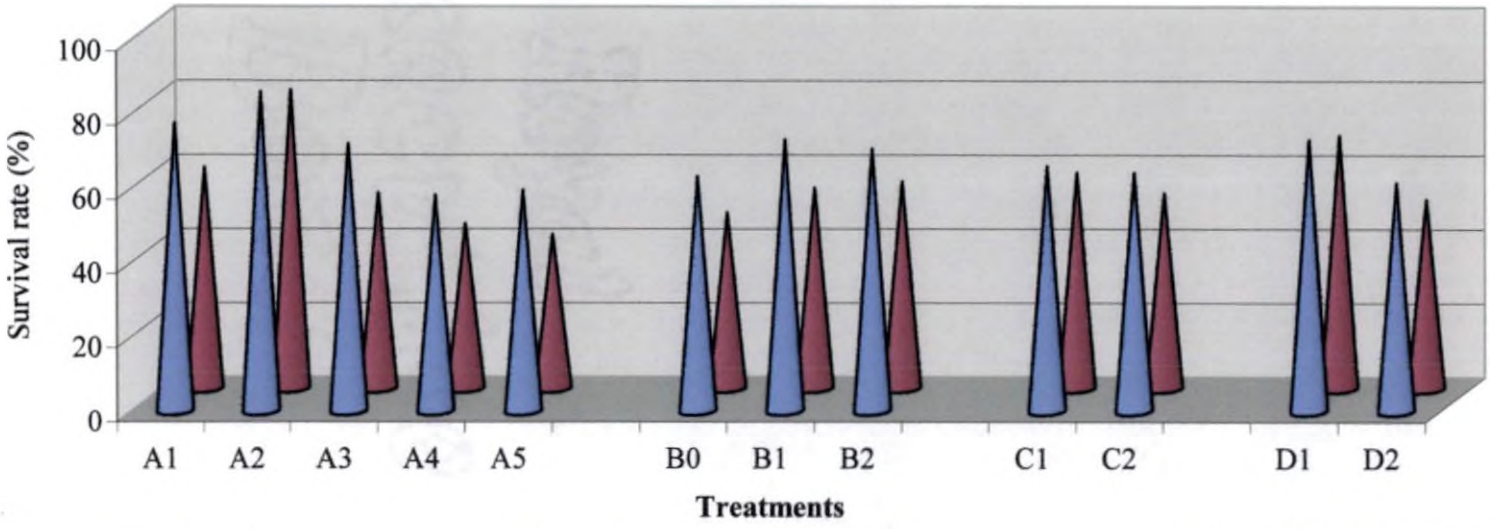


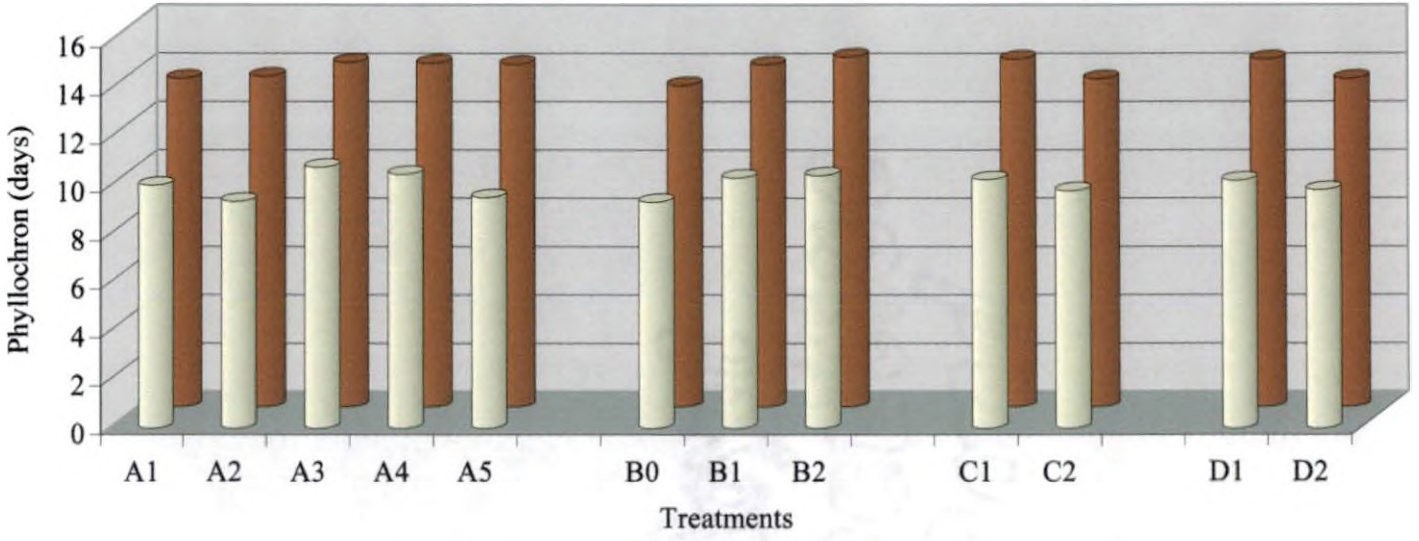
Fig. 1. Survival rate of plantlets as influenced by potting media, triazole, mycorrhizae and height of the potting media in the container

decreased stomatal density and aperture (Romano and Martins–Loucao, 2003).

Height of the potting media in the container did not show significant effect on treatments. According to Tilt *et al.* (1987) the key to plant growth responses may not lie in the size of container or type of media, but in the matching of medium and container geometry with plant growth habit. Media and container are physical components of growing systems. However, plants respond to the microenvironment created by the potting media, irrigation methods, containers and nutrients. The effects of all these reflect in plant growth.

Triadimefon, a triazole compound which is a plant growth retardant, was tried in the *ex vitro* establishment study. Plantlets treated with triazole at a concentration of 4 mg/l showed a higher survival rate of 73.83 per cent 15 days after planting, whereas one month after planting, those treated with 8 mg/l concentration showed 55.83 per cent survival followed by 4 mg/l with 54.16 per cent survival. Control plants recorded 47.50 per cent survival after one month of planting (Fig.1). It is evident that triazole treatment recorded higher survival per cent than untreated plants. Davis *et al.* (1986) obtained better survival of ornamental plants treated with triazole while transplanting to greenhouse. Samasya (2000) also obtained similar results during the *ex vitro* establishment stage of micropropagated orchids.

Height of the plant was found to be affected significantly by triazole treatment one month after planting (Fig. 3). Untreated plants recorded maximum plant height of 15.81 cm while plants treated with triazole at the higher concentration of 8 mg/l recorded minimum plant height of 14.86 cm. This shows the retarding effect of triazoles. Similar result was obtained by Samasya (2000) in micropropagated orchids. The reduced plant height is attributed to the inhibitory action of triazole on gibberellic acid biosynthesis (Hazarika, 2003). In the present



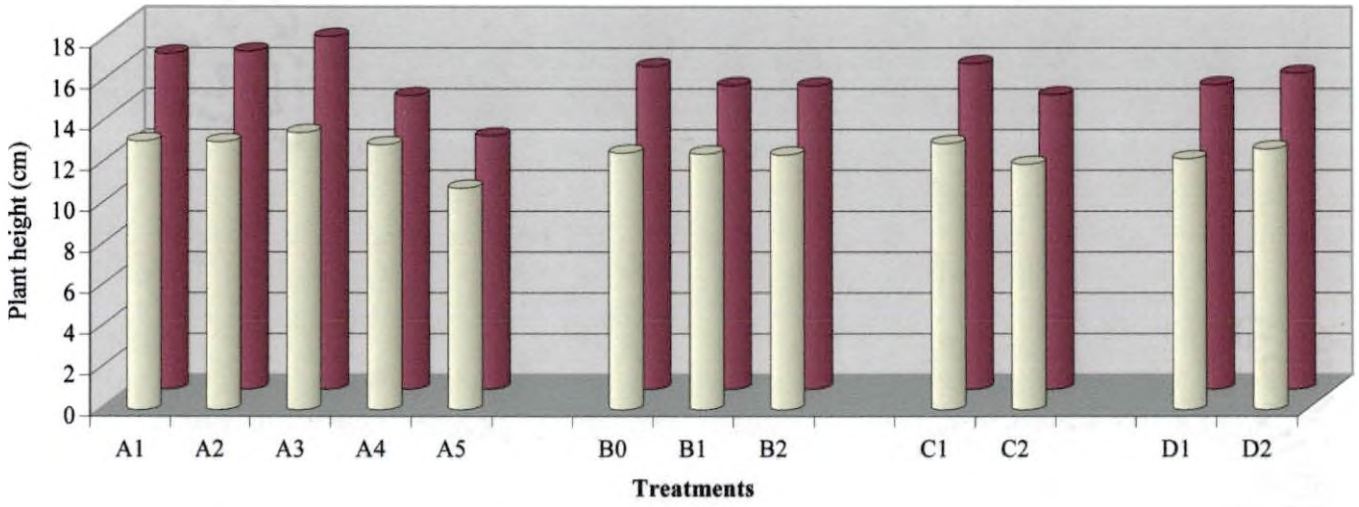
Potting media
- A_1 Sand
- A_2 Soilrite
- A_3 Sand : Soil
- A_4 Sand : Soil : coirpith
- A_5 Sand : Soil : Leaf mould

Triazole
- B_0 - control
- B_1 - 4 mg/l
- B_2 - 8 mg/l

Mycorrhizae
- C_1 *Glomus fasciculatum*
- C_2 *Glomus monosporum*

Height of potting media
- D_1 4 cm
- D_2 6 cm

Fig. 2. Main effects of potting media, triazole, mycorrhizae and height of potting media on phyllochron



Potting media

A_1 Sand
A_2 Soilrite
A_3 Sand : Soil
A_4 Sand : Soil : coirpith
A_5 Sand : Soil : Leaf mould

Triazole

B_0 - control
B_1 - 4 mg/l
B_2 - 8 mg/l

Mycorrhizae

C_1 *Glomus fasciculatum*
C_2 *Glomus monosporum*

Height of potting media

D_1 4 cm
D_2 6 cm

15 DAP
30 DAP

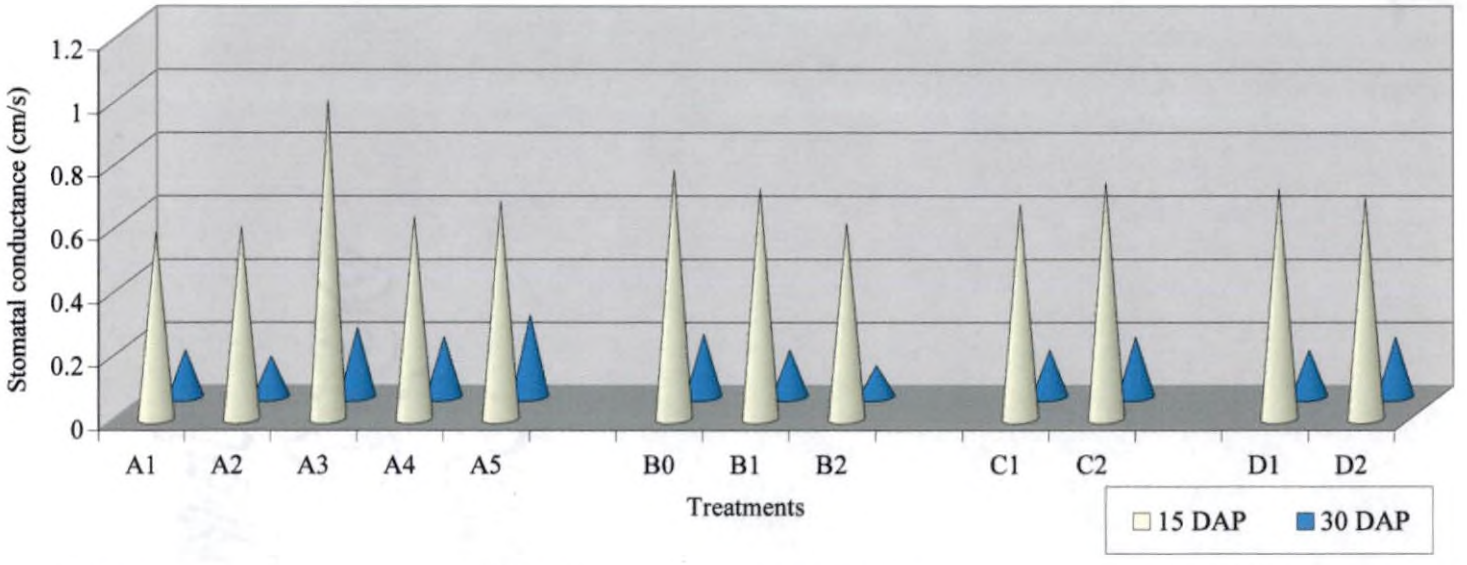
Fig. 3. Main effects of potting media, triazole, mycorrhizae and height of potting media on plant height

study it is observed that plants with greater height tend to be lanky with long and narrow leaves that are difficult to establish.

One month after planting, plants subjected to triazole application at a concentration of 8 mg/l showed highest root number (9.38) while untreated plants showed lowest root number (8.23). Similar results were obtained in micropropagated *Prunus serotina* by Eliasson *et al.* (1994) and in micropropagated sugarcane by Dhaliwal *et al.* (1997). This might be due to the shift in partitioning of assimilates from the leaves to the roots due to the action of triazoles (Steffens *et al.*, 1985).

Triazole treatment affected leaf emergence of the plantlets during the *ex vitro* establishment stage (Fig. 2). In plants treated with triazole at a concentration of 8 mg/l, the first leaf emerged after 10.45 days and second leaf emerged after 14.48 days of transplanting. At the same time untreated plants took 9.35 and 13.30 days for emergence of first and second leaf respectively (Table 12). From this it is clear that triazole treated plants showed a higher leaf production interval (phyllochron) compared to that of untreated plants. This might be due to the growth retarding action of triazoles. In accordance with this, Tari (2003) reported inhibition of primary leaf expansion in bean seedlings by triazole treatment. Ziv *et al.*(2003) also reported inhibition of leaf expansion *in vitro* as a result of addition of triazoles to the culture media.

Triazole treatment influenced the stomatal conductance significantly (Fig.4). After the first fortnight, plants treated with triazole at 8 mg/l concentration showed low stomatal conductance of 0.62 cm/s followed by 4 mg/l concentration with a value of 0.73 cm/s whereas untreated plants exhibited a higher stomatal conductance of 0.79 cm/s. The same trend was seen one month after planting even if the values ranged between 0.10 and 0.20 cm/s. This indicates that triazole decreases the stomatal conductance. Similar results were obtained by Bishnoi *et al.* (1994). Low stomatal conductance indicates reduced water loss and it is important in



Potting media

A_1 Sand
A_2 Soilrite
A_3 Sand : Soil
A_4 Sand : Soil : coirpith
A_5 Sand : Soil : Leaf mould

Triazole

B_0 - control
B_1 - 4 mg/l
B_2 - 8 mg/l

Mycorrhizae

C_1 *Glomus fasciculatum*
C_2 *Glomus monosporum*

Height of potting media

D_1 4 cm
D_2 6 cm

Fig. 4. Main effects of potting media, triazole, mycorrhizae and height of potting media on stomatal conductance

the maintenance of plant water status. High rate of water loss incurred by *in vitro* grown plants impose severe limitation in acclimatization. But application of triazole during *ex vitro* establishment stage helps to overcome this limitation by reducing the water loss from the plant. This is in accordance with the findings of Hazarika *et al.* (2002b). According to Tari (2003) the decrease in stomatal conductance was due to the reduced water loss from triazole treated leaves which in turn was caused by the reduced leaf area due to the growth retarding effect of triazole.

In the present study, untreated plants showed higher leaf area index of 1.08 followed by a reduction in leaf area index by the triazole treated plants (Table 20). This is contradictory to the findings of Dhaliwal *et al.* (1997) and Rajendiran and Ramanujan (2003). The reduced leaf area index might be due to the reduction in plant height and leaf size.

The plantlets were inoculated with two arbuscular mycorrhizal fungi during the *ex vitro* establishment stage. The two mycorrhizal fungi tried in the present study exerted differential effect on survival of plantlets. *Glomus fasciculatum* inoculated plantlets recorded 58.33 per cent survival and those inoculated with *Glomus monosporum* recorded 52.77 per cent survival of the plantlets one month after planting. Various workers reported improved survival of micropropagated crops during the *ex vitro* establishment stage due to the inoculation of arbuscular mycorrhizal fungi in several crops (Puthur *et al.*, 1988; Ramesh, 1990; Sreelatha, 1992; Vidal *et al.*, 1992; Wang *et al.*, 1993; Schultz *et al.*, 1998; Sato *et al.*, 1999; Lins *et al.*, 2003; Trindade *et al.*, 2003).

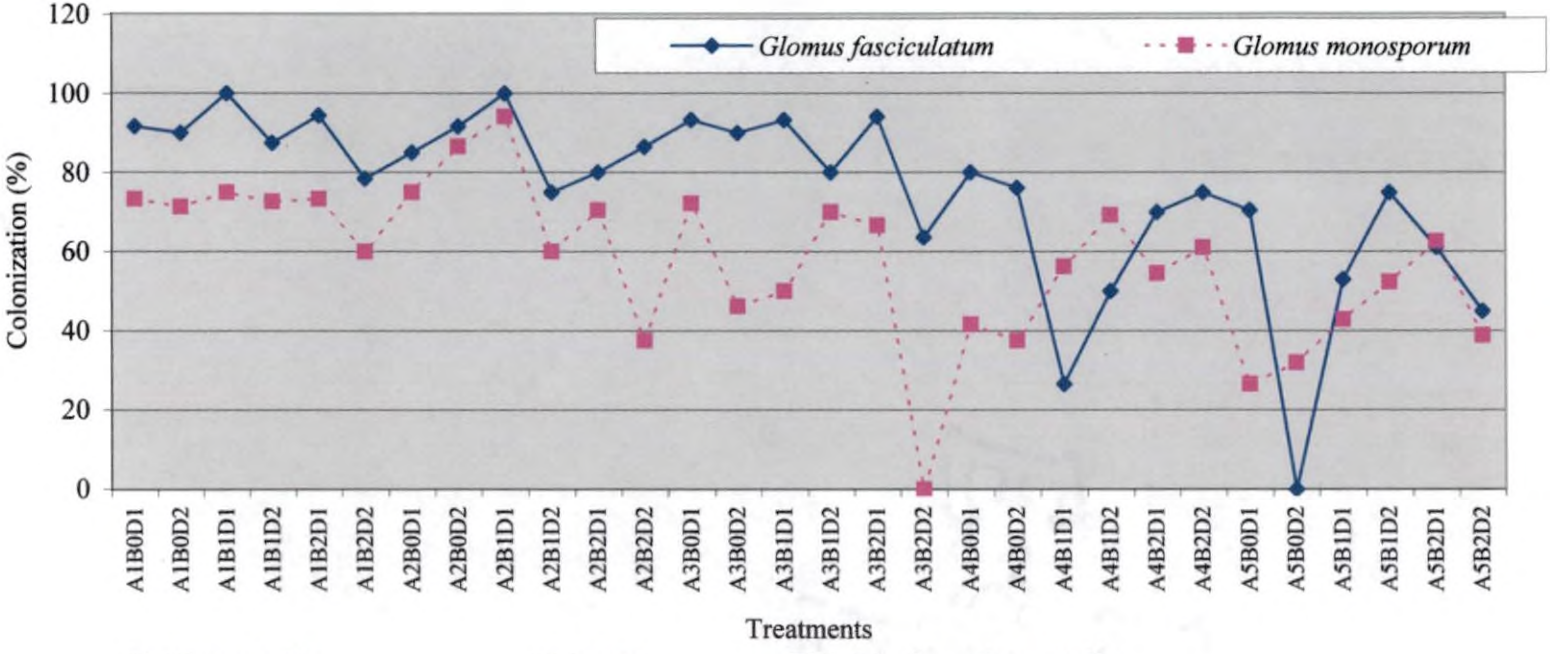
Glomus fasciculatum showed 100 per cent colonization in the treatments with sand : triazole 4 mg/l; potting media at 4 cm height and soilrite; triazole 4 mg/l; potting media at 4 cm height whereas *Glomus monosporum* recorded highest colonization of 94.10 per cent in the treatment combination with soilrite; triazole 8 mg/l; potting media at 4 cm height. Treatments with sand: soil; triazole 8 mg/l; potting media at 6 cm height

and sand : soil : leaf mould; triazole 0; potting media at 6 cm height recorded no colonization (Fig. 5). This shows that the substrate used for *ex vitro* establishment is important for growth of plants and development of AMF. This is in accordance with the findings of You-Shan *et al.* (2001), who reported the varying effect of different substrates with difference in nutrient status, aeration and water holding capacity on development of AMF.

Plant height was significantly different due to the inoculation of the two mycorrhizal fungi. *Glomus fasciculatum* inoculated plants recorded a height of 15.94 cm while *Glomus monosporum* inoculated plants showed a height of 14.41 cm one month after planting. Number of roots were also found to be high for *Glomus fasciculatum* inoculated plants compared to *Glomus monosporum* inoculated plants (Table 5).

Time taken for emergence of the first and second leaf varied with inoculation of the two mycorrhizal fungi. *Glomus monosporum* inoculated plants produced the first leaf in 9.82 days and second leaf in 13.57 days where as *Glomus fasciculatum* inoculated plants produced the first leaf in 10.28 days and second leaf in 14.38 days. In micropropagated rose inoculated with *Glomus fasciculatum* the first leaf emerged in 4.80 days and second leaf in 10.60 days (Wilson, 1993).

Glomus fasciculatum inoculated plants showed a stomatal conductance of 0.68 and 0.15 cm/s 15 and 30 DAP respectively, whereas plants inoculated with *Glomus monosporum* showed a stomatal conductance of 0.75 and 0.19 cm/s respectively 15 and 30 DAP. According to Auge *et al.* (2004) stomatal conductance is influenced by AMF colonization of the roots. At high irradiances stomatal conductance tends to increase and at lower irradiance, AMF promotion of stomatal conductance will be minimum. In the acclimatization conditions the irradiance is lower than that of field conditions and thus the stomatal conductance values also tend to be less.



Potting media

A_1 Sand
A_2 Soilrite
A_3 Sand : Soil
A_4 Sand : Soil : coirpith
A_5 Sand : Soil : Leaf mould

Triazole

B_0 - control
B_1 - 4 mg/l
B_2 - 8 mg/l

Height of potting media

D_1 4 cm
D_2 6 cm

Fig. 5. Mycorrhizal colonization of Gladiolus plantlets inoculated with *Glomus fasciculatum* and *Glomus monosporum*

Inoculation of AMF during the *ex vitro* establishment stage of micropropagated plantlets significantly improved their survival and growth due to improved absorption of water and nutrients. These benefits of AMF are attributed to the development of extensive network of hyphae around the root, which acts as an extension of the root surface and supplies more nutrients to the plant (Azcon-Aguilar and Barea, 1997; Jaizme-Vega *et al.*, 1997). The increase in phytochrome production (Allen *et al.*, 1980) and improved photosynthetic efficiency (Sivaprasad and Rai, 1984) also helped the plants in successful acclimatization during the *ex vitro* establishment.

Fresh weight was found to be highest in the treatment $A_1B_1C_2$ (sand; triazole 4 mg/l; *Glomus monosporum*) and it was on par with $A_1B_1C_1$ (sand; triazole 4 mg/l; *Glomus fasciculatum*) and $A_4B_2C_2$ (sand : soil : coirpith ; triazole 8 mg/l; *Glomus monosporum*) after the first fortnight. The values were 1.302, 1.254 and 1.218 g plant^{-1} respectively. After the second fortnight also the treatment $A_1B_1C_2$ recorded higher fresh weight followed by $A_4B_2C_2$ with values of 1.697 and 1.621 g plant^{-1} respectively (Table 19).

Dry weight was also found to be highest in the treatment $A_1B_1C_2$ (0.508 g plant^{-1}) followed by $A_3B_2C_1$ (sand : soil ; triazole 8 mg/l; *Glomus monosporum*) with a value of 0.419 g plant^{-1} after the first fortnight. After the second fortnight highest value for dry weight was recorded by the treatment $A_2B_1C_2$ (soilrite; triazole 4 mg/l; *Glomus monosporum*) and $A_2B_2C_1$ (soilrite; triazole 8 mg/l; *Glomus fasciculatum*) with a value of 0.918 g plant^{-1} (Table 19). In the present study it was observed that irrespective of the potting media and mycorrhizal fungi, triazole treated plants exhibited higher fresh and dry weights. The reduced water loss and increased photosynthate production due to the higher chlorophyll accumulation as a result of triazole treatment might be the reason for the increase in dry weight. This is in accordance with the findings of

Frederick *et al.* (2003). Higher dry matter accumulation is important for greater productivity of the plant. triazole induced higher fresh and dry weights was also reported by Samasya (2000). In the present study plants treated with triazole and mycorrhizal fungi showed greater plant weight than plants treated with triazole only. This is in accordance with the findings of Reis *et al.* (1994).

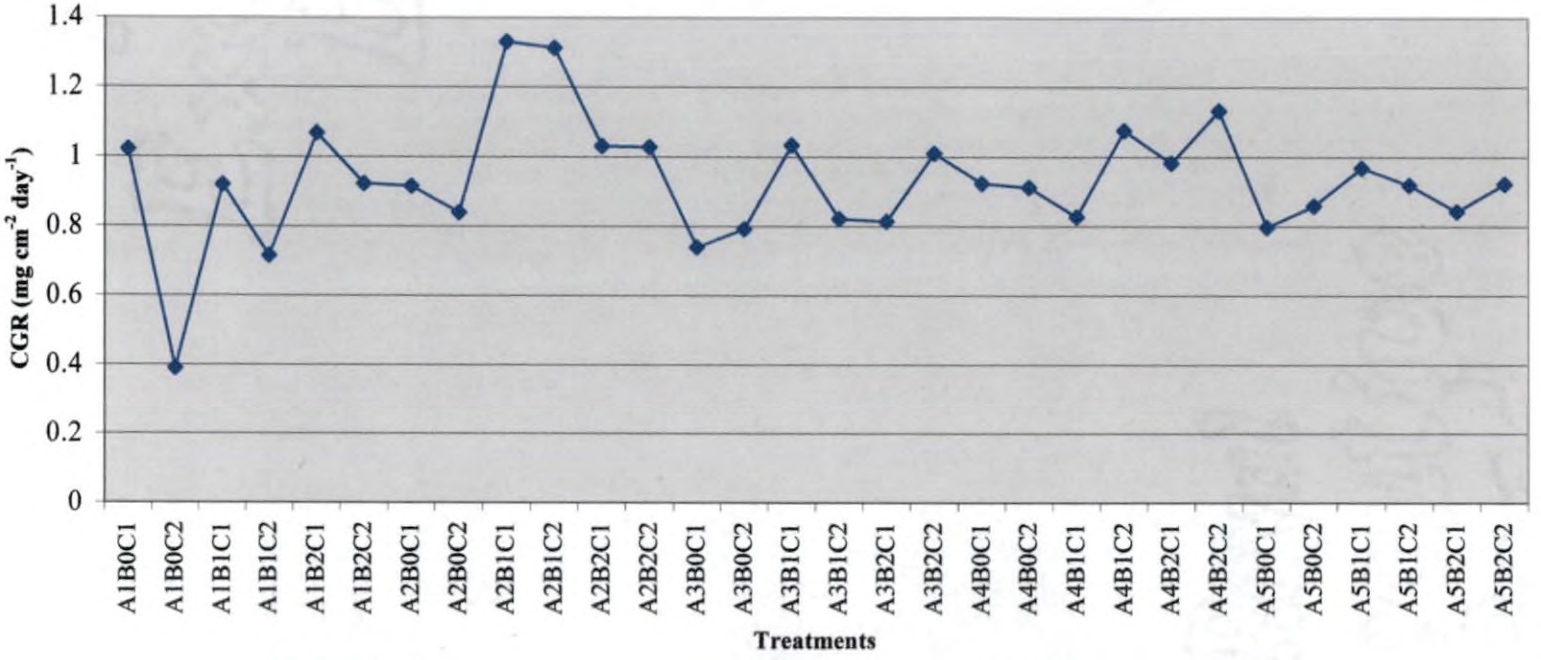
CGR values were found to be influenced by the treatments (Fig. 6). The highest value was obtained in the treatment $A_2B_1C_1$ (soilrite; triazole 4 mg/l; *Glomus fasciculatum*) followed by $A_2B_1C_2$ (soilrite; triazole 4 mg/l; *Glomus monosporum*) with values of 1.330 and 1.312 mg cm^{-2} day^{-1} respectively (Fig. 5). The lowest value of 0.391 mg cm^{-2} day^{-1} was obtained in the treatment $A_1B_0C_2$ (sand; triazole 0; *Glomus monosporum*).

In the case of RGR, the treatment $A_2B_1C_2$ (soilrite; triazole 4 mg/l; *Glomus monosporum*) recorded the highest value of 0.089 mg g^{-1} day^{-1} which was on par with the treatment $A_2B_1C_1$ (soilrite; triazole 4 mg/l; *Glomus fasciculatum*) with a value of 0.086 mg g^{-1} day^{-1}. The lowest value was observed for the treatment $A_1B_0C_2$ (sand; triazole 0; *Glomus monosporum*) with a value of 0.027 mg g^{-1} day^{-1}.

In the present study CGR and RGR values were found to be higher in treatments with triazole. Untreated plants recorded lower CGR and RGR. This might be due to the increased dry weight as a result of triazole application. This is in agreement with the findings of Samasya (2000).

In the present study the two factor interactions between potting media and triazole, potting media and mycorrhizae, type of potting media and height of potting media, triazole and mycorrhizae, triazole and height of potting media, mycorrhizae and height of potting media have been discussed wherever they were found to be significant.

Leaf number was significantly affected by the interaction effect between triazole and mycorrhizae after the first fortnight (Table 9). The



Potting media

A_1 Sand
A_2 Soilrite
A_3 Sand : Soil
A_4 Sand : Soil : coirpith
A_5 Sand : Soil : Leaf mould

Triazole

B_0 - control
B_1 - 4 mg/l
B_2 - 8 mg/l

Mycorrhizae

C_1 *Glomus fasciculatum*
C_2 *Glomus monosporum*

Fig. 6. CGR values as influenced by potting media, triazole and mycorrhizae one month after planting

maximum number of leaves was recorded in the interaction B_1C_2 (triazole 4 mg/l; *Glomus monosporum*). The value was 5.25. Lowest leaf number was recorded by the interaction B_2C_2 (triazole 8 mg/l; *Glomus monosporum*) with a value of 3.95. This can be explained by the fact that the growth retardant action of triazole is compensated by the growth promotory action of AMF. Varshney *et al.* (2002) obtained increased number of leaves in micropropagated onion bulblets due to inoculation of AMF. This interaction was not significant one month after planting. Leaf number was not influenced by any other interaction.

Plant height was significantly influenced by the interaction between potting media and triazole after the first fortnight. But the interaction was not significant at 30 DAP. The highest plant height obtained 15 DAP was due to the interaction A_5B_0 (sand : soil : leaf mould; triazole 0) and it recorded a value of 14.36 cm. This was on par with the treatments A_1B_0 (sand; triazole 0) and A_3B_0 (sand : soil : leaf mould; triazole 8 mg/l) with values of 13.64 cm and 13.30 cm respectively. Lowest plant height was recorded by the interaction A_5B_2 (sand: soil: leaf mould; triazole 8 mg/l) with a value of 8.95 cm. The growth retarding effect of triazole was dominant irrespective of the potting media tried. This is due to the effect of triazoles on isopropanoid pathway which produces gibberellin, a plant growth regulator, responsible for elongation of plants. triazoles block the isopropanoid pathway and thus affect gibberellin biosynthesis (Graebe, 1987), which in turn lead to reduction in plant height.

Interaction between potting media and mycorrhizae affected the plant height significantly 15 DAP (Table 7). But the effect was not significant one month after planting. The interaction A_2C_1 (soilrite; *Glomus fasciculatum*) recorded the highest value of 13.83 cm after the first fortnight. It was on par with A_1C_1 (sand; *Glomus fasciculatum*) with 13.5 cm height. The lowest value of 9.22 cm was obtained for the interaction A_5C_2 (sand : soil : leaf mould; *Glomus monosporum*).

Difference in nutrient status, aeration and water holding capacity of the media have different influence on growth of AMF and plant growth. Improvement in height due to mycorrhizal inoculation might be due to the enhanced nutrient uptake by the roots.

Interaction effect between triazole and mycorrhizae was significant on the plant height 15 DAP (Table 9). The interaction B_0C_1 (triazole 0; *Glomus fasciculatum*) recorded a value of 14.21 cm while the interaction B_2C_2 (triazole 8 mg/l; *Glomus monosporum*) recorded the lowest value of 10.88 cm. The interaction was not significant 30 DAP. This indicates that irrespective of the mycorrhizal fungi, triazole application suppressed the plant height.

Interaction between type of potting media and height of potting media, triazole and height of potting media, mycorrhizae and height of potting media did not affect the plant height significantly.

Interaction between triazole and mycorrhizae affected the root number significantly (Table 16). The interaction B_2C_1 (triazole 8 mg/l; *Glomus fasciculatum*) produced highest root number (6.75) after the first fortnight. Lowest value of 4.90 was recorded by B_0C_2 (triazole 0; *Glomus monosporum*). One month after planting, the interaction B_1C_1 (triazole 4 mg/l; *Glomus fasciculatum*) recorded highest root number (9.65) which was closely followed by B_2C_2 (triazole 8 mg/l; *G. monosporum*) and B_1C_2 (triazole 4 mg/l; *Glomus fasciculatum*) with values of 9.35 and 9.20 respectively. The interaction B_0C_1 (triazole 0; *Glomus fasciculatum*) recorded the lowest number of roots (7.80) after 30 days of planting. The interaction effect of triazole and AMF on root number was positive. The increased partition of assimilates to roots due to the action of triazole is enhanced by inoculation of mycorrhizae. AMF inoculated roots were found to be more branched (Nowak, 2004) thus improving the nutrient uptake.

Interaction of potting media and triazole affected the leaf production interval (Fig. 7). Highest interval to produce first leaf was observed in the interaction A_5B_2 (sand : soil : leaf mould; triazole 8 mg/l) which took 11.63 days. It was on par with A_5B_1 (sand : soil : leaf mould; triazole 4 mg/l) with a value of 11.25 days. Earliest emergence of first leaf was recorded in the interaction A_2B_0 (soilrite; triazole 0) with a value 9.38 days. The interaction A_4B_2 (sand : soil : coirpith; traizole 8 mg/l) recorded the highest interval to produce the second leaf with a value of 15.38 days. Interaction A_1B_0 (sand; triazole 0) took the shortest interval of 11.25 days for emergence of the second leaf. Irrespective of potting media used, triazole was found to significantly affect the leaf production interval. triazole treated plants showed greater delay in emergence of leaves compared to that of untreated plants.

Interaction between potting media and mycorrhizae did not affect the emergence of first leaf but the effect was significant on production of second leaf (Table 14). Interaction A_5C_1 (sand : soil : leaf mould; *Glomus fasciculatum*) took 15.67 days for emergence of second leaf while A_1C_2 (sand; *Glomus monosporum*) took 13.42 days.

Interaction effect of triazole and mycorrhizae was significant on production of second leaf (Table 16). Interaction B_2C_1 (triazole 8 mg/l; *Glomus fasciculatum*) recorded the highest interval of 14.45 days while B_0C_1 (triazole 0; *Glomus fasciculatum*) recorded 12.15 days for emergence of second leaf.

All other interactions did not significantly influence phyllochron.

Stomatal conductance was significantly affected by the interaction of potting media and triazole both 15 and 30 DAP. Highest value of 1.48 cm/s was recorded for the interaction A_4B_0 (sand : soil : coirpith; triazole 0) and lowest value of 0.16 cm/s was obtained for A_5B_2 (sand : soil : leaf mould; triazole 8 mg/l) after the first fortnight. One month after planting, the interaction A_4B_0 recorded the highest value of 0.29 cm/s. The value

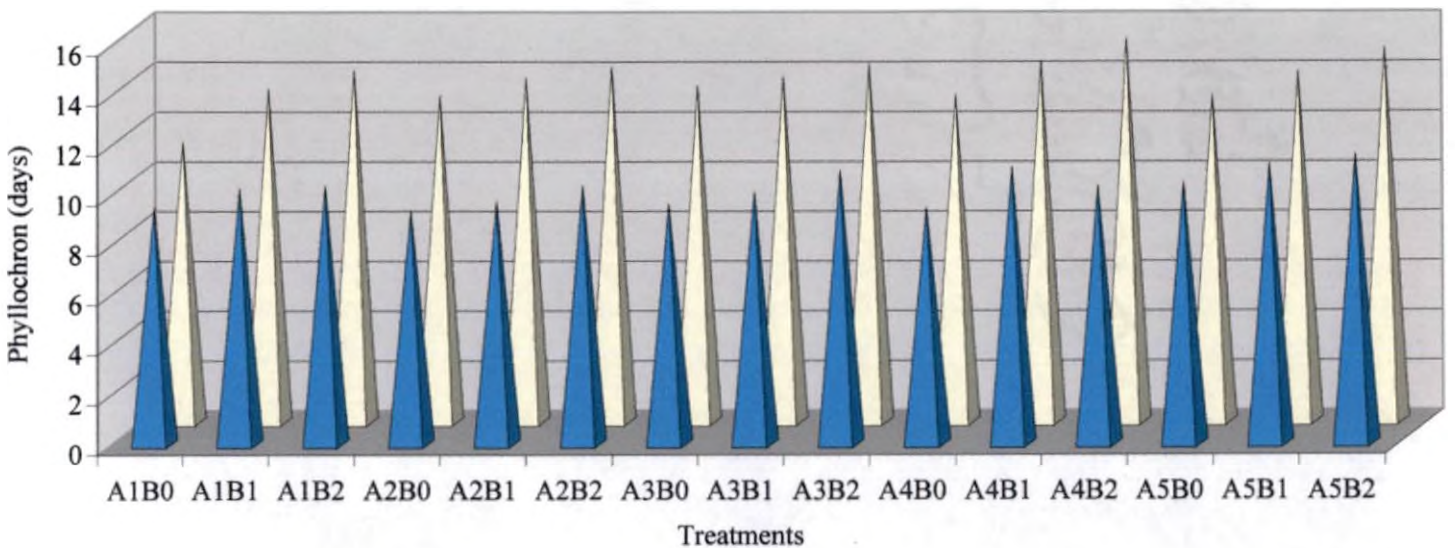


Fig. 7. Interaction effect of potting media and triazole on phyllochron

was lowest for A_1B_2 (sand; triazole 8 mg/l) which was on par with A_1B_1 (sand; triazole 4 mg/l) and A_2B_1 (soilrite; triazole 4 mg/l). *In vitro* developed stomata are unable to close and fail to control water loss. Thus during initial stages of acclimatization the plantlets show higher water loss. Inability of the plantlets to regulate the water loss may lead to their death. So a reduced stomatal conductance towards the later stages of acclimatization is essential for proper plant establishment. In the present study, sand and soilrite in combination with triazole was found to be better in decreasing the stomatal conductance.

Interaction of potting media and mycorrhizae significantly affected stomatal conductance (Table 22). The interaction A_3C_2 (sand: soil; *Glomus monosporum*) recorded the highest value both 15 and 30 DAP. The values were 1.13 and 0.23 cm/s respectively. The interaction A_2C_1 (soilrite; *Glomus fasciculatum*) recorded lowest value of 0.45 cm/s after the first fortnight and A_1C_2 (sand; *Glomus monosporum*) recorded lowest value of 0.09 cm/s after the second fortnight.

Stomatal conductance was significantly affected by interaction between type of potting media and height of potting media in the container (Table 23). The interaction A_2D_2 (soilrite; 6 cm) gave highest value of 1.10 cm/s after 15 days of planting. The same interaction recorded the highest value of 0.24 cm/s after 30 days of planting. Lowest values were recorded by the interactions A_5D_1 (sand : soil : leaf mould; 4 cm) 15 DAP and A_1D_1 (sand ; 4 cm) 30 DAP. Stomatal conductance was found to be positively influenced by height of potting media. Potting media with greater height was found to produce plants with higher stomatal conductance. Higher water holding capacity of soilrite may also have influence on the stomatal conductance of the plants (Bohne, 2004).

Interaction of triazole and mycorrhizae significantly affected the stomatal conductance 15 DAP. But it was not significant after one month

of planting (Table 24). Stomatal conductance was not significantly affected by interaction between triazole and height of potting media.

Interaction effect of mycorrhizae and height of potting media in the container was significant on stomatal conductance (Table 26). The interaction C_2D_1 (*Glomus monosporum*; 4 cm) gave highest value of 0.87 cm/s after first fortnight and C_1D_1 (*Glomus fasciculatum*; 4 cm) recorded lowest value of 0.58 cm/s. After one month of planting, the interaction C_1D_2 (*Glomus fasciculatum*; 6 cm) recorded the highest value of 0.23 cm/s and C_2D_2 (*Glomus monosporum*; 6 cm) recorded the lowest value of 0.14 cm/s.

Leaf area index was found to be affected by the interaction between potting media and triazole (Table 21). The interaction A_2B_0 (soilrite; triazole 0) recorded the highest value of 1.24 and A_4B_2 (sand : soil : coirpith; triazole 8 mg/l) recorded the lowest value of 0.64. The increased LAI in soilrite might be due to the optimum plant growth regulating conditions of this medium. Similar results were obtained for micropropagated anthurium during the acclimatization stage by Ajithkumar (1993). The inhibitory effect of triazole might be the reason for the poor leaf area index shown by the interaction with high concentration of triazole.

Interaction between potting media and mycorrhizae significantly influenced the leaf area index (Table 22). Highest value of 1.08 was recorded by A_2C_1 (soilrite; *Glomus fasciculatum*) and it was on par with A_1C_1 (sand; *Glomus fasciculatum*) and A_3C_1 (sand : soil; *Glomus fasciculatum*) with values of 1.04 and 1.03 respectively. The interaction A_5C_2 (sand: soil : leaf mould; *Glomus monosporum*) recorded the lowest value of 0.78. Increased leaf area index due to AMF inoculation was reported by several workers (Setua *et al.*, 1999; Yano-Melo *et al.*, 1999; Estrada-Luna, 2000; Varshney *et al.*, 2002). This might be due to the improved penetration of plant roots in the potting media due to the proli-

mycelium of AMF which increases nutrient uptake and in turn improves the plant growth (Thaker and Jasrai, 2003).

Leaf area index was significantly affected by the interaction between triazole and mycorrhizae (Table 24). Interaction B_1C_2 (triazole 4 mg/l; *Glomus monosporum*) recorded the highest value of 1.05 while B_2C_2 (triazole 8 mg/l; *Glomus monosporum*) recorded the lowest value of 0.90. It was found that triazole at lower concentration of 4 mg/l and *Glomus monosporum* showed a positive effect on leaf area index.

All other interactions did not affect the leaf area index.

Economics of acclimatization was computed by combining the production cost of *in vitro* plantlets and the cost of *ex vitro* establishment. The cost of micropropagation was the same for all the plantlets and it was found to be approximately 3.41 Rs./plantlet. The cost of acclimatization showed slight differences since different treatments were involved using different potting media and varying concentrations of triazole. The cost of mycorrhiza was constant for all the treatments. Cost of acclimatization was found to be highest for the treatments $A_2B_2C_1D_2$ (soilrite; triazole 8 mg/l; *Glomus fasciculatum*; 6 cm height) and $A_2B_2C_2D_2$ (soilrite; triazole 8 mg/l; *Glomus monosporum*; 6 cm height) with 1.84 Rs/plantlet. This is due to the higher cost of soilrite, higher concentration of triazole involved and greater quantity of potting media required to maintain a height of 6 cm inside the container for both the treatments. The lowest cost of acclimatization was estimated for the treatments $A_3B_0C_1D_1$ (sand : soil; triazole 0; *Glomus fasciculatum*; 4 cm) and $A_3B_0C_2D_1$ (sand : soil; triazole 0; *Glomus monosporum*; 4 cm) with 1.09 Rs./plantlet. This is due to the lower cost of sand : soil combination, absence of triazole and lesser quantity of potting media required.

The cost of a hardened plantlet was computed by combining the *in vitro* production cost and acclimatization cost. As mentioned before, the production cost is constant for all the plantlets. But the differences in

acclimatization cost have brought about slight variation in the total cost of the individual plantlets (Table 28). The cost per plantlet was found to range from a maximum of Rs. 5.25 to a minimum of Rs. 4.50. Eventhough there is a marginal increase in cost when soilrite and high concentration of triazole are used, the better survival and overall growth obtained will compensate for the slight increase in cost.

Survival rates obtained in this study was moderate and the growth rate was not satisfactory. The shift in partition of assimilates from leaves to the *ex vitro* developed corm might be the reason for the initial slow growth of the plantlets. Production of cormels *in vitro* and planting out of these cormels may help to overcome this situation. Future works should be oriented in this direction.

SUMMARY

6. SUMMARY

Attempts were made in the Department of Pomology and Floriculture, College of Agriculture, Vellayani during 2000-2004 to standardize the *ex vitro* establishment techniques in gladiolus variety Vinks Glory.

The effects of different potting media, triazole, arbuscular mycorrhizal fungi and height of potting media inside the container on *ex vitro* establishment of gladiolus plantlets were studied.

The salient findings of the above studies are summarized below:

Among the different potting media tried, soilrite recorded the highest survival of 86.11 and 80.55 per cent after the first and second fortnight respectively. Sand : Soil : leaf mould recorded the lowest survival per cent.

Triazole treated plants exhibited a higher survival per cent than non treated plants. Plantlets treated with triazole at a concentration of 4 mg/l showed highest survival rate of 73.83 per cent 15 DAP. However plantlets treated with 8 mg/l concentration recorded a higher survival rate of 55.83 per cent 30 DAP.

Among the two arbuscular mycorrhizal fungi tried for *ex vitro* establishment, *Glomus fasciculatum* inoculated plants recorded higher survival per cent both 15 and 30 DAP. *Glomus fasciculatum* recorded a higher colonization of 100 per cent in the treatment combination of sand; triazole 4 mg/l; 4 cm height of potting media and soilrite; triazole 4 mg/l; 4 cm height of potting media. *Glomus monosporum* recorded highest colonization of 94.10 per cent in the treatment combination of soilrite; triazole 4 mg/l; 4 cm height of potting media.

Regarding the leaf number, soilrite recorded the highest value both 15 and 30 DAP. Triazole treated plants showed a decrease in leaf number compared to that of the control plants probably due to the growth retarding effects of triazole. The two mycorrhizal fungi used did not cause any significant difference in leaf number. Interaction effect of triazole and mycorrhizae affected leaf number significantly after the first fortnight. Triazole 4 mg/l in combination with *Glomus monosporum* recorded the highest leaf number. The growth retardant action of triazole at lower level might be compensated by the growth promoting action of AMF. But at higher concentration of triazole (8 mg/l) this effect was not observed.

After the first and second fortnight the highest plant height was recorded when sand: soil was used as the potting media. The lowest values during both periods were recorded in sand: soil : leaf mould medium. The effect of triazole application on height of the plantlets was evident. The lowest plant height was obtained for plants treated with triazole at a concentration of 8 mg/l with a value of 14.86 cm one month after planting and the value was highest for untreated plants (15.81 cm). Among the two mycorrhizal fungi tried in the experiment, *Glomus fasciculatum* treated plants showed higher plant height both 15 and 30 DAP as compared to those inoculated with *Glomus monosporum*.

Plant height was significantly affected by the interaction effect between potting media and triazole after the first fortnight. The growth retarding effect of triazole was found to be dominant irrespective of the potting media used. The interaction between potting media and mycorrhizae also influenced the plant height after the first fortnight. It was also observed that irrespective of the *Glomus* sp. used, triazole application suppressed the plant height.

Treatments with sand and sand : soil recorded highest number of roots both 15 and 30 DAP. Triazole application was found to influence the root number significantly only during the second fortnight. The highest

value was observed for 8 mg/l concentration while control plants recorded the lowest value. *Glomus fasciculatum* treated plants recorded higher root number as compared to those treated with *Glomus monosporum* after one month of planting. Interaction between triazole and mycorrhizae affected the root number. Triazole application and mycorrhizal inoculation improved the number of roots.

Phyllochron was not affected by the potting media used. But application of triazole delayed the leaf production of the plants compared to that of control plants. *Glomus monosporum* enhanced the leaf production when compared to *Glomus fasciculatum*. Interaction effect of potting media and triazole affected the phyllochron. The delay in leaf production as a result of application of triazole was visible in the treatments irrespective of the potting media.

Inspite of the differences in the potting media and mycorrhizal fungi used, triazole application resulted in higher fresh and dry weights. Similarly CGR and RGR values were also found to be highest in treatments with triazole due to the increase in dry matter accumulation as a result of triazole application. For both CGR and RGR, the highest values were noted for the treatments involving soilrite and triazole at 4 mg/l concentration regardless of the mycorrhizal species used.

Stomatal conductance was significantly affected by the different potting media used. The values of all the treatments were found to be higher in the first fortnight and gradually decreased by the end of the second fortnight. Triazole application was found to lower the stomatal conductance. Among the two mycorrhizae tried for *ex vitro* establishment, *Glomus monosporum* recorded higher values both 15 and 30 DAP.

Interaction effect of potting media and triazole was evident on the stomatal conductance of the plantlets. Sand and soilrite in combination with triazole was found to be better in decreasing the stomatal conductance. The interaction effect between type of potting media and

height of potting media showed that stomatal conductance was positively influenced by the height of the potting media. Interaction of potting media and mycorrhizae significantly affected stomatal conductance. The decrease in stomatal conductance during the later stages in all the interactions indicates the better acclimatization of the plantlets to the *ex vitro* conditions.

Among the different potting media used, soilrite recorded the maximum leaf area index of 1.13 after one month of planting and the lowest value recorded was 0.84 for sand : soil : leaf mould. Triazole treated plants showed a reduced leaf area index due to the reduced leaf number and plant height. Triazole concentration of 8 mg/l recorded the lowest value of 0.94 whereas untreated plants recorded a value of 1.08. The two mycorrhizal species did not have any significant effect on the leaf area index.

Cost of acclimatization was found to be highest in the treatments with soilrite and higher concentration of triazole irrespective of the *Glomus* species used. However survival and growth parameters were found to be better in these treatments.

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* Original not seen

APPENDICES

APPENDIX – I

Composition of Murashige and Skoog (1962) medium

Particulars	Weight taken	Volume made up	Volume pipetted
Solution A			
1) NH_4NO_3	16.5 g		
2) KNO_3	19.0 g	250 mg	25 ml
3) $MgSO_4 . 7 H_2O$	3.7 g	(10 x)	
4) KH_2PO_4	1.7 g		
Solution B			
1) $CaCl_2 . 2H_2O$	8.00 g	100 ml (20 x)	5 ml
Solution C			
1) H_3BO_3	920 mg		
2) $MnSO_4 . 4 H_2O$	1.69 g	100 ml	1 ml
3) $ZnSO_4 . 7 H_2O$	860 mg	(100 x)	
4) KI	83 mg		
5) $Na_2MoO_4 . 2 H_2O$	25 mg		
Solution D			
1) $FeSO_4 . 7H_2O$	745 mg	100 ml	5 ml
2) Na_2EDTA	556 mg	(20 x)	
Solution E			
1) $CoCl_2 . 6 H_2O$	12.5 mg	250 ml	0.5 ml
2) $CuSO_4 . 5H_2O$	12.5 mg	(500 x)	
Solution F			
1) Glycine	200 mg		
2) Nicotine acid	50 mg	100 ml	1 ml
3) Pyridoxine HCl	50 mg	(100 x)	
4) Thiamine HCl	10 mg		

Inositol 100 g
Sucrose 30 g
Agar 8 g

APPENDIX – II

Survival percentage of plantlets, 15 and 30 DAP

Treatments	15 DAP		30 DAP	
	Number of plants survived	% Survival	Number of plants survived	% Survival
$A_1B_0C_1D_1$	5	83.33	5	83.33
$A_1B_0C_1D_2$	5	83.33	4	83.33
$A_1B_0C_2D_1$	6	100.00	6	100.0
$A_1B_0C_2D_2$	6	100.00	5	83.33
$A_1B_1C_1D_1$	5	83.33	5	83.33
$A_1B_1C_1D_2$	6	83.33	4	66.66
$A_1B_1C_2D_1$	5	83.33	3	50.00
$A_1B_1C_2D_2$	4	66.66	2	33.33
$A_1B_2C_1D_1$	4	66.66	2	33.33
$A_1B_2C_1D_2$	4	66.66	2	33.33
$A_1B_2C_2D_1$	4	66.66	3	50.00
$A_1B_2C_2D_2$	3	50.00	2	33.33
$A_2B_0C_1D_1$	6	100.0	6	100.00
$A_2B_0C_1D_2$	5	83.33	4	66.66
$A_2B_0C_2D_1$	6	100.0	6	100.00
$A_2B_0C_2D_2$	4	66.66	4	66.66
$A_2B_1C_1D_1$	5	83.33	5	83.33
$A_2B_1C_1D_2$	6	100.00	6	100.00
$A_2B_1C_2D_1$	5	83.33	4	66.66
$A_2B_1C_2D_2$	5	83.33	5	83.33
$A_2B_2C_1D_1$	6	100.0	5	83.33
$A_2B_2C_1D_2$	5	83.33	5	83.33
$A_2B_2C_2D_1$	4	66.66	4	66.66
$A_2B_2C_2D_2$	5	83.33	4	66.66
$A_3B_0C_1D_1$	4	66.66	3	50.00
$A_3B_0C_1D_2$	5	83.33	4	66.66
$A_3B_0C_2D_1$	5	83.33	4	66.66
$A_3B_0C_2D_2$	4	66.66	3	50.00
$A_3B_1C_1D_1$	3	50.00	2	33.33
$A_3B_1C_1D_2$	3	50.00	2	33.33
$A_3B_1C_2D_1$	6	100.00	5	83.33
$A_1B_1C_2D_2$	5	83.33	5	83.33
$A_1B_2C_1D_1$	6	100.00	3	50.00
$A_3B_2C_1D_2$	5	83.33	3	50.00
$A_3B_2C_2D_1$	4	66.66	2	33.33
$A_3B_2C_2D_2$	2	33.33	2	53.33

APPENDIX – II Continued

Treatments	15DAP		30DAP	
	Number of plants survived	% Survival	Number of plants survived	% Survival
$A_4B_0C_1D_1$	4	66.66	3	50.00
$A_4B_0C_1D_2$	5	83.33	2	33.33
$A_4B_0C_2D_1$	3	50.00	2	33.33
$A_4B_0C_2D_2$	2	33.33	2	33.33
$A_4B_1C_1D_1$	4	66.66	3	50.00
$A_4B_1C_1D_2$	4	66.66	4	66.66
$A_4B_1C_2D_1$	4	66.66	3	50.00
$A_4B_1C_2D_2$	3	50.00	3	50.00
$A_4B_2C_1D_1$	3	50.00	2	33.33
$A_4B_2C_1D_2$	2	33.33	2	33.33
$A_4B_2C_2D_1$	4	66.66	3	50.00
$A_4B_2C_2D_2$	4	66.66	3	50.00
$A_5B_0C_1D_1$	5	83.33	3	50.00
$A_5B_0C_1D_2$	4	66.66	3	50.00
$A_5B_0C_2D_1$	5	83.33	2	33.33
$A_5B_0C_2D_2$	3	50.00	2	33.33
$A_5B_1C_1D_1$	2	33.33	2	33.33
$A_5B_1C_1D_2$	3	50.00	3	50.00
$A_5B_1C_2D_1$	3	50.00	2	33.33
$A_5B_1C_2D_2$	4	66.66	3	50.00
$A_5B_2C_1D_1$	3	50.00	2	33.33
$A_5B_2C_1D_2$	4	66.66	2	33.33
$A_5B_2C_2D_1$	3	50.00	3	50.00
$A_5B_2C_2D_2$	4	66.66	3	50.00

APPENDIX – III

Mean values of biometric and physiological characters as influenced by potting media, triazole, mycorrhizae and height of potting media in the container

Treatments	No. of leaves /plant		Plant height (cm)		No. of roots/plant		Phyllochron (days)		Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	First leaf	Second leaf	15 DAP	30 DAP	30 DAP
$A_1B_0C_1D_1$	4.50	5.50	17.45	21.65	7.50	9.50	9.00	14.50	1.35	0.50	1.15
$A_1B_0C_1D_2$	4.00	5.00	12.55	15.75	5.00	9.00	9.50	13.50	2.25	1.94	0.87
$A_1B_0C_2D_1$	5.50	5.50	12.55	15.80	4.50	9.50	10.5	13.50	1.27	1.40	1.12
$A_1B_0C_2D_2$	5.50	5.50	12.00	16.15	6.50	8.00	9.00	11.50	1.86	0.29	0.88
$A_1B_1C_1D_1$	4.50	6.50	10.35	14.00	6.50	9.50	10.00	14.00	1.87	0.95	1.09
$A_1B_1C_1D_2$	4.50	7.00	11.60	13.30	6.00	9.00	10.00	13.00	0.82	0.60	0.95
$A_1B_1C_2D_1$	6.00	5.00	13.20	16.95	6.50	8.00	10.50	13.00	1.31	0.87	1.08
$A_1B_1C_2D_2$	6.50	6.50	15.00	18.40	7.50	8.50	10.50	13.50	1.12	0.85	0.85
$A_1B_2C_1D_1$	4.50	6.00	12.50	15.90	5.50	9.00	11.00	15.00	0.89	0.46	1.07
$A_1B_2C_1D_2$	6.00	5.00	16.70	20.50	7.50	9.00	9.50	12.50	0.78	0.39	1.10
$A_1B_2C_2D_1$	4.00	5.50	11.60	13.70	5.50	9.00	10.00	15.50	0.89	0.51	1.14
$A_1B_2C_2D_2$	4.50	5.50	12.35	15.00	5.50	9.00	11.00	13.50	0.69	0.12	1.05
$A_2B_0C_1D_1$	5.00	6.00	11.35	17.10	6.00	8.50	10.00	14.50	1.55	0.37	0.93
$A_2B_0C_1D_2$	4.00	6.50	11.35	15.70	4.50	9.50	8.50	13.50	1.41	0.60	1.38
$A_2B_0C_2D_1$	5.00	6.00	13.15	15.50	4.50	8.00	10.50	15.00	1.37	0.64	1.49
$A_2B_0C_2D_2$	5.00	6.00	10.80	13.15	3.50	9.50	9.00	14.00	1.39	0.63	1.15
$A_2B_1C_1D_1$	4.00	5.00	12.70	14.16	6.00	9.50	9.00	14.50	1.28	0.58	0.76
$A_2B_1C_1D_2$	5.00	6.00	12.45	14.85	6.50	9.00	10.50	12.50	1.03	0.38	1.23
$A_2B_1C_2D_1$	6.50	7.00	12.00	14.25	4.00	8.50	10.00	13.50	0.74	0.45	1.13
$A_2B_1C_2D_2$	5.50	6.50	12.20	14.55	4.50	8.00	9.50	12.00	2.83	0.89	1.28
$A_2B_2C_1D_1$	5.50	6.50	12.25	15.20	5.00	8.50	8.50	12.50	1.39	0.34	1.14
$A_2B_2C_1D_2$	6.00	6.50	14.85	18.30	5.50	8.50	9.50	13.50	0.75	0.43	1.06
$A_2B_2C_2D_1$	5.50	6.50	9.25	17.75	7.00	9.00	9.00	13.50	2.10	1.34	0.95
$A_2B_2C_2D_2$	3.50	4.50	13.05	16.00	3.00	9.50	10.50	15.00	2.02	0.71	1.06
$A_3B_0C_1D_1$	4.50	6.00	15.15	17.90	3.50	9.00	9.50	13.00	1.63	0.85	1.12
$A_3B_0C_1D_2$	5.00	6.00	11.55	17.10	4.00	8.50	10.00	15.00	2.55	1.30	0.95
$A_3B_0C_2D_1$	4.50	5.50	13.00	16.50	5.50	7.00	10.50	14.50	2.20	1.76	0.94
$A_3B_0C_2D_2$	5.00	6.00	13.50	17.86	5.00	8.50	11.50	15.50	1.49	2.01	1.07
$A_3B_1C_1D_1$	4.50	6.00	11.00	14.65	6.00	9.00	11.00	15.00	1.44	0.66	1.23
$A_3B_1C_1D_2$	5.00	6.00	16.05	19.65	4.50	8.00	9.50	13.00	2.69	1.66	0.97
$A_3B_1C_2D_1$	4.50	5.50	13.55	17.20	4.50	10.00	10.50	14.00	2.07	1.28	1.06
$A_3B_1C_2D_2$	5.00	6.00	14.10	17.20	3.50	8.50	9.50	13.50	1.18	0.67	1.05
$A_3B_2C_1D_1$	4.50	6.00	12.45	15.05	6.50	9.50	12.00	15.00	0.91	0.55	0.75
$A_3B_2C_1D_2$	4.50	6.00	16.80	20.20	5.00	9.00	11.50	14.00	0.47	0.28	0.92
$A_3B_2C_2D_1$	3.50	5.50	12.65	16.20	5.00	9.50	10.50	15.00	1.12	0.39	1.02
$A_3B_2C_2D_2$	4.00	6.00	13.15	16.35	6.00	8.50	10.00	13.50	1.20	0.70	1.02
$A_4B_0C_1D_1$	4.50	5.50	12.35	15.30	6.50	7.00	11.50	15.00	1.01	1.02	0.93
$A_4B_0C_1D_2$	5.00	5.50	10.70	11.75	4.50	9.00	11.50	15.00	0.87	0.39	0.97

APPENDIX – III Continued

Treatments	No. of leaves /plant		Plant height (cm)		No. of roots/plant		Phyllochron (days)		Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	30 DAP
$A_4B_0C_2D_1$	4.00	5.00	13.10	16.16	4.50	10.00	11.50	15.00	0.53	0.27	1.08
$A_4B_0C_2D_2$	4.50	5.00	11.90	14.70	4.50	8.50	12.00	15.00	1.19	1.05	0.82
$A_4B_1C_1D_1$	5.00	6.00	12.50	16.00	7.00	7.50	11.50	14.00	1.13	0.37	1.05
$A_4B_1C_1D_2$	4.00	5.50	11.50	14.60	5.00	8.00	9.50	13.50	1.21	0.69	0.86
$A_4B_1C_2D_1$	4.00	5.00	10.75	16.20	7.00	8.50	12.50	15.50	0.91	0.68	0.75
$A_4B_1C_2D_2$	5.00	6.00	15.55	18.85	4.50	7.50	11.00	15.00	0.73	0.40	1.15
$A_4B_2C_1D_1$	5.00	6.00	16.35	19.00	5.50	9.00	11.00	16.50	0.50	0.29	1.03
$A_4B_2C_1D_2$	5.50	5.50	14.00	15.90	5.50	9.50	11.00	15.00	1.10	0.69	0.96
$A_4B_2C_2D_1$	5.50	2.50	11.65	7.25	7.00	9.00	9.00	16.50	1.58	1.01	0.80
$A_4B_2C_2D_2$	5.50	5.50	15.45	16.75	6.00	8.00	10.50	14.50	0.71	0.46	1.20
$A_5B_0C_1D_1$	4.50	6.00	11.30	12.65	6.00	7.50	12.00	16.50	0.38	0.21	1.07
$A_5B_0C_1D_2$	4.00	6.50	10.75	14.15	6.50	7.00	11.50	15.50	0.36	0.16	0.95
$A_5B_0C_2D_1$	4.50	3.50	12.15	15.20	3.50	8.50	10.50	15.00	0.26	0.15	0.85
$A_5B_0C_2D_2$	5.50	6.00	13.25	16.15	7.00	8.00	11.00	14.50	0.19	0.11	1.15
$A_5B_1C_1D_1$	5.00	5.50	12.45	15.90	6.50	7.50	12.50	15.50	0.96	0.75	0.82
$A_5B_1C_1D_2$	3.50	6.00	14.35	17.05	5.50	8.00	12.50	16.00	1.27	0.86	0.78
$A_5B_1C_2D_1$	5.00	6.00	8.50	11.85	5.50	9.00	11.00	16.00	0.68	0.46	0.97
$A_5B_1C_2D_2$	4.50	5.00	11.75	7.80	5.50	8.00	6.00	16.00	1.08	0.66	0.94
$A_5B_2C_1D_1$	3.50	4.50	12.25	9.10	5.00	8.00	9.50	16.00	1.47	0.72	0.95
$A_5B_2C_1D_2$	5.50	6.50	13.90	15.95	6.50	8.50	10.00	14.50	1.27	1.19	0.86
$A_5B_2C_2D_1$	3.50	6.00	9.65	13.00	6.50	7.00	10.00	14.50	1.99	1.85	0.75
$A_5B_2C_2D_2$	2.50	6.00	10.03	11.05	4.50	7.50	10.00	13.00	1.01	0.08	0.70
SE	0.88	0.45	1.04	1.04	1.09	1.12	1.45	1.1.0	0.08	0.04	0.06
CD (0.05)	NS	NS	2.95	NS	NS	2.90	NS	3.11	0.23	0.11	0.16

APPENDIX – IV

Interaction effect of potting media, triazole and mycorrhizae on biometric and physiological characters

Treatments	No. of leaves /plant		Plant height (cm)		No. of roots /plant		Phyllochron (days)		Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	First leaf	Second leaf	15 DAP	30 DAP	30 DAP
$A_1B_0C_1$	4.25	5.25	15.00	18.70	6.25	9.25	9.25	14.00	1.27	0.39	1.01
$A_1B_0C_2$	5.50	5.50	12.28	15.98	5.50	8.75	9.75	12.50	0.85	0.08	1.00
$A_1B_1C_1$	4.50	5.50	10.98	13.65	6.25	9.25	10.00	13.50	0.77	0.16	1.02
$A_1B_1C_2$	6.25	6.75	14.10	17.67	7.00	8.25	10.50	13.25	0.86	0.06	0.96
$A_1B_2C_1$	5.25	5.75	14.60	18.20	6.50	9.00	10.25	13.75	0.43	0.08	1.08
$A_1B_2C_2$	4.25	5.25	11.98	14.35	5.50	9.00	10.50	14.50	0.31	0.12	1.09
$A_2B_0C_1$	4.50	5.50	11.35	16.40	5.25	9.00	9.25	14.00	0.48	0.17	1.15
$A_2B_0C_2$	5.00	6.00	11.98	14.33	4.00	8.75	9.75	14.50	0.64	0.08	1.32
$A_2B_1C_1$	4.50	5.50	12.58	14.50	5.75	9.25	9.75	13.50	0.48	0.23	0.99
$A_2B_1C_2$	6.00	6.75	12.10	14.40	4.25	8.25	9.75	12.75	0.66	0.05	1.20
$A_2B_2C_1$	5.75	6.50	13.55	16.75	5.25	8.50	9.00	13.00	0.38	0.20	1.10
$A_2B_2C_2$	4.50	5.50	11.15	16.88	5.00	9.25	9.75	14.25	1.02	0.06	1.01
$A_3B_0C_1$	4.75	6.00	13.35	17.50	3.75	8.75	9.75	14.00	1.08	0.17	1.04
$A_3B_0C_2$	4.75	5.75	13.25	17.20	5.25	9.50	11.00	15.00	1.88	0.37	1.00
$A_3B_1C_1$	4.75	6.00	13.53	17.15	5.25	9.50	10.25	14.00	1.16	0.23	1.10
$A_3B_1C_2$	4.75	5.75	13.82	17.20	4.00	9.50	10.00	13.75	0.97	0.11	1.05
$A_3B_2C_1$	4.50	5.50	14.63	17.63	5.75	9.00	11.75	14.50	0.41	0.21	0.84
$A_3B_2C_2$	3.75	5.00	12.90	16.28	5.50	9.75	10.25	14.25	0.54	0.21	1.02
$A_4B_0C_1$	4.75	5.75	11.53	13.50	5.50	10.00	11.50	15.00	0.71	0.28	0.95
$A_4B_0C_2$	4.25	5.50	12.50	15.43	4.50	9.75	11.75	15.00	0.66	0.30	0.95
$A_4B_1C_1$	4.50	5.75	12.00	15.30	6.00	10.50	10.50	13.75	0.53	0.12	0.95
$A_4B_1C_2$	4.50	4.00	13.15	12.53	5.75	9.75	11.75	15.25	0.54	0.18	0.95
$A_4B_2C_1$	5.25	6.25	15.18	17.45	5.50	6.50	11.00	15.75	0.49	0.10	1.00
$A_4B_2C_2$	5.50	4.75	13.55	12.00	6.50	9.00	8.25	10.50	0.73	0.17	1.00
$A_5B_0C_1$	4.25	5.25	11.03	13.40	5.25	9.50	9.75	16.00	0.19	0.10	1.01
$A_5B_0C_2$	5.00	6.25	12.70	15.68	6.25	9.50	10.75	14.75	0.13	0.05	1.00
$A_5B_1C_1$	4.25	5.50	13.40	16.48	5.50	10.50	12.50	15.75	0.81	0.20	0.80
$A_5B_1C_2$	4.75	4.50	10.13	9.83	5.50	7.00	8.50	16.00	0.56	0.22	0.95
$A_5B_2C_1$	4.50	4.50	13.08	12.53	5.75	7.00	9.75	15.25	0.96	0.22	0.90
$A_5B_2C_2$	4.75	2.50	14.83	11.50	5.50	8.75	10.50	14.25	0.92	0.18	0.38
SE	0.62	0.72	0.74	1.65	0.77	0.79	0.27	0.78	0.06	0.03	0.04
CD (0.05)	NS	NS	1.21	4.67	NS	NS	NS	2.20	0.16	0.08	0.12

APPENDIX – V

Interaction effect of potting media, triazole and height of potting media in the container on biometric and physiological characters

Treatments	No. of leaves /plant		Plant height (cm)		No. of roots /plant		Phyllochron (days)		Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	First leaf	Second leaf	15 DAP	30 DAP	30 DAP
$A_1B_0D_1$	5.00	5.50	15.00	18.73	6.00	9.50	9.75	14.00	1.00	0.15	1.14
$A_1B_0D_2$	4.75	5.25	12.28	15.95	5.75	8.50	9.25	12.50	1.11	0.32	0.87
$A_1B_1D_1$	5.25	6.00	11.78	15.48	6.50	8.75	10.25	13.50	0.91	0.09	1.08
$A_1B_1D_2$	5.50	6.25	13.30	15.85	6.75	8.75	10.25	13.25	0.73	0.13	0.90
$A_1B_2D_1$	4.25	5.00	12.05	14.80	5.50	9.00	10.50	15.25	0.48	0.05	1.10
$A_1B_2D_2$	5.25	6.00	14.53	17.75	6.50	9.00	10.25	13.00	0.26	0.15	1.07
$A_2B_0D_1$	5.00	6.00	12.25	16.30	5.25	8.25	10.25	14.75	0.51	0.14	1.21
$A_2B_0D_2$	4.50	5.50	11.08	14.43	4.00	9.50	8.75	1375	0.61	0.11	1.26
$A_2B_1D_1$	5.25	6.00	12.35	14.20	4.50	9.00	9.50	14.00	0.51	0.17	0.94
$A_2B_1D_2$	5.25	6.25	12.33	14.70	5.50	8.50	10.00	12.25	0.64	0.11	1.25
$A_2B_2D_1$	5.50	6.50	10.75	16.48	6.00	8.75	8.75	13.00	0.84	0.06	1.05
$A_2B_2D_2$	4.75	5.50	13.95	17.15	4.25	9.00	10.00	14.25	0.57	0.20	1.06
$A_3B_0D_1$	4.50	5.75	14.08	17.22	4.50	9.25	10.00	13.75	1.31	0.28	1.03
$A_3B_0D_2$	5.00	6.00	12.53	17.47	4.50	9.00	10.75	15.25	1.66	0.26	1.01
$A_3B_1D_1$	4.50	5.75	12.28	15.93	5.25	9.50	9.50	14.50	0.97	0.16	1.14
$A_3B_1D_2$	5.00	6.00	15.07	18.42	4.00	9.50	11.25	13.25	1.16	0.22	1.01
$A_3B_2D_1$	4.00	5.25	12.55	15.63	5.75	9.50	10.75	15.00	0.47	0.22	0.89
$A_3B_2D_2$	4.25	5.25	14.98	18.28	5.50	9.00	11.50	13.75	0.49	0.21	0.97
$A_4B_0D_1$	4.25	5.50	12.73	15.73	5.50	9.75	11.75	15.00	0.64	0.23	1.01
$A_4B_0D_2$	4.75	5.75	11.30	13.23	4.50	10.00	12.00	15.00	0.72	0.35	0.89
$A_4B_1D_1$	4.50	4.25	11.63	11.10	7.00	11.00	10.25	14.75	0.52	0.18	0.90
$A_4B_1D_2$	4.50	5.50	13.53	16.73	4.75	9.25	8.50	14.25	0.54	0.12	1.00
$A_4B_2D_1$	5.25	4.75	14.00	13.13	6.25	7.00	10.75	11.50	0.64	0.12	0.92
$A_4B_2D_2$	5.50	6.25	14.73	16.33	5.75	9.25	11.25	14.75	0.57	0.15	1.08
$A_5B_0D_1$	4.50	5.75	11.73	13.93	4.25	9.50	11.25	15.75	0.19	0.07	0.96
$A_5B_0D_2$	4.75	5.75	12.00	15.15	6.25	10.50	11.75	15.00	0.13	0.09	1.04
$A_5B_1D_1$	5.00	6.00	10.48	13.88	5.50	10.25	9.25	15.75	0.61	0.19	0.89
$A_5B_1D_2$	4.00	4.00	13.05	12.42	5.50	7.25	10.75	16.00	0.76	0.22	0.86
$A_5B_2D_1$	3.50	3.75	10.95	11.05	5.75	7.00	9.75	15.25	1.28	0.22	0.85
$A_5B_2D_2$	2.75	3.25	10.95	12.10	5.50	4.75	11.25	11.25	0.60	0.17	0.43
SE	0.62	0.72	0.74	1.65	0.77	0.79	1.03	0.78	0.06	0.03	0.04
CD (0.05)	NS	NS	2.09	NS	NS	NS	NS	2.20	0.16	0.08	0.12

APPENDIX – VI

Interaction effect of potting media, mycorrhizae and height of potting media in the container on biometric and physiological characters

Treatments	No. of leaves /plant		Plant height (cm)		No. of roots/plant		Phyllochron (days)		Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	First leaf	Second leaf	15 DAP	30 DAP	30 DAP
$A_1C_1D_1$	4.50	5.33	13.43	17.18	6.50	9.33	10.00	14.50	0.67	0.13	1.10
$A_1C_1D_2$	4.83	5.67	13.62	16.52	6.17	9.00	9.67	13.00	0.97	0.29	0.97
$A_1C_2D_1$	5.17	5.67	12.45	15.48	5.50	8.83	10.33	14.00	0.92	0.05	0.97
$A_1C_2D_2$	5.50	6.00	13.12	16.52	6.50	8.50	10.17	12.83	0.42	0.11	1.11
$A_2C_1D_1$	4.83	5.83	12.10	15.48	5.33	8.83	9.17	13.83	0.43	0.20	0.93
$A_2C_1D_2$	5.00	5.83	12.88	16.28	5.50	9.00	9.50	13.17	0.47	0.20	0.94
$A_2C_2D_1$	5.67	6.50	11.47	15.83	5.17	8.50	9.83	14.00	0.81	0.05	1.22
$A_2C_2D_2$	4.67	5.67	12.02	14.57	3.67	9.00	9.67	13.67	0.74	0.08	1.19
$A_3C_1D_1$	4.50	5.83	12.87	15.87	5.33	9.33	10.83	14.00	0.69	0.14	1.16
$A_3C_1D_2$	4.83	5.83	14.80	18.98	4.50	8.83	10.33	14.50	1.08	0.26	1.03
$A_3C_2D_1$	4.17	5.83	13.07	16.65	5.00	9.50	10.50	14.17	1.18	0.26	0.95
$A_3C_2D_2$	4.67	5.67	13.58	17.13	4.83	9.50	10.33	15.17	1.12	0.20	1.01
$A_4C_1D_1$	4.83	6.00	13.73	16.77	6.33	9.50	11.33	14.50	0.56	0.18	1.05
$A_4C_1D_2$	4.83	5.83	12.07	14.08	5.00	9.83	10.67	12.33	0.59	0.16	1.01
$A_4C_2D_1$	4.50	3.67	11.83	9.87	6.17	9.67	10.00	14.83	0.65	0.18	0.93
$A_4C_2D_2$	5.00	5.83	14.30	16.77	5.00	8.67	11.17	16.00	0.64	0.26	0.88
$A_5C_1D_1$	4.33	4.67	12.00	12.55	5.17	9.33	10.00	15.33	0.57	0.10	0.06
$A_5C_1D_2$	4.33	5.50	13.00	15.72	5.83	8.33	10.00	15.17	0.74	0.25	0.94
$A_5C_2D_1$	4.33	5.67	10.10	13.35	5.17	9.50	11.33	15.33	0.82	0.22	0.86
$A_5C_2D_2$	3.33	3.17	8.33	11.98	5.67	5.00	10.00	10.17	0.25	0.07	0.69
SE	0.51	0.59	0.60	1.35	0.63	0.65	0.65	0.64	0.05	0.02	0.03
CD (0.05)	NS	1.66	1.70	3.81	NS	1.83	2.38	1.80	0.13	0.06	0.09

APPENDIX - VII

Interaction effect of triazole, mycorrhizae and height of potting media in the container on biometric and physiological characters

Treatments	No. of leaves /plant		Plant height (cm)		No. of roots/plant		Phyllochron (days)		Stomatal conductance (cm/s)		Leaf area index
	15 DAP	30 DAP	15 DAP	30 DAP	15 DAP	30 DAP	First leaf	Second leaf	15 DAP	30 DAP	30 DAP
$B_0C_1D_1$	4.60	5.80	13.52	16.92	5.70	9.20	10.40	14.70	0.62	0.20	1.04
$B_0C_1D_2$	4.40	5.30	11.38	14.89	4.70	9.50	10.20	14.50	0.88	0.24	1.02
$B_0C_2D_1$	4.70	5.60	12.79	16.84	4.50	9.30	10.70	16.60	0.85	0.15	1.09
$B_0C_2D_2$	5.10	6.00	12.29	15.60	5.30	9.50	10.50	14.10	0.82	0.21	1.01
$B_1C_1D_1$	4.60	5.70	11.80	14.94	6.00	10.00	10.80	14.60	0.66	0.15	0.99
$B_1C_1D_2$	4.40	5.60	13.19	15.89	5.50	9.30	10.40	13.60	0.84	0.23	0.96
$B_1C_2D_1$	5.20	5.50	11.60	13.29	5.50	9.40	10.90	14.40	0.74	0.15	0.99
$B_1C_2D_2$	5.30	5.60	13.72	15.36	5.10	8.00	9.30	14.00	0.69	0.10	1.05
$B_2C_1D_1$	4.60	5.10	13.16	14.85	5.50	8.20	9.60	15.00	0.47	0.10	0.99
$B_2C_1D_2$	5.50	6.30	15.25	18.17	6.00	9.10	10.3	13.90	0.60	0.22	0.98
$B_2C_2D_1$	4.40	5.00	10.96	13.58	6.20	8.30	9.10	13.00	1.02	0.17	0.93
$B_2C_2D_2$	3.50	4.20	10.80	12.82	5.00	7.30	10.4	11.30	0.39	0.13	0.87
SE	0.39	0.45	0.47	1.04	0.49	0.5	0.65	0.49	0.04	0.02	0.03
CD (0.05)	1.11	NS	1.32	NS	1.38	NS	NS	NS	0.10	0.05	NS

APPENDIX – VIII

Cost of production of micropropagated gladiolus plantlets

Capacity - 20000 plants/year

Sl. No.	Item	Total cost(Rs.)/ life span	Cost per year (Rs.)
1	Glasswares		
	a) Conical flasks (100 ml, 1000 nos.)	60,000/5 year	12000.00
	b) Jam bottles (4000 nos.)	20,000/5 year	4000.00
2	Distillation unit	20,000/10 year	2000.00
3	Laminar air flow chamber	50,000/10 year	5000.00
4	Balance	35,000/10 year	3500.00
5	Laboratory and culture room	2,00,000/50 year	4000.00
6	Air conditioner	30,000/15 year	2000.00
7	Autoclave	90,000/15 year	6000.00
8	Culture rack	8,000/20 year	400.00
9	pH meter	6,000/10 year	600.00
10	Refrigerator	12,000/15 year	800.00
11	Heating mantle	1,000/5 year	200.00
12	Power / fuel	6,000/ year	6000.00
13	a) Chemicals	1,500/ year	1500.00
	b) Sucrose	2,400/ year	2400.00
	c) Agar	3,000/ year	3000.00
14	Hot air oven	12,000/ 10 year	1000.00
15	Skilled labourers (4 nos.)	800/ month	9600.00
16	Pressure cooker	2,500/10 year	210.00
17	Miscellaneous	4,000/year	4000.00

Total = Rs. 68,210

Per plant cost = Rs. 3.41

EX VITRO ESTABLISHMENT OF GLADIOLUS (*Gladiolus grandiflorus* L.)

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Abstract of the
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ABSTRACT

Studies were conducted to standardize *ex vitro* establishment techniques in *Gladiolus grandiflorus* L. variety Vinks Glory during 2002-2004 in the Department of Pomology and Floriculture, College of Agriculture, Vellayani.

The effects of various potting media (sand, soilrite, sand: soil, sand: soil: coirpith, sand: soil: leaf mould), triazole (0, 4 and 8 mg/l), mycorrhizae (*Glomus fasciculatum* and *Glomus monosporum*) and height of potting media in the container (4 and 6 cm) on *ex vitro* establishment of the micropropagated plantlets were studied.

Among the different potting media used for *ex vitro* establishment, soilrite recorded higher survival rates of 86.11 and 80.55 per cent after the first and second fortnight respectively. Triazole application improved the survival rate over non-treated plants. Triazole at 4 and 8 mg/l concentrations recorded 54.16 and 55.83 per cent survival respectively after one month of planting. The two mycorrhizal fungi tried in the study exerted differential effect on survival.

Colonization of the roots by mycorrhizal inoculation was found to be high and this enhanced the survival rate of plantlets.

Leaf number and plant height was found to be affected by triazole application. Triazole treated plants exhibited a decrease in number of leaves and a reduced plant height due to its growth retarding action which in turn resulted in reduced leaf area index.

Triazole application delayed leaf production of plants due to inhibition of leaf expansion. Fresh weight, dry weight, CGR and RGR were found to be high in treatments with triazole application.

Growth of mycorrhizal fungi was affected by different potting media. But triazole treatment did not affect the colonization of AMF. Among the two mycorrhizal fungi, *Glomus fasciculatum* inoculated plants exhibited higher plant height, increased number of roots, delayed emergence of leaf and low stomatal conductance.

A decrease in stomatal conductance observed during the later stages of planting out indicate the acclimatization of the plantlets to the *ex vitro* conditions.

Potting media, triazole, mycorrhizae and their interactions affected the stomatal conductance significantly.

Height of potting media in the container did not influence the survival and growth parameters.

The estimated cost of hardened plants ranged from Rs. 4.50 to 5.25. Eventhough the cost is higher than that of conventional propagation method, this can satisfy the need for large scale production of disease free planting material.