Effects of the Growth Retardant Triadimefon on the *Ex vitro* Establishment of Gladiolus (*Gladiolus grandiflorus* L.) cv. Vinks Glory

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Abstract

Triadimefon, a triazole compound increased survival per cent of micropropagated Gladiolus (*Gladiolus grandiflorus* L.) plantlets. Application of triadimefon caused a retarding effect on height of the plant (14.86 cm) compared to the untreated plants one month after planting to *ex vitro* conditions. Triazole application increased root number of the plants. Plants treated with triazole showed delayed leaf production interval of 10.45 and 14.48 days for first and second leaves, respectively. Triazole treated plants exhibited a lower stomatal conductance which indicated the reduced water loss from the leaves. Leaf area index was high for untreated plants(1.08) compared to treated plants.

Introduction

Gladiolus (*Gladiolus grandiflorus* L.), a member of Iridaceae is one of the important bulbous ornamental plants. It is a high value cut flower crop mainly grown for its elegant cut spikes. It is popular in many parts of the world due to the beauty and vase life of the inflorescence. The genus *Gladiolus* includes 180 species with more than 10,000 cultivars (Sinha and Roy 2002). Dormancy of the cormels coupled with diseases in storage as well as under field condition creates problems in multiplication of planting material. Tissue culture tech-nique, 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 researchers (Ziv et al. 1970, Hussey 1977, Bajaj et al.

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1983, Misra and Singh 1999, Hussain et al. 2001, Pathania et al. 2001, Priyakumari and Sheela 2005, Aftab et al. 2008, Kumar et al. 2010).

The technique for micropropagation in Gladiolus is standardized. However, problems are being encountered in *ex vitro* establishment of plantlets. Hardening and *ex vitro* establishment of plantlets are the most difficult stages in the micropropagation of gladiolus (Razdan 2003). The benefit of any micropropagation system can only be fully realized by the successful transfer of plantlets from tissue culture vessels 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 and grow vigorously when transferred to soil (Hazarika 2003). Normal development of micropropagated plantlets during acclimatization and hardening stage is mandatory to ensure a high percentage of survival after transplanting to *ex vitro* conditions. Acclimatizing, hardening-off, or conditioning plantlets from the *in vitro* to the ambient environment can be a challenge that may result in death or damage to a large percentage of micropropagated plants (Preece 2010). 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.

The present study was carried out to investigate the effect of triazole compound on the *ex vitro* establishment of micropropagated gladiolus plantlets.

Materials and Methods

In vitro rooted plantlets of Gladiolus (*Gladiolus grandiflorus* L.) cv. Vinks Glory were used for the experiment. The plantlets were produced based on the following protocol. Cormels collected from field grown gladiolus plants were dehusked and immersed in 1000 times diluted Labolene solution for 30 minutes. It is washed thoroughly in running tap water for five minutes and then in distilled water. These cormels were surface sterilized with 0.08 per cent $HgCl_2$ for ten min with intermittent shaking inside a laminar air flow. The solution is drained and cormels are washed four to five times with sterile distilled water. Cormels were inoculated in MS with BA 2.00 mg/l and NAA 0.50 mg/l and multiple shoot initiation is obtained. After two - three subcultures, individual shoots measuring 2.50 - 3.50 cm length excised from the shoot proliferating cultures are subjected to *in vitro* rooting in IBA 2.00 mg/l.

The experiment was laid out in completely randomized design with six replications.

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

The culture vessels with *in vitro* rooted plantlets were opened and plantlets were taken out using a pair of sterilized forceps. The agar adhering to the roots was completely removed by thorough washing 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. The plants were kept inside a humidity chamber covered with polythene sheets of 350 gauge thickness for 30 days.

After 15 days of planting 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.

Survival of the plantlets was observed at fortnightly and they were observed for further study. Height of the plant was measured from the collar region to the tip and the mean length was expressed in centimeter. The total number of fully opened leaves developed per plantlet was counted and the mean value was expressed. The total number of roots per plantlet was counted and the mean value was calculated.

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

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

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

Stomatal conductance was expressed in cm/s.

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.

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

Results and 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.

Triadimefon [1-(4-chlorophenoxy)-3, 3-dimethyl-1*H*-(1, 2, 4-triazol-1-yl) butan-2-one], a triazole compound which is a plant growth retardant, was tried in the *ex vitro* establishment study. Survival per cent was calculated at fortnightly interval. 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, individuals treated with 8 mg/l showed 55.83 per cent survival followed by 4 mg/l with 54.16 per cent survival. Untreated plants recorded 47.50 per cent survival after one month of planting. 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.

Leaf number, plant height, root number, leaf production interval (phyllochron), stomatal conductance and leaf area index was given in Table 1.

Table 1. Leaf number, plant height, root number , leaf production interval (phyllochron), stomatal conductance and leaf area index of triadimefon treated micropropagated gladiolus plantlets.

Treat-ment	Leaf number		Plant height (cm)		Root number		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
Triazole											
B_0	4.88	5.68	12.58	15.81	5.05	8.23	9.35	13.30	0.79	0.20	1.08
B_1	4.70	5.60	12.54	14.87	5.53	9.18	10.35	14.15	0.73	0.15	1.00
B_2	4.50	5.37	12.49	14.86	5.68	9.38	10.45	14.48	0.62	0.10	0.94
SE	0.20	0.23	0.23	0.28	0.24	0.25	0.33	0.25	0.02	0.01	0.01
CD (0.05)	NS	NS	NS	0.79	NS	0.71	0.92	0.70	0.05	0.02	0.04

Height of the plant was found to be affected significantly by triazole treatment one month after planting. Untreated plants recorded maximum plant height of 15.81 cm while plants treated with triazole at 8 mg/l recorded minimum plant height (14.86 cm). This shows the retarding effect of triazoles. Similar result was obtained by Samasya (2000) in micropropagated orchids. This is due to the effect of triazoles on isopropanoid pathway which produces gibberellin responsible for elongation of plants. Triazoles block the isopropanoid pathway and thus affect gibberellin biosynthesis (Graebe 1987), which in turn led to reduction in plant height. The reduced plant height due to the inhibitory action of triazole on gibberellic acid biosynthesis was also reported by Hazarika (2003). In the present study plants with greater height tended to be lanky with long and narrow leaves that are difficult to establish.

One month after planting, plants subjected to triazole at 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. In plants treated with triazole at 8 mg/l, the first leaf emerged after 10.45 days and second leaf 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. 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. After the first fortnight, plants treated with triazole at 8 mg/l showed low stomatal conductance of 0.62 cm/s followed by 4 mg/l 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 the maintenance of plant water status. High rate of water loss incurred by *in vitro* grown plants imposed severe limitation in acclimatization. But application of triazole during *ex vitro* establishment helped to overcome this limitation by reducing the water loss from the plant. This was in accordance with the findings of Hazarika et al. (2002). 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 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 leaves of *in vitro* grown plants showed open stomata and collapsed guard cells, while acclimatized leaves presented closed stomata as well as decreased stomatal density and aperture (Romano and Martins-Loucao 2003).

In the present study, untreated plants showed higher leaf area index of 1.08 followed by a reduction (0.94) by the triazole treated plants. 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.

Survival rate obtained in this study was moderate and the initial growth rate was slow. 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 of these cormels may have helped to overcome this situation. Future work should be oriented in this direction.

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