Journal of Agricultural Technology 2012 Vol. 8(7): 2331-2351
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

In vitro regeneration of gladiolus propagules

N. Memon[1*], A. Yasmin[1], V. M. Pahoja[1], Z. Hussain[2] and I. Ahmad[3]

[1]Sindh Agriculture University, Tandojam, Sindh Pakistan, [2]University College of Agriculture, University of Sargodha, Pakista, [3]College of Agriculture D.G. Khan sub-campus University of Agriculture, Faisalabad

N. Memon, A. Yasmin, V. M. Pahoja, Z. Hussain and I. Ahmad (2012) *In vitro* regeneration of gladiolus propagules. Journal of Agricultural Technology 8(7):2331-2351.

Gladiolus is a potential cut flower grown throughout the world for its elegant attractive spikes of different hues and good keeping quality. Commercially it is propagated by vegetative mean by corms and cormels. However the multiplication rate of corms and cormels is slow and the conventional methods are insufficient to meet the demand of planting material (corms and cormels). *In vitro* techniques are applicable for the propagation of corm producing species. These techniques are adopted at commercial level in order to fulfill supply gap of huge demand. A number of *in vitro* protocols have been developed for regeneration of gladiolus plantlets by using various explants sources of the plant. However, literature is rather scanty on *in vitro* cormel formation and acclimatization of *in vitro* propagules.

Key words: cormel propagation, *In vitro*, Gladiolus, shoot regeneration

Introduction

Gladiolus is cultivated for its elegant and attractive spikes of different hues throughout the world (Sinha and Roy, 2002). The major gladiolus producing countries are the United States (Florida and California), Holland, Italy, France, Poland, Bulgaria, Brazil, India, Australia and Israel. It is the best selling bulb of the United States with an estimated annual sale of more than 370 million corms (Narain, 2004). Gladiolus is a member of the Iris family having short life cycle of 110-120 days and requires temperature regime between 10-25^{0}C for optimal growth. Commercially, gladiolus is propagated by natural multiplication of new corms and cormels (Hartman *et al*., 1990; Ziv and Lilien-Kipnis, 1990; Singh and Dohare, 1994) whereas seed propagation is only used to evolve new and improved varieties by hybridization. However, Memon *et al*. (2009) reported few improved conventional techniques including divisions of the corms and leaf/flower clippings for enhancement of corm and cormel production in gladiolus. Usually, the bulbous plants are perpetuated by using

* Corresponding author: N. Memon; e-mail: norimemon@gmail.com

their underground storage organs such as rhizomes of tuberose, corms of gladiolus and bulbs of lilies. Although there are other methods which are applied to these underground storage organs such as chipping, scooping, scaling and scoring, these are not suitable for the propagation of the corms of gladiolus because predominantly bulb is a fleshy leaf scales and a corm is a compressed solid thickened stem with distinct nodes and internodes (Hartman *et al*., 1990).

Very few improved conventional techniques were reported for the multiplication of corms and cormels including cutting of the corms and leaf clippings by the Memon *et al*. (2009). Additionally, commercial production of corms and cormels is affected by *Fusarium* corm rot during storage. Besides, one mother corm normally produces about 25 cormels each season (Sinha and Roy, 2002). These cormels require three to four seasons to attain standard size of flowering spike and daughter corms. The dormancy of the corms and cormels is another problem in this regard (Priyakumari and Sheela, 2005). Thus its commercial cultivation is limited by low rate of multiplication and does not fulfill the local demand of planting material which eventually affects the final cost of corms. Therefore novel cultivars need to be rapidly mass multiplied by using modern in vitro technologies in order to fulfill the supply gap of huge demand of our local market which is of course not possible through conventional methods.

Mass propagation of corms and cormels through modern technologies such as tissue culture techniques have adopted at commercial level. Advanced countries are using highly sophisticated modern technologies for the commercial production of desired varieties in order to compete in the international markets. This technology makes also possible to produce disease free and true to type planting material. However, in many developing countries the establishment cost of facilities and unit production cost of *in vitro* propagated plants is high, and often the return on investment is not in proportion to the potential economic advantages of the technology (Savangikar, 2004; Jo *et al*., 2008). Hence, it is emphasized that all plants should not be commercially produced from tissue culture. This technology works in a way when tissue culture methods are superior to conventional propagation, produce pathogen free plants in huge quantities, compete with conventional method and used for cloning.

In vitro techniques are useful for the propagation of corm producing species. As most of the hybrid cultivars of gladiolus have a very low rate of multiplication. *In vitro* propagation is an alternative to the conventional methods; it increases multiplication rates (Novak and Petru, 1981; Takayama and Misawa, 1983; Wickremesinhe *et al*., 1994) and also generates material

free from viruses and other pathogens (Blom-Barnhoorn and Van Aartrijk, 1985; Van Aartrijk *et al*., 1990).

Since the pioneering efforts, a lot of data were generated and a number of papers have been published on different aspects of *in* vitro studies of gladiolus with a greater emphasis on micropropagation. However, literature is rather scanty on *in vitro* cormel formation. *In vitro* propagation of gladiolus plantlets and cormels is possible to achieve through direct or indirect (callogenesis) mode of regeneration. A consolidated account of tissue culture studies on gladiolus is dealt with in the present review.

Direct mode of regeneration

In vitro direct mode of regeneration is a faster method for mass cloning of bulbous plants. However, the rate of regeneration is mainly depends on a number of factors, including the type of the explant, composition of the culture medium, type and concentration of plant growth regulators and genotype of the plant material.

Choice of explant

The choice of appropriate explant is critical in tissue culture (Ascough *et al*., 2009). The specific differences in the regeneration potential of different organs and explants have various explanations. In gladiolus, the most popular tissue source has been corms (Nhut et al., 2004) or inflorescence explants (Ziv and Lilien-Kipnis, 2000). Various explant types such as shoot tips or cormel sprouts (Ziv *et al*., 1970; Logan and Zettler, 1985; Sutter, 1986; Hussain *et al*., 2001; Memon *et al*., 2010; Memon *et al*., 2012), axillary buds (Priyakumari and Sheela, 2005; Begum and Haddiuzaman, 1995; Dantu and Bhojwani, 1995; Ahmad *et al*., 2000, Boonvanno and Kanchanapoom, 2000), basal leaf tissues (Prasad and Gupta, 2006), meristem (Aftab, *et al*., 2008); explants from inflorescence stalk (Ziv *et al*., 1970; Ziv, 1979; Grewal *et al*., 1995; Kumar *et al*., 1999; Ziv and Lilien-Kipnis, 2000; Memon *et al*., 2010), cormel and cormel segments (Nagaraju and Parthasarathy, 1995; Kumar *et al*., 1999; Babu and Chawla, 2000; Aftab *et al*., 2008; Memon *et al*., 2010) have also been reported for *in vitro* direct shoot regeneration of gladiolus.

Different responses from different explants sources and even from the same explant were reported by a number of researchers. Memon *et al*. (2010) used different explants at different stages/sizes including nodal cultures from different developmental stages of flower spike, various sizes of whole cormels and cormel sprouts obtained at different intervals in cultivar *White Friendship* of gladiolus. Memon *et al*. (2010) found heading stage the best explant for

efficient shoot regeneration on MS medium supplemented with BAP (4 mg L^{-1}) from nodal cultures taken at various developmental stages of flower spike as compared to one bud or three bud opened stage in *White Friendship* variety of gladiolus. They also used different sizes of whole cormels and reported the best size of 0.6 g cormel for efficient shoot regeneration on the same MS medium as compared to 0.2 and 0.4 g sized cormels. Babu and Chawla (2000) used corm slices and observed the better shoot induction from top slice of the cormels as compared to medium and bottom slices of the cormels. They also observed an average of 90% response for shooting from *in vitro* bisected shoot tips having no base of the cormel when inoculated on the medium containing kinetin (18.6 µM) in the cut-side down orientation. Aftab *et al*. (2008) used cormel and meristem explants and recorded cormel as a better source for shoot multiplication as compared to meristem. Nhut *et al*. (2004) depicted highest shoot formation from basal plates (30 mm diameter) as compared to shoot tip (5 mm in length), longitudinal corm section (20 mm in width) and daughter corm (15-20 mm diameter) in the medium containing BA (4.5 µM). Ahmad *et al*. (2000) achieved maximum number of shoots from nodal explants rather than terminal and axillary buds of cormel. Similar findings were observed by Grewal *et al*. (1995). They found better response from nodal segments as compared to internodal, rachis and floret segments.

Composition of culture medium

The response of *in vitro* cultures for efficient direct shoot regeneration varies according to the strength of the MS medium and combination of the plant growth regulators. In gladiolus, multiple shoot regeneration was generally found on full strength MS medium (Memon et al., 2010; 2012) However successful multiple shoot induction was also observed in half strength MS medium when containing BAP (0.75 mg L^{-1}) by Begum and Haddiuzaman (1995). Aftab *et al*. (2008) also observed multiple shoot induction response (up to 90%) on both full as well as half strength MS basal medium. Half strength of MS salts supplemented with 3-9% sucrose in order to produce multiple shoots was also reported by Ahmad *et al*. (2000). High sucrose concentrations (8, 10 or 12%) significantly increased shoot production in G. hybridus cultures.

Optimum concentration of plant growth regulators

The two most important plant growth regulators -cytokinins and auxins have been reported for *in vitro* regeneration and proliferation of shoots in gladiolus. In the class of cytokinins, beneficial effect of Benzyl aminopurine (BAP) or Benzyladenine (BA) over other cytokinins have been reported (Dantu

and Bhojwani, 1987; De Bruyn and Ferreira, 1992; Memon *et al*., 2010; Memon *et al*., 2012). Regarding auxins, naphthalene acetic acid (NAA) has also been found beneficial for the establishment of bud and meristem tip cultures (Ziv, 1979; Logan and Zettler, 1985; Lilien-Kipnis and Kachba, 1987). Usually high levels of cytokinnis and low levels of auxins are recommended for *in vitro* shoot proliferation. Whereas, at lower levels of cytokinins or in its absence, shoot elongation occurs (Ahmad *et al*., 2000; Arora *et al*., 1996; Dantu and Bhojwani, 1987;) so organ initiation depend on the manipulation of auxin and cytokinins levels in the media. The optimum concentration of plant growth regulators for shoot proliferation varies from cultivar to cultivar (Hussey, 1977; Dantu and Bhojwani, 1987; Grewal *et al*., 1990). Priyakumari and Sheela (2005) used intact cormels of cultivar "*Peach Blossom*" on MS medium supplemented with different levels of BA and Kinetin (1, 2, 4 mg L^{-1} each) alone and in combination with NAA (0.1 and 0.5 mg L^{-1}) and IAA (1 and 2 mg L^{-1}). The highest rate of multiple shoots (33.7) was found in response to BA (4 mg L^{-1}) supplemented with NAA (0.5 mg L^{-1}). BA at low concentrations (1 or 2 mg L^{-1}) was however, suitable for further shoot multiplication. Memon *et al*. (2010) and Memon et al. (2012) also reported efficient shoot regeneration from different explants sources at BAP 4 mg L^{-1}. Prasad and Gupta (2006) studied comparative performance of three different culture systems viz; semi-solid agar (0.8% w/v), liquid media supported with membrane raft (MR) and duroplast foam (DF) in order to produce *in vitro* shoot regeneration from basal parts of leaves in *Gladiolus hybridus* Hort. cv. *Wedding Bouquet*. They used MS basal salt mixture along with different concentrations of NAA (0, 0.2, 0.5 and 1.0 mg L^{-1}) and BAP (0, 1, 2, and 4 mg L^{-1}) either alone or in combination. NAA and BAP alone didn't exhibit any response in all the three culture systems. Whereas a combination of NAA (1 mg L^{-1}) and BAP (2 mg L^{-1}) induced maximum (33.15) shoots/cluster in MR system followed by DF (24.74) with NAA (0.5 mg L^{-1}) and BAP (4.0 mg L^{-1}) and AS (21.34) with NAA (0.2 mg L^{-1}) and BAP (2.0 mg L^{-1}). Shoot multiplication was rapid in AS and MR systems as compared to DF system. BAP 2 to 4 mg L^{-1} along with GA_3 was optimal for culture establishment of gladiolus cvs. '*Eurovision*' and '*Wine* and *Roses*' as reported by Pathania *et al*. (2001). Beura and Singh (1998) demonstrated a higher rate of bud proliferation in gladiolus on MS medium supplemented with high concentration of BAP (4 mg L^{-1}). Same results were observed by De Bruyan and Ferreira (1992). They reported *in vitro* multiple shoot induction with BAP as single growth regulator. Direct shoot regeneration was observed from segments of cormels and inflorescence axes cultured on MS basal medium containing 5.0 µM each of BA and 2,4-D, besides callus formation (Kumar *et al*., 1999). Ahmad *et al*. (2000) cultured axillary buds, cormel tips and nodal

buds on MS basal medium supplemented with BA or KIN alone and BA with IBA for shoot production in gladiolus. They observed maximum number of shoots on MS basal medium supplemented with BAP (18 µM) and IBA (36 µM). Nodal buds were found better explant for efficient shoot regeneration as compared to other types of the explants. Grewal *et al*. (1995) obtained single shoot per explant from nodal segments in cultivars viz. *Mayur*, *Sylvia*, *Spic* and *Span* on MS medium supplemented with BAP (1 mg L^{-1}) and developed 14-20 shoot primoridia within 4 weeks from these developed shoots when these were cultured on MS medium containing BAP (5 mg L^{-1}). These clump sections of shoot primordia were successfully multiplied on the same medium.

From the works presented under this section, it is evident that direct shoot induction and proliferation is mainly dependent on the use of cytokinins especially BA (1-5 mg L^{-1}) alone or in combination with low levels of auxins. Among auxins, NAA (0.5-1 mg L^{-1}) is the most commonly used plant growth regulator. Although, IBA or 2,4-D along with BA is also reported in few reports for successful regeneration and proliferation of shoots.

Regarding genotype a thorough study is required for screening and selection of genotypes in gladiolus for productive *in vitro* regeneration of plantlets. It is not necessary that *in vitro* optimized protocol of one genotype is applicable for another even in the same species.

Indirect Mode of regeneration (Through Callogenesis)

Choice of explant

The bulbous ornamental plants are monocotyledons and among this group, callus is generally initiated from meristematic tissue such as embryos, basal meristems or shoot tips. However, in gladiolus, callus initiation is also reported from various explants sources such as segments of inflorescence stalk (Ziv *et al*., 1970, Kumar *et al*., 1999), cormel tips (Simonsen and Hildebrandt, 1971; Goo *et al*., 2003), cormel slices or basal leaf regions (Kamo, 1994; Kamo, 1995; Kumar *et al*., 1999; Emek and Erdag, 2007; Remotti and Loffler, 1995; Aftab *et al*., 2008), axillary buds (Lilien-Kipnis and Kochba, 1987; Boonvanno and Kanchanapoom, 2000) and slices of cormel sprouts (Sinha and Roy, 2002). The shoot tip of the cormel was considered the best source for callus initiation (Simonsen and Hildebrandt, 1971; Goo *et al*., 2003).

The callus initiation and regeneration from any explant source is mainly based on cultivated varieties, concentrations and combinations of plant growth regulators used in the culture media (Kamo, 1994, 1995). The first published report on tissue culture of gladiolus was by Ziv *et al*. (1970) who used inflorescence discs to induce callus (Ascough and Erwin, 2009) followed by

Simonsen and Hildebrandt (1971), who used stem tip of cormel to induce callus. A series of experiments by Hussey (1977) showed that corms and inflorescence explants were superior to leaves or ovaries for callus production. Flower stalk as explants produced more callus compared to bracts, denuded flowers or inflorescence segments (Bajaj et al., 1983). Segments of inflorescence stalk (Kumar *et al*., 1999), cormel tips (Goo *et al*., 2003), cormel slices or basal leaf regions (Kamo, 1994; Kamo, 1995; Kumar *et al*., 1999; Emek and Erdag, 2007; Remotti and Loffler, 1995; Aftab *et al*., 2008), axillary buds (Lilien-Kipnis and Kochba, 1987; Boonvanno and Kanchanapoom, 2000) and slices of cormel sprouts (Sinha and Roy, 2002) have also been reported for successful regeneration of callus.

Hormonal requirement for callus initiation

The hormonal requirement for callus initiation and subsequent plant regeneration from the monocotyledonous bulbous crops in the floral industry are largely unknown (Kamo, 1994). Various results reported that the best callusing occurs in gladiolus in the presence of NAA (Goo *et al*., 2003; Emek and Erdag, 2007, Memon *et al*., 2009). 2,4-D alone (Kumar *et al*., 1999 and Remotti and Loffler, 1995) or in combination with NAA was also reported by Aftab *et al.*, 2008. Callus induction was also reported in the presence of BAP supplemented with 2,4-D (Kumar *et al*., 1999, Aftab *et al*., 2008). Media supplemented with either 1-10 mg L^{-1} naphthalene acetic acid (NAA), 0.5-2.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 2 mg L^{-1} dicamba or 1 mg L^{-1} picloram have also been used for callus induction and maintenance (Kamo and Joung, 2007).

The variations in concentration of plant hormones may vary from hormone to hormone and genotype to genotype even for the same explant as reported by Aftab *et al*. (2008). They used cormel slices (2-3 mm thick) and leaf sections (1 to 1.5 cm^2) for callus induction on MS basal media supplemented with NAA or 2,4-D alone or in combination with BAP. They found positive response for callus induction from slices of cormel at lower concentrations of 2,4-D (2 mg L^{-1}) supplemented with BAP (1 mg L^{-1}). They also reported callus induction from the same explant on MS basal medium containing NAA (3 or 4 mg L^{-1}). Kim *et al*. (1988) observed the best callus formation from cormel explants on a medium containing 2,4-D (10 ppm) in about 40 days. Kamo (1995) achieved the best callus induction from cormel segments at higher levels of NAA (10 mg L^{-1}) or MS medium with 2,4-D (0.5 mg L^{-1}) or dicamba (2.0 mg L^{-1}). Kumar *et al*. (1999) induced better callus formation from cormel segments of gladiolus on MS basal media containing BAP (5.0 µM) and 2,4-D (5.o to 10.0 µM) from '*Her Majesty* and *Aldebaran*'

culivars. In the same way Remotti and Loffler (1995) reported the best callus induction from cormel slices on a medium containing 2,4-D (9 mM) in cultivar *Peter Pears*. They also reported that middle part of the cormel had the highest competence for callus initiation. Emek and Erdag (2007) optimized NAA at the rate of 8.5 mg L^{-1} for maximum callus production from longitudinal corm slices in *Gladiolus anatolicus*. Cormel segments responded better for callus induction as compared to inflorescences axes (Kumar *et al*., 1999) and leaf explant (Aftab *et al*., 2008). It is clear from the above findings that NAA or 2,4-D alone or in combination had major role in initiation of callus in different genotypes.

For most of the explants, callus initiation and regeneration was reported by using same type of growth regulators such as NAA, 2,4-D, BAP and KIN. When explants from inflorescence stalk (flower stalks, debudded flower, bract, perianth) and leaf segments cultured on MS basal medium containing NAA and KIN showed the best callus formation from the segments of flower stalks (Bajaj *et al*., 1983). Callus formation from leaf sections was observed on MS basal medium supplemented with 2,4-D (3.0 mg L^{-1}) (Aftab *et al*., 2008). Seven days old sprouts of *in* vitro cultured cormels of *Gladiolus primulinus* cv. Golden wave initiated callus on MS medium supplemented with NAA (4 mg L^{-1}) (Sinha and Roy, 2002). Goo *et al*. (2003) also observed 100% callus formation ratio from cormel tip of *Topaz* on MS medium supplemented with NAA (1.0 mg L^{-1}) Kasumi *et al*. (1998) observed callus induction from all explants of ovaries on MS medium containing NAA (5 mg L^{-1}) and BAP (5 mg L^{-1}).

Kinetin (KIN) induced callus formation from explants of Inflorescence stem and the shoot tip on a medium containing 26.9 µM or 53.8 µM NAA and 2.3 µM KIN (Ziv *et al*., 1970; Bajaj *et al*., 1983). Simonsen and Hildebrandt (1971) achieved the best callus induction on agar as compared to liquid medium in the presence of 2.3 µM KIN. On the basis of their results they suggested the best callus growth at lower levels of KIN.

Callus induction was demonstrated as genotype dependent phenomenon and among the tested cultivars , 'Peter Pears' and 'White Prosperity' were found superior with respect to callus production on the media with either 2,4-dichloropbenoxyacetic acid or picloram (Remoti and Loffler, 1995).

Regeneration of in vitro cultures

In vitro regeneration of plantlets has been successfully carried out from callus derived either from slices of cormel sprouts (Sinha and Roy, 2002), cormel slices and basal leaf region (Kamo, 1994; Emek and Erdag *et al*., 2004) comel tips (Simonsen and Hildebrandt, 1971; Goo *et al*., 2003), and inflorescence stalk (Ziv *et al*., 1970). Additionally, direct organogenesis was also reported from cormel tips (Misra and Singh, 1999; Arora *et al*., 1996),

corm and corm slices (Babu and Chawla, 2000; Nhut *et al*., 2004; Aftab *et al*., 2008) nodal segments (Grewal *et al*., 1995; Arora *et al*., 1996;) axillary and lateral buds (Lilien-kipnis and Kachba, 1987; Begum and Hadiuzzaman, 1995; Dantu and Bhojwani, 1995; Priyakumari and Sheela, 2005) and basal plates (Nhut *et al*., 2004). However, Cormel tips were found most suitable explant for culture establishment than leaf and inflorescence segments (Misra and Singh, 1999).

Regeneration through callogenesis is mainly based on the quality of callus, its age and regeneration potential. In cultivar *Gladiolus primulinas* Baker, Sinha and Roy (2002) regenerated shoots from approximately one gram of the callus on MS media supplemented with 0.5-2.0 mg L^{-1} KIN or/and 0.5-2.0 mg L^{-1} BAP. The maximum number of shoots (28) regenerated on MS medium with BAP (2.0 mg L^{-1}) from one month old callus and in the five month old callus, no regeneration of shoots was observed on any medium. On the other side this is not the case with direct regeneration of *in vitro* cultures. The rate of regeneration depends on the combination of growth regulators used in the medium and on the cultivar. Cytokinins play major role in this regard. Earlier, Dantu and Bhojwani (1987) and De Bruyn and Ferreira (1992) had reported the beneficial effect of BAP over other cytokinins for shoot multiplication and regeneration. when cytokinins are used their optimal concentration depend on the cultivar. In cultivar *Gladiolus grandzilorus* "pink" more shoots (6.1 per explant) were recorded from the basal plates on medium containing BAP 1 mg L^{-1}. In cultivar *Eurovision* 5-8 shoots obtained per sub culture on a medium containing KIN (2 mg L^{-1}) (Ziv, 1979), whereas 11-15 shoots produced on medium having KIN (1 mg L^{-1}) for a series of cultivars. Dantu and Bhojwani (1995) reported maximum number of shoots from axillary buds of gladiolus on MS medium containing BAP (0.5 mg L^{-1}). Kamo (1995) cultured cormel slices on MS medium supplemented with BAP (1.0 mg L^{-1}) and regenerated plants from all six tested cultivars. Remotti and Loffler (1995) regenerated plants from yellow compact cells of all genotypes on media containing zeatin and BA in various concentrations.

The use of NAA alone or in combination with BAP or KIN has also been found beneficial for the establishment of bud and meristem tip cultures (Ziv, 1979; Lilien-kipnis and Kachba, 1987; Boonvanno and Kanchanapoom, 2000). Emek and Erdag (2007) got more shoots per explant (4.71) at the combination of low level of BA (0.2 mg L^{-1}) and high level of NAA (2 mg L^{-1}) in *Gladiolus anatolicus.* The same observations were recorded by Kumar *et al*. (1999); they noted shoot differentiation from callus cultures at lower concentration of BA (0.2 mg L^{-1}) supplemented with higher concentration of NAA (1.9 mg L^{-1}) in three cultivars viz. *Her Majesty*, *Aldebaran* and *Bright Eye*. Boonvanno and

Kanchanapoom (2000) cultured axillary buds on MS medium supplemented with KIN (0.5 mg L^{-1}) or NAA (0.4 mg L^{-1}) to produced multiple shoots initiated from axillary buds of the corms. Callus induced from buds in the presence of sole NAA (0.4 mg L^{-1}) and multiple shoots were developed from the callus on MS medium containing BA (1 mg L^{-1}).

It is very obvious from the cited reports here that in vitro direct or indirect regeneration of gladiolus is possible through different kind of explants, however regeneration potential depends on plant genotype and choice of growth regulators. KIN, BAP and NAA are the most important growth regulators which alone or in combination effect the regeneration of gladiolus plantlets.

Rooting of in vitro regenerated cultures

Better rooting plays major role in acclimatization. The poor survival rate of transplanted plants is usually the result of poorly developed roots (Ziv *et al*., 1970). Thus a major change in the pre-transplant stage is the change in culture environment to promote root initiation and shoot elongation.

Various factors such as low concentrations or completely elimination of cytokinins and high concentrations of auxins (Logan and Zettler, 1985; Lilien-Kipnis and Kochba, 1987; Ziv, 1979), low concentrations of inorganic salts (Sriskandarajal and Mullins, 1981), addition of sucrose (Kumar *et al*., 1999), addition of activated charcoal (Lilien-Kipnis and Kochba, 1987) and addition of vermiculite (Logan and Zettler, 1985) instead of agar in the medium is reported for better rooting response in *in vitro* cultures. Rooting in simple basal MS medium without plant growth regulators was also observed by Goo *et al.* (2003).

The role of various auxins (NAA, IBA and IAA) has been reported for initiation and regeneration of roots in various cultivars of gladiolus and other bulbous plants such as *Snowdrop* bulblets (Tipirdamaz, 2003) and Narcissus (Hosaki and Asahira, 1980; Chow *et al*., 1992). In gladiolus, better rooting response was demonstrated in the presence of NAA and IBA. Root length, number and its morphology was greatly affected with the increasing levels of NAA (Lilien-Kipnis and Kochba, 1987). Ahmad *et al*. (2000) also observed better rooting response on MS medium supplemented with NAA (0.5 mg L^{-1}) or IBA (3.9 mg L^{-1}) but the best survival rate was found in the medium containing NAA in *White prosperity*. IBA (2 mg L^{-1}) was also observed beneficial in to induce rooting in *Golden Wave* as documented by Sinha and Roy (2002). Similar reports were published by Priyakumari and Sheela (2005). They found earlier rooting (within 7 days) and longer roots (5 cm) on MS medium supplemented with IBA (2 mg L^{-1}) in cultivar *Peach Blossom*.

However more number of roots (24) was produced by NAA (1 mg L^{-1}) in the same cultivar. Hussain *et al*. (1994) observed extensive growth of roots on MS medium supplemented with IBA (2 mg L^{-1}) in cultivar *White Friendship*. Although cytokinins induce shoots Emek and Erdag (2007) observed rooting (20%) on MS medium with BA (0.1 mg L^{-1}) in *Gladiolus anatolicus* and no rooting was observed with NAA (0.5 or 2 mg L^{-1}). Memon et al. (2010) found good rooting response from different explant sources by using IBA at 2 mg L^{-1} plus sucrose (3%) in *White Friendship* variety of gladiolus.

According to published reports besides auxins, use of activated charcoal and sucrose also promote rooting positively. Activated charcoal can have two effects in rooting formation; it can adsorption all organic compounds with the exception of sugars and block light supply to the nutrient medium creating similar conditions as soil do in nature. Lilien-Kipnis and Kochba (1987) observed rooting in the presence of auxins and activated charcoal. They reported white branched roots and differentiation of roots in the presence of activated charcoal and observed no effect of the increasing levels of NAA on rooting.

Low concentrations of salts have also been reported for successful rooting in number of plants (Sriskandarajal and Mullins, 1981). The species in which induction of shoot multiplication requires a full strength MS medium, reduction of the salt concentration to half or one-quarter (Sinha and Roy, 2002) is found satisfactory for rooting. Aftab *et al*. (2008) found 100% rooting response in gladiolus on half strength MS medium supplemented with IBA (2 mg L^{-1}) after 5 days of inoculation. Kumar *et al.* (1999) reported that sucrose concentration without use of the plant growth regulators had a direct affect on both the quality of the roots and induction of rooting in *Her Majesty* and *Aldebaran*. The beneficial effect of sucrose on rooting was also reported as Sinha and Roy (2002) used half strength MS medium supplemented with IBA (2 mgL^{-1}) and sucrose (6%) that promoted roots in 100% cultures within two weeks. Kumar *et al*. (1999) replaced sucrose by equimolar concentrations of mannitol had no beneficial effect on the rooting in gladiolus.

Vermiculite is an alternative method to ensure good root system for easy transplanting to soil (Logan and Zettler, 1985). They used horticultural grade vermiculite instead of agar; the vermiculite was enriched with a half strength liquid MS medium, NAA and activated charcoal which resulted in extensive root development.

Heat shock is also useful to induce rooting in gladiolus (Kumar *et al*. 1999). They tested heat shock at temperature 35, 40, 45 and 50^0C and found best rooting response at 50^0C in cvs. *Her Majesty* and *Aldebaran.*

Rengeneration of cormels

The ultimate goal of the successful *in vitro* propagation of gladiolus is mass production of cormels in gladiolus (Steinitz, *et al*., 1991; Dantu and Bhojwani, 1995; Sen and Sen, 1995; Nagaraju *et al*., 2002). The *in vitro* raised cormels can be easily stored and sown like seeds in plantation season (Wang and Hu, 1982; Ziv and Lilien Kipnis, 1990). This may also reduce the transplantation difficulties occurred during acclimatization (Ziv, 1979; Sengupta *et al*., 1984).

Various explants such as nodal buds (Grew *et al*., 1990; Arora *et al*., 1996), cormel tips (Arora *et al*., 1996), inflorescence stalk (Ziv *et al*., 1970), axillary buds of corm (Dantu and Bhojwani, 1987; Ahmad *et al*., 2000; Begum and Haddiuzaman, 1995) and slices of cormel sprouts (Sinha and Roy, 2002) have been utilized for *in vitro* cormel production in gladiolus on MS basal medium with different concentrations of growth hormones and sucrose.

Sucrose requirement for cormel formation

Sucrose plays an important role for *in vitro* cormel formation in gladiolus (Dantu and Bhojwani, 1987; Arora *et al*., 1996; Sinha and Roy, 2002). It effect multiplication of shoots (Kumar *et al*., 1999; De Bruyn and Ferreira, 1992), somatic embryogenesis (Loiseau *et al*., 1995) and rooting of microshoots (Rahman *et al*., 1992; Romano *et al*., 1995). The increased growth of tuberous organs needs a relatively high (> 50 g L^{-1}) concentration of sucrose in the medium (Dantu and Bhojwani, 1987; Nagaraju *et al*., 2002). Roy *et al*. (2006) compared agar-gelled medium with liquid medium supported with coir as the matrix at two different concentrations of sucrose (3 and 6%) using basal portion of innermost leaves as an explant. They obtained large number of microcorms in liquid medium at higher concentration (6%) of sucrose as compared to agar-gelled medium.

The addition of sucrose had a positive effect on cormel weight and number in gladiolus as reported by Nagaraju *et al*. (2002). Other works (Ziv, 1979; Sutter 1986; Steinitz and Yahel, 1982) showed that the sucrose is totally utilized for corm filling as indicated by weight. Nagaraju *et al*. (2002) further reported that the presence of 12% sucrose in MS basal medium exhibited elongated leaves but small cormels. This suggests that higher concentration of sucrose limit growth in general due to osmotic effect of sugars. According to Ziv (1979), the growth of these longer leaves need not be related to the synthesis of more food by photosynthesis for the development of cormels. This might be poor photosynthetic rate of *in vitro* cultures under low irradiance. Sinha and Roy (2002) produced three categories of corms viz. small (5-10 mm), medium (10-15 mm) and large (16-22 mm) from rooted shoots cultured in half strength of MS supplemented with IBA (2 mg L^{-1}) and sucrose (6%). Better

corm production also obtained on a medium supplemented with sucrose (6-9%) and cultured at 15^0C (De- Bruyn and Ferreira 1992). They also reported that sucrose that replaced by mannitol had no beneficial effect on corm production. Goo and Kim (1994) also reported *in vitro* cormel formation from the shoot base of gladiolus cv. *Topaz* ; that was greatest (90%) with 9% sucrose. Dantu and Bhojwani (1995) reported cormel formation from 96% of shoots on liquid MS medium supplemented with sucrose (6%). Kumar *et al*. (1999) observed cormel formation on medium containing high sucrose concentration (> 6% and up to 12%). Memon et al. (2010) reported successful cormel formation on medium containing IBA (1 mg L^{-1}) with high levels of sucrose (7%) in variety *White Friendship* of gladiolus. They also reported cormel formation using same medium in variety *Traderhorn* and *Peter Pears* (Memon et al., 2012).

Use of growth retardants for cormel formation

Growth retardants such as chloromequat (Kim and Han, 1993), paclobutrazol (Courduroux, 1967 and El-Antalby *et al*., 1967), daminozide and ancymidol (Ziv, 1990) play major role in *in vitro* cormel formation in gladiolus. Ziv (1990) produced cormels by using bud explants propagated in agitated liquid medium and supplemented with growth retardants like daminozide, ancymidol and paclobutrazol. The regeneration of buds was proliferated without leaves and these buds developed into procorms and after sub-culture to a hardening agar solidified medium, formed cormels 8-10 mm in diameter. Supplementation of paclobutrazol and sucrose also found benefical in the medium for *in vitro* cormel formation. Formation of bigger cormels was favored with paclobutrazol at 10 mg L^{-1} and sucrose (120 g L^{-1}) in MS medium (Nagarju *et al*., 2002).

Reduced *in vitro* growth due to the presence of growth retardant such as paclobutrazol was also reported by Nagarju *et al*. (2002). The absence of paclobutrazol resulted in elongated leaves and the formation of smaller comels. Same responses were also reported by Ritchie *et al*. (1991) in chrysanthemum. Coulston and Shearing (1985) observed reduction in stem elongation due to paclobutrazol in several ornamental species due to the anti-gibberellin activity of paclobutrazol (Graebe, 1987) and promoted corm formation (Ziv, 1989, Steinitz and Lilien-Kipnis, 1989) when grown in media enriched with sucrose. Steinitz *et al*. (1991) obtained shoots on agar solidified media, whereas corm regeneration was obtained in subsequent liquid cultures. They also reported that paclobutrazol at 10 mg L^{-1} and sucrose promoted corm formation in liquid media.

Role of cytokinins in cormel formation

There are a number of reports on the role of cytokinins in *in vitro* stimulation of tuberization (Palmer and Smith, 1970; Koda and Okazawa, 1983; Hussey and Stacey, 1984). There is apparent ambiguity about their role in the regulation of gladiolus corm formation. Emek and Erdag (2007) reported corm formation on MS basal media containing BA (0.1 mg L^{-1}). Kinetin also found inducing cormel formation on excised stolon tips (Ginzburg and Ziv, 1973). Memon et al. (2010) reported the role of kinetin in cormel formation. BA adversely affects corm formation at the shoot base (Steinitz and Lilien-Kipnis, 1989). Ginzburg and Ziv (1973) used four plant hormones viz. kinetin, gibberellin, abscisic acid and naphthalene acetic acid for cormel development in gladiolus. Kinetin induced cormel formation, whereas, other three had no effect on tuberization. While on other side Kumar *et al*. (2002) reported corm formation on MS media without growth regulators.

According to published reports on cormel formation we can conclude that this process of tuberization can be boost up by optimizing concentration sucrose, growth retardants and concentration of cytokinins in the growth medium.

Acclimatization of *in vitro* propagules

Acclimatization of *in vitro* propagated plantlets to the *ex vitro* environment is a critical step for successful propagation. It is necessary because i) *in vitro* plantlets are not autotrophic (McCartan *et al.*, 2004) ii) poor development of leaf cuticle and iii) impaired stomatal functioning (Preece and Sutter, 1991; Hazarika, 2006). *In vitro* grown plants also have poor photosynthetic efficiency and vascular connection between the shoots and roots. This abnormal morphology, anatomy and physiology of *in vitro* plantlets (Kozai, 1991; Pospisilova *et al*., 1992; Buddendorf-Joosten and Woltering, 1994; Desjardins, 1995; Kozai and Smith, 1995) make difficult to survive the plantlets *ex vivo*. In gladiolus, there are very few but varied reports of transplanting *in vitro* grown plants either from direct or indirect regeneration. No optimized protocol has yet been developed for acclimatization process in gladiolus. Ziv *et al*. (1970) reported poor survival of transplanted plantlets in gladiolus. In 1979, he faced difficulty in transplanting gladiolus plants of the cultivar *Eurovison* to the soil until the plants were cultured on half-strength MS medium supplemented with a reduced sucrose concentration (1.5%), 0.4 mg L^{-1} thiamine, 0.5 mg L^{-1} NAA and 0.3% activated charcoal, and gown under a higher light intensity than used for maintaining the microporpagated plants. Ziv (1991) also reported that the addition of paclobutrazol to the medium resulted

in the formation of cormels with 100% survival following transfer to the greenhouse, whereas 58% was observed without paclobutrazol. Priyakumari and Sheela (2005) reported successful acclimatization of the gladiolus plantlets planted in 2:1 of sand and soil in plastic pots. Earlier Jager *et al*. (1998) also reported the similar results.

In *Lilium speciosum* Thunb. var. gloriosoides Baker, 98% survival rate of rooted plantlets was recorded in 35 cavity growing trays under mist condition for first four weeks (Chang *et al*., 2000). Hannweg *et al*. (1996) also found almost same results in *Bowiea volubilis*. They transplanted *in vitro* regenerated plantlets in sterilized soil and washed coarse river sand under three different conditions i) covered tightly for seven days to achieve high relative humidity ii) used loose covering for two to three weeks to acquire medium relative humidity iii) plantlets uncovered and mist sprayed twice a daily. Mist sprayed plantlets gave maximum survival rate (90.9%) as compared to other conditions. This phase of transplantation from *in vitro* to *in vivo* usually needs some weeks of acclimatization with gradual lowering in air humidity (Preece and Sutter, 1991; Bolar *et al*., 1998).

In gladiolus, a successful acclimatization is possible at three different stages of in vitro propagation; i) when *in vitro* regenerated plantlets have optimal shoot/root ratio but no cormel formation ii) after cormel formation and before their dormancy iii) when cormels goes under dormant period and plant shoot dries up. Generally the first option is practiced more in which *in vitro* regenerated shoots are planted into rooting medium and then placed into high humidity environment with low irradiance and temperature for acclimatization. However, to reduce the losses occur during the hardening process of *in vitro* grown plants, it is better especially in bulbous plants to induce shoots to form storage organs such as cormels in gladiolus and bulbs of lilies. These underground storage organs are generally resilient and can be planted or stored when desired. The survival of *in vitro* plantlets with cormels/bulblets is usually based on the size of the cormels as reported by Naik and Nayak (2005) in *Ornithogalum virens*; Slabbert and Niederwieser (1999) in *Lachenalia.* Smaller bulbs (2-3 mm diameter) showed low survival as compared to large one (4-10 mm diameter) (Naik and Nayak, 2005). Paek and Murthy (2002) reported 100% survival of *in vitro* rooted bulblets those had a diameter of more than 10 mm. Cormels usually undergo dormancy and thus do not sprout upon planting. To overcome dormancy and with the idea of producing plants year round, a cold treatment is followed to break the dormancy before the cormels are sown in the soil (Stimart, *et al*., 1982). Gladiolus require cold treatment for a period of four weeks at a temperature range of 2-5^{0}C (Hussey 1977). He also reported that the dormancy can also be broken when *in vitro* produced cormels are subcultured

on a medium containing BA. A period of 4-8 weeks at 0-5^{0}C was required to break dormancy in bulblets (Bacchetta *et al*., 2003). Paek and Murthy (2002) employed cold treatment for 5 weeks at 5^{0}C in *Fritillaria thunbergii*.

Role of corm size in acclimatization

Corm size plays major role in the acclimatization of the bulbous plants as poor survival rate was observed within five to seven days from bulblets having diameter smaller than 4 mm whereas the survival rate of larger bulblets was high (Hannweg *et al*., 1996). Paek and Murthy (2002) planted *in vitro* regenerated bulblets of *Fritillaria thunbergii* of different sizes in equal ratio of peat moss, vermiculite and perlite. They recorded survival rate after five weeks of planting, it was 17.6% for bulblets having a diameter less than 5 mm whereas bulblets having a diameter 6-10 mm or more showed 86% to 100% survival . In an experiment Naik and Nayak (2005) reported that bulblets of small size (2-3 mm diameter) showed a survival rate of 40-50%, whereas the larger bulblets (4-10 mm diameter) had a 70 to 80% survival rate. This is also supported by Slabbert and Niederwieser (1999) in Lachenalia.

Conclusion

The broad expansion in the international trade of floriculture products is forcing researchers and growers to establish new methods for rapid multiplication of elite clones, production of disease-free plants, introduction of new exotic varieties or novel cultivars with desirable traits and improvement of regular varieties as a part of gladiolus improvement program. In our opinion micropropagation techniques can play a vital role in this regard. We have combined most of the published work on the micropropagation of gladiolus as reference for future studies in this paper. Additionally, most of the critically important factors affecting successful microprogation of gladiolus are discussed here in detail. Although it is obvious from literature that there are many reproducible protocols for micropropagation of gladiolus, there are many challenges being faced by the tissue culture industry at present. These include research for cost effective protocols, applying automation for commercialization and optimizing microenvironment etc. Therefore, it is important to bring further improvements in the existing tissue culture protocols to go for commercialization and to maintain the clonal fidelity of elite clones.

References

Aftab, F., Alam, M. and Afrasiab, H. (2008). *In vitro* shoot multiplication and callus induction in *Gladiolus hybridus* Hort. Pak. J. Bot. 40(2):517-522.

Ahmad T, Ahmad, M.S., Nasir, I.A. and Riazuddin, S. (2000). *In vitro* production of cormels in gladiolus. Pak. J. Biol. Sci. 3:819-821.

Arora, J.S., Singh, K., Grewal, H.S., Gosal, S.S. and Chanana. Y.R. (1996). *In vitro* cormel production from nodal buds and cormel tips in gladiolus. *In*: Islam AS (Ed.) Plant tissue culture. Oxford and IBH publishing Co. Pvt. Ltd. New Delhi, Calcutta, pp. 50-53.

Ascough, G.D., Erwin, J.E. and Staden, J.V. (2009). Micropropagation of Iridaceae-A review. Plant Cell Tissue Org. Cult. 97:1-19.

Babu, P., and Chawla, H.S. (2000). *In vitro* regeneration of *Agrobacterium* mediated transformation in gladiolus. J. Hort. Sci. Biotechnol. 75:400-404.

Bacchetta, L., Remotti, P.C., Bernardini, C. and Saccardo, F. (2003). Adventitious shoot regeneration from leaf explants and stem nodes of Lilium. Plant Cell Tissue Org. Cult. 74:37-44.

Bajaj, Y.P.S. (1992). A suggested method for *in vitro* long-term storage at 40^0C of chrysanthemum and petunia germplasm. Plant Tiss. Cult. 3:57-58.

Bajaj, Y.P.S., Sidhu, M.M.S. and Gill, A.P.S. (1983). Some factors affecting the *in vitro* propagation of gladiolus. Sci. Hort. 18:269-275.

Begum, S. and Haddiuzaman, S. (1995). *In vitro* rapid shoot proliferation and corm development in glaiolus grandiflorus cv. Redbrand. Plant Tissue Cult. 5:7-12.

Beura, S. and Singh R. (1998). *In vitro* corm production in gladiolus. Souvenir, National Seminar on Plant Biotechnology for sustainable Hill Agriculture. Defence Agric. Res. Lab., Pithoragarh, Uttar Pradesh, pp. 10.

Blom-Barnhoorn, G.J. and Van Aartrijk, J. (1985). The regeneration of plants free of LSV and TBV from infected *Lilium* bulb-scale explants in the presence of virazole. Acta Hort. 164:163-168.

Bolar, J.P., Norelli, J.L., Aldwinckle, H.S. and Hanke, V. (1998). An efficient method for rooting and acclimation of micropropagated apple cultivars. Hort. Sci. 37: 1251-1252.

Boonvanno, K. and Kanchanapoom, K. (2000). *In vitro* propagation of *gladiolus*. Suranaree J. Sci. Technol. 7:25-29.

Buddendorf-Joosten, J.M.C. and Woltering, E.J. (1994). Components of the gaseous environment an their effects on plant growth and development *in vitro*. Plant Growth Regul. 15:1-16.

Chang, C., Chen, C., Tsai, Y. and Chang, W. (2000). A tissue culture protocol for propagation of a rare plant, *Lilium speciosum* Thunb. Var. *Gloriosoides Baker. Bot.* Bull. Acad. Sin. 41:139-142.

Chow, Y.N., Selby, C. and Harvey, B.M.R. (1992). Stimulation by sucrose of narcissus bulbil formation *in vitro*. J. Hort. Sci. 67:289-293.

Coulston, G.H. and Shearing, S.J. (1985). Review of the effects of paclobutrazol on ornamental pot plants. Acta Hort. 167:339-348.

Courduroux, J.C. (1967). Etude du mechanisme physiologique de la tuberization chez le topinambour (*Helianthus tuberosus* L.) Ann. Sci. Nat. Bot. 8:215-356.

Dantu, P.K. and Bhojwani, S.S. (1987). *In vitro* propagation and corm formation in *gladiolus*. Gartenbauwissenchaft 52:90-93.

Dantu, P.K. and Bhojwani, S.S. (1995). *In vitro* corm formation and field evaluation of corm-derived plants of *gladiolus*. Sci. Hort. 61:115-129.

De Bruyn, M.H. and Ferreira, D.I. (1992). *In vitro* corm production of *Gladiolus dalenii* and *G. tritis.* Plant Cell Tissue. Org. Cult. 31:123-128.
Desjardins, Y. (1995). Photosynthesis *in vitro*-on the factors regulating CO_2 assimilation in micropropagation systems. Acta Hort. 393:45-61.
El-Antalby, H.M.M., Wareing, P.F. and Hillmarn, J. (1967). Some physiological responses to d-1 abscisin (dormin). Planta 73:74-90.
Emek, Y. and Erdag, B. (2007). *In vitro* propagation of *Gladiolus anatolicus* (Boiss.) Stapf. Pak. J. Bot. 39:23-30.
Ginzburg, C. and Ziv, M. (1973). Hormonal regulation of corm formation in *gladiolus* stolons grown *in vitro*. Ann. Bot. 37: 219-224.
Goo, D.H. and Kim, K.W. (1994). Influence of sucrose, ABA and daylength on cormlet formation of *gladiolus in vitro*: Histological observation. J. Kor. Soc. Hort. Sci. 35:400-405.
Goo, D.H., Joung, H.Y. and Kim, K.W. (2003). Differentiation of *gladiolus* plantlets from callus and subsequent flowering. Acta Hort. 620:339-342.
Graebe, J.E. (1987). Gibberellin biosynthesis and control. Ann. Rev. Plant Physiol. 38:419-465.
Grewal, H.S., Gosal, S.S., Arora J.S. and Singh, K. (1990). Mass propagation of carnation, chrysanthemum and *gladiolus* through tissue culture. 23rd Int. Hort. Cong., Florence, Italy.
Grewal, M.S., Arora, J.S. and Gosal, S.S. (1995). Micropropagation of *gladiolus* through *in vitro* cormel production. Plant Tissue. Cult. 5:27-33.
Hannweg, K., Watt, M.P. and Berjak, P. (1996). A simple method for the micropropagation of *Bowiea volubilis* from inflorescence explants. Bot. Bull. Acad. Sin. 37:213-218.
Hartman, H.T., Kester, D.E. and Davis, F.T. (1990). Plant propagation: Principles and practices. Prentice/Hall International, Inc. Englewood Cliffs, New Jersey.
Hazarika, B.N. (2006). Morpho-physiological disorders in *in vitro* culture of plants. Sci. Hort. 108:105-120.
Hosaki, T. and Asahira, T. (1980). *In vitro* propagation of narcissus. Hort. Sci. 15:602-603.
Hussain, S.C.T., Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K. (1994). Plant regeneration from root derived callus in *gladiolus*. J. Ornam. Hort. 2:46-50.
Hussain, I., Muhammad, A., Rashid, H. and Quraishi, A. (2001). In vitro multiplication of *gladiolus* (*Gladiolus crassifolius)*. Plant Tissue Cult. 11:121-126.
Hussey, G. (1977). *In vitro* propagation of *gladiolus* by precocious axillary shoot formation. Sci. Hort. 6:287-296.
Hussey, G. and Stacey, N.J. (1984). Factors affecting the formation of *in vitro* tubers of potato (*Solanum tubersoum* L.*)* Ann. Bot. 53:565-578.
Jager, A.K., Alister, B.G.Mc. and Staden, J.V. (1998). *In vitro* culture of *Gladiolus carneus.* South African J. Bot. 64:146-147.
Jo, U.A., Murthy, H.N., Hahn, E.J. and Paek, K.Y. (2008). Micropropagation of *Alocasia amazonica* using semisolid and liquid cultures. In vitro Cell Dev. Biol-Plant. 44:26-32.
Kamo, K. (1994). Effect of phytohormones on plant regeneration from callus of *gladiolus* cultivar "Jenny Lee". In vitro Cell Dev. Biol-Plant. 30:26-31.
Kamo, K. (1995). A cultivar comparison of plant regeneration from suspension cells, callus, and cormel slices of *gladiolus*. In vitro Cell Dev. Biol- Plant. 31:113-115.
Kamo, K. and Joung, Y.H. (2007). *Gladiolus*. In: *Biotechnology in Agriculture and Forestry*, (Vol. 61). E.C., Pua, and M.R. Davey (eds.). Transgenic Crops VI. Springer-Verlag Berlin Heidelberg., pp. 289-298.

Kasumi, M., Takatsu, Y., Tomotsune, H. and Sakuma, F. (1998). Callus formation and plant regeneration from developing ovaries in *gladiolus*. J. Jpn. Soc. Hort. Sci. 67:951-957.

Kim, K., Jung-Bun, C. and Kee-Young, K. (1988). Rapid multiplication of *gladiolus* plants through callus culture. J. Kor. Soc. Hort. Sci. 29:312-318.

Kim, K.W. and Han, S.Y. (1993). Cormlet formation in *gladiolus* shoot base by growth retardants *in vitro*. J. Kor. Soc. Hort. Sci. 34:136-144.

Koda, Y. and Okazawa, Y. (1983). Influence of environmental, hormonal and nutritional factors on potato tuberization *in vitro*. Jpn. J. Crop Sci. 52:582-591.

Kozai, T. (1991). Micropropagation under Photoautotrophic Conditions. In: *Micropropagation*: Technology and Application. P.C. Debergh, and R.H. Zimmerman (eds.) Kluwer Academic Pub., Dordrecht, Boston, London, pp. 447-469.

Kozai, T., Smith, M.A.L. (1995). Environmental control in plant tissue culture-general introduction and overview. In: Automation and Environmental Control in Plant Tissue Culture. J. Aitken-Christie, T. Kozai, M.L. Smith. (eds.). Kluwer Academic Pub., Dordrecht, Boston, London. pp. 301-318.

Kumar, A., Sood, A., Palni, L.M.S. and Gupta, A.K. (1999). *In vitro* propagation of *gladiolus* hybridus hort.: Synergistic effect of heat shock and sucrose on morphogenesis. Plant Cell Tissue Org. Cult. 57:105-112.

Kumar, A., Palni, L.M.S., Sood, A., Sharma, M., Palni, U.T. and Gupta, A.K. (2002). Heat-shock induced somatic embryogenesis in callus cultures of *gladiolus* in the presence of high sucrose. J. Hort. Sci. Biotechnol. 77:73-78.

Lilien-Kipnis, H., and Kochba, M. (1987). Mass propagation of *Gladiolus hybrids*. Acta Hort. 212:631-638.

Logan, A.E. and Zettler, F.W. (1985). Rapid *in vitro* propagation of virus-indexed gladioli. Acta. Hort. 164:169-175.

Loiseau, J., Marche, C. and Deunff, Y.L. (1995). Effect of auxins, cytokinins, carbohydrates and amino acid on somatic embryogenesis induction from shoot apices of pea. Plant Cell Tissue Org. Cult. 41:267-275.

McCartan, S.A., Beckett, R.P., Ivanova, V. and Staden, J.V. (2004). *In vitro* hardening: the role of ventilation on acclimation stress in *Kniphofia leucocephala*. Plant Growth Reg. 43:49-55.

Memon, N., Qasim, M., Jaskani, M.J., Ahmad, R. and Ahmad, I. (2009). Enhancement of corm and cormel production in gladiolus. New Zealand J. Crop and Hort. Sci. 37:319-325.

Memon, N., Qasim, M., Jaskani, M.J. and Ahmad, R. (2010). *In vitro* cormel production of gladiolus. Pak. J. Agri. Sci. 47:115-123.

Memon, N., Qasim, M., Jaskani, M.J., Awan, F.S., Khan, A.I., Sadia, B. and Hussain, Z. (2012). Assessement of somaclonal variation in *In vitro* propagated cormels of *Gladiolus*. Pak. J. Bot. 44:769-776.

Misra, S. and Singh, R. (1999). *In vitro* propagation of *gladiolus* cv. *American Beauty*. J. Ornam. Hort. 2:67-70.

Nagaraju, V. and Parthasarthy, V.A. (1995). Effect of growth regulators on *in vitro* shoots of *Gladiolus hybridus*. Folia Hort. 7:93-100.

Nagaraju, V., Bhowmik, G. and Parthasarathy, V.A. (2002). Effect of paclobutrazol and sucrose on *in vitro* cormel formation in *gladiolus*. Acta Bot. Croat. 61:27-33.

Naik, P.K. and Nayak, S. (2005). Different modes of plant regeneration and factors affecting *in vitro* bulblet production in *Ornithogalum virens*. Sci. Asia. 31:409-414.

Narain, K. (2004). Garden life-Glorious gladioli. In: The Tribune magazine. Spectrum. http://www.tribuneindia.com/2004/20040905/spectrum/garden.htm.

Nhut, D.T., Jaime, A. and Silva, T.D. (2004). The importance of explant source on regeneration and micropropagation of *gladiolus* by liquid shake culture. Sci. Hort. 102:407-414.
Novak, F.J. and Petru, E. (1981). Tissue culture propagation of *Lilium* hybrids. Sci. Hort. 14:191-99.
Paek, K.Y. and Murthy, H.N. (2002). High frequency of bulblet regeneration from bulb scale sections of *Fritillaria thunbergii.* Plant Cell Tissue Org. Cult. 68:247-252.
Palmer, C.E. and Smith, O.E. (1970). Effect of kinetin on tuber formation on isolated stolons of *Sozanum tuberosum* L. cultured *in vitro*. Plant Cell Physiol. 11:303-314.
Pathania, N.S., Misra, R.L. and Raghava, S.P.S. (2001). Precocious shoot proliferation and microcorm production in gladiolus through tissue culture. J. Omam. Hort. 4:69-73.
Pospisilova, J., Solarova, J. and Catsky, J. (1992). Photosynthetic responses to stresses during *in vitro* cultivation. *Photosynthetica*. 26:3-18.
Prasad, V.S.S. and Gupta, S.D. (2006). *In vitro* shoot regeneration of *gladiolus* in semi-solid agar versus liquid cultures with support systems. Plant Cell Tissue Org. Cult. 87:263-271.
Preece, J.E. and Sutter, E.G. (1991). Acclimatization of micropropagated plants to the greenhouse and field. In: Micropropagation. Technology and Application. P.C. Debergh, R.H. Zimmerman (eds.). Kluwer Academic Pub., Dordrecht, Boston, London, pp.71-93.
Priyakumari, I. and Sheela, V.L. (2005). Micropropagation of *gladiolus* cv. 'Peach Blossom' through enhanced release of axillary buds. J. Trop. Agric. 43:47-50.
Rahman, S.M., Islam, A.K.M.R. and Joarder, O.I. (1992). Effect of media composition and culture condition on *in vitro* rooting of rose. Sci. Hort. 52:163-169.
Remotti, P.C. and Loffler, H.J.M. 1995. Callus induction and plant regeneration from *gladiolus*. Plant Cell, Tissue Org. Cult. 42:171-178.
Ritchie, G.A., Short, K.C. and Davey, M.R. (1991). *In vitro* acclimatization of chrysanthemum and sugar beet plantlets by treatment with paclobutrazol and exposure to reduced humidity. J. Exp. Bot. 42:1557-1563.
Romano, A., Noronha, C. and Martin-Loucoo, M.A. (1995). Role of carbohydrate in micropropagation of cork oak. Plant Cell Tissue Org. Cult. 40:159-167.
Roy, S.K., Gangopadhyay, G., Bandyopadhyay, T., Modak, B.K., Datta, S., Mukherjee, K.K. (2006). Enhancement of *in vitro* micro corm production in *gladiolus* using alternative matrix. African J. Biotechnol. 5:1204-1209.
Savangikar, V.A. (2004). Role of low cost options in tissue culture. In: *Low Cost Options for Tissue Culture Technology in Developing Countries*. Proc. Food and Agric. Int. Atomic Energy Agency, Vienna, pp. 11-15.
Sen, J. and Sen, S. (1995). Two step bud culture technique for a high regeneration of *gladiolus* corm. Sci. Hort. 64:133-138.
Sengupta, J., Mitra, G.C. and Sharma, A.K. (1984). Organogenesis and tuberization in cultures of *Dioscorea floribunda*. Plant Cell Tissue Org. Cult. 3:325-331.
Simonsen, J., and Hildebrandt, A.C. (1971). *In vitro* growth and differentiation of *gladiolus* plants from callus cultures. Can. J. Bot. 49:1817-1819.
Singh, A.P. and Dohare, S.R. (1994). Maximization of corms and cormel production in Gladiolus. In: Floriculture-Technology, Trades and Trends. J. Prakash and K.R. Bhandary (eds.). Oxford & IBH Pub.Co. Pvt. Ltd. India. pp. 205-208.
Sinha, P. and Roy, S.K. (2002). Plant regeneration through *In vitro* cormel formation from callus culture of *Gladiolus primulinus* Baker. Plant Tisssue Cult. 12:139-145.
Slabbert, M.M. and Niederwieser, J.G. (1999). *In vitro* bulblet production of *Lachenalia.* Plant Cell Rep. 18:620-624.

Sriskandarajal, C. and Mullins, M.G. (1981). Micropropagation of granny smith apple: Factors affecting root formation *in vitro*. J. Hort. Sci. 56:71-76.

Steinitz. B. and Yahel, H. (1982). *In vitro* propagation of *narcissus.* Hort. Sci. 17:333-334.

Steinitz, B. and Lilien-Kipnis, H. (1989). Control of precocious *gladiolus* corm and cormel formation in liquid shake cultures. J. Plant Physiol. 135:495-500.

Steinitz, B., Cohen, A., Goldberg, Z., Kochba, M. (1991). Precocious *Gladiolus* corm formation in liquid shake culture. Plant Cell Tissue Org. Cult. 26:63-70.

Stimart, D.P. and Ascher, P.D. (1982). Overcoming dormancy of Lilium longiflorum bulblets produced in tissue culture. J. Amer. Soc. Hort. Sci. 107:1004-1007.

Sutter, E.G. (1986). Micropropagation of *Ixia viridifolia* and *Gladiolus X Homoglossum hybrid*. Sci. Hort. 29:181-189.

Takayama, S. and Misawa, M.M. (1983). A scheme for mass propagation of *Lilium in vitro*. Sci. Hort. 18:353-362.

Tipirdamaz, R. (2003). Rooting and acclimatization of *in vitro* mciropropagated snowdrop (*Galanthus ikariae* Baker) bulblets. Akdeniz Univ. Ziraat Fakultesi Dergisi. 16:121-126.

Van Aartrijk, J., Blom-Barnhoorn, G.J. and Van der Linde, P.C.G. (1990). Lilies. In: *Handbook of Plant Cell Culture, Vol-5*. P.V. Ammirato, D.A. Evans, W.R. Sharp and Y.P.S. Bajaj. (eds.). Collier Macmillian Publishers, London, pp. 535-576.

Wang, P.J. and Hu, C.Y. (1982). *In vitro* mass tuberization and virus-free seed-potato production in Taiwan. Am. Potato J. 59:33-37.

Wickremesinhe, E.R.M., Holcomb, E.J. and Arteca, R.N. (1994). A practical method for the production of flowering Easter lilies from callus cultures. Sci. Hort. 60:143-152.

Ziv, M. (1979). Transplanting gladiolus plants propagated *in vitro*. Sci. Hort. 11(3):257-260.

Ziv, M. (1989). Enhanced shoot and cormlet proliferation in liquid cultured gladiolus buds by growth retardants. Plant Cell Tissue Org. Cult., 17(2-3):101-110.

Ziv, M. (1990). The effect of growth retardants on shoot proliferation and morphogenesis in liquid cultured gladiolus plants. Acta Hort., 280:207-214.

Ziv, M. (1991). Plant Growth Substances. In 1991 Prceedings of 14th international conference, Amesterdam, 21-26 July. Agric. Univ. 19:142-143.

Ziv, M., Halevy, A.H., Shilo, R. (1970). Organs and plantlet regeneration of *gladiolus* through tissue culture. Ann. Bot. 34:671-676.

Ziv, M., Lilien- Kipnis, H. (1990). *Gladiolus*. In*: Handbook of Plant Cell Culture,* Vol. 5. P.A. Ammirato, D.A. Evans, W.R. Shark, Y.P.S. Bajaj (Eds.). Mcgraw Hill Publishing Co. New York, pp. 461-478.

Ziv, M. and Lilien-Kipnis, H. (2000). Bud regeneration from inflorescence explants for rapid propagation of geophytes *in vitro*. Plant Cell Rep. 19:845-850.

(Received 6 November 2012; accepted 30 November 2012)