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Review

Propagation of *Gladiolus* corms and cormels: A review

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***Gladiolus* is an important estimated 8th in the world cut flower trade's cut flower grown throughout the world for its elegant attractive spikes of different hues and good keeping quality. The commercial cultivation of *Gladiolus* is based on natural multiplication of corms and cormels. However, multiplication rate of corms and cormels is slow and the conventional method of propagation is insufficient to meet the demand of planting material and eventually affect the final cost of corms. A number of improved conventional techniques including division of the corms, removal of leaf and flower spikes, use of standard corm size, and mechanical removal of sprouts can increase the multiplication rate of corms and cormels. These improved conventional methods of propagation are insufficient to meet the demand of planting materials. *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 using different media 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: Corms, cormels, galdiolus, propagation.

INTRODUCTION

Gladiolus is an important estimated 8th in the world cut flower trade grown for its elegant attractive spikes of different hues and good keeping quality (Sinha and Roy, 2002). The major producing countries are the United States (Florida and California), Holland, Italy, France, Poland, Bulgaria, Brazil, India, Australia and Israel. In the United States, the best-selling bulb is the *Gladiolus* with an estimated annual sale of more than 370 million corms (Narain, 2004). *Gladiolus*, a member of the Iris family with short life cycle of 110 to 120 days, require temperature regime between 10 and 25°C. *Gladiolus* comes under the category of bulbous plants. The bulbous plants are commercially perpetuated by using their underground storage organs such as rhizomes of tuberose, corms of *Gladiolus* and bulbs of lilies. However, there are other methods which are applied to these underground storage organs such as chipping, scooping, scaling, and scoring. These methods used for bulbs are not applicable for the propagation of corms as in *Gladiolus*. Unlike a bulb, which is predominantly fleshy leaf scales, a corm is a compressed solid thickened stem with distinct nodes and internodes (Hartman et al., 1990). Propagation of *Gladiolus* is principally by the natural multiplication of new corms and cormels (Hartman et al., 1990; Ziv and Lilien-

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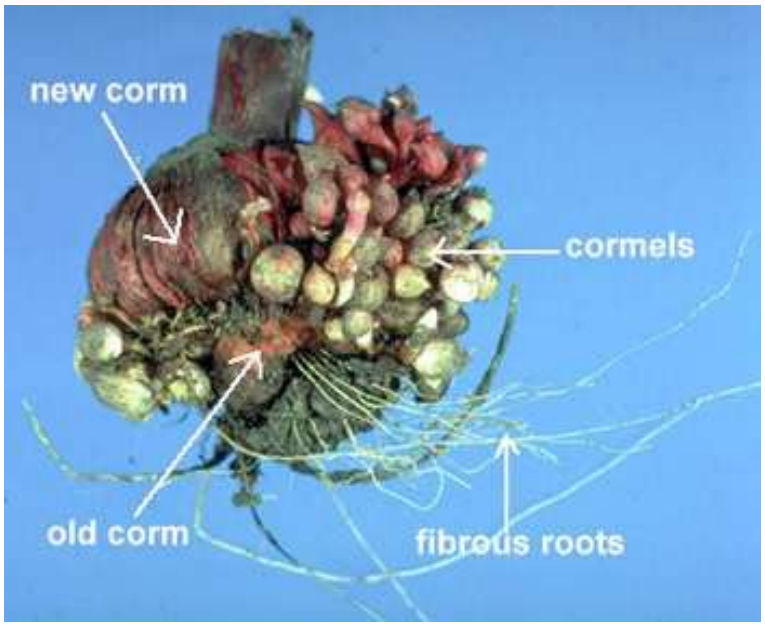

Figure 1. The *gladiolus* corm with cormels (http://montananaturalist.blogspot.com/2010/04/yellowbells-fritillaria-pudica.html).

Kipnis, 1990; Singh and Dohare, 1994; Bose et al., 2003). However, its commercial cultivation is limited by low rate of multiplication. One mother corm normally produces 1 to 2 daughter corms and about 25 cormels each season (Figure 1) (Misra, 1994; Sinha and Roy, 2002). However, daughter cormels developed from the axillary buds of the mother corm after one month of planting (Teixeira da Silva, 2003), require three to four seasons to attain standard size of flowering spike and daughter corms. The commercial production of corms and cormels is also greatly affected by *Fusarium* corm rot and high percentage of spoilage of corms during storage (Sinha and Roy, 2002; Riaz et al., 2010). This commercial production of corms and cormels does not fulfill the local demand of planting material and eventually affects corm cost. The dormancy of the corms and cormels is another problem in this regard (Priyakumari and Sheela, 2005).

Gladiolus commercial cultivation is not dependent on seed propagation as seed propagation is only used to evolve new and improved varieties by hybridization (Singh, 1992). Due to very low natural propagation rate, *Gladiolus* takes many years of growth before the cultivar can be released. Therefore, novel cultivars need to be rapidly mass multiplied by using *in vitro* propagation techniques in order to fulfill supply gap of huge demand of our local market which is not possible through conventional methods. These techniques increase multiplication rates (Novak and Petru, 1981; Takayama and Misawa, 1983; Wickremesinhe et al., 1994; Shabbir et al., 2009) and also generate material free from viruses and other pathogens (Blom-Barnhoorn and Van Aartrijk, 1985; Van Aartrijk et al., 1990). 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). This technology works in a way only when tissue culture methods are superior to conventional propagation, produce pathogen free plants in huge quantities, competition with conventional method and used for cloning.

A number of protocols have been developed on *in vitro* regeneration of plantlets in *Gladiolus*. However, literature is rather scanty on *in vitro* cormel formation. Since these pioneering efforts, a lot of data were generated and a number of papers have been published on different aspects of corm propagation and production in *Gladiolus*. A consolidated account of corm production techniques used in *Gladiolus* propagation is presented in this review.

PROPAGATION OF CORMS AND CORMELS THROUGH IMPROVED CONVENTIONAL METHODS

A number of improved techniques are used to promote corm and cormel production in *Gladiolus*. The research work was reported on divisions of the corms (Singh and Dohare, 1994; Memon et al., 2009a), removal of flower spikes (El-Gamassy and Sirry, 1967; Wilfret and Raulston, 1974; Mukhopadhyay and Das, 1977; Singh et

Figure 2. Sprouting on the basis of number of sprouts (Source: http://www.shutterstock.com/pic-3075215/stock-photo-one-gladiolus-flower-bulb-brightly-lit-isolated-on-white.html).

al., 1978; Misra, 1994; Singh and Dohare, 1994; Memon et al., 2009a), leaf clippings (Misra, 1994; Memon et al., 2009a), corm sizes (Farid Uddin et al., 2002; Memon et al., 2009b), manual removal of apical buds (Singh and Dohare, 1994) and mechanical removal of sprouts in succession during storage (Sharga and Basario, 1976; Misra, 1994). Each technique has its own merits and limitations to act as a satisfactory technique. The end users (growers of the *Gladiolus*) are unaware about these techniques. Very few and old references are available on these improved techniques used as conventional methods for the corm and cormel propagation of *Gladiolus*.

CONVENTIONAL IMPROVED TECHNIQUES

Corm divisions and sizes

The low rate production of corms and cormels is one of the major constrains in commercial cultivation of *Gladiolus*. Division of the corms in this regard is one of the best and economical alternatives to increase the yield of corms and cormels. Corm division is mainly based on the size of the mother corms and existing buds on the corm (Gromov, 1972). The size of the corms may be determined on the basis of minimum and maximum circumference or diameter. The North American *Gladiolus* Council (Wilfret, 1980) grouped corms into three grades on the basis of their circumference/diameter: Large, medium, and small. Jumbo (>5.1 cm) and No. 1 (>3.8 to ≤5.1 cm) categories come under the “large” category, whereas No. 2 (>3.2 to ≤3.8 cm) and No. 3 (>2.5 to ≤3.2 cm) are in the “medium” category. “Small” corms include No. 4 (>1.9 to ≤2.5 cm), No. 5 (>1.3 to ≤1.9 cm), and No. 6 (>1.0 to ≤1.3 cm). Circumference or diameter means the greatest dimension of the corm at right angles to a line running from the stem to the center of the basal portion.

Commercially, growers use whole corms of medium size (>2.5 to ≤ 3.8 cm) for getting the flower spike of standard size and daughter corms and cormels. However, when the objective is to get maximum production of corms and cormels, then it is better to use jumbo (>5.1 cm) and large size (>3.8 to ≤5.1 cm) corms. Commercial producers may be able to cut large corms instead of using whole corms for getting maximum corm and cormel production. Gromov (1972) reported that small corms are divided into 3 to 4 parts, large into 7 to 10 and very large ones may be divided into 12 to 15 parts depending on the number of the buds (Figure 2). Each division should have a bud and a portion of root zone. McKay et al. (1981) reported that division of large or number 3 corm sizes exhibited greater yield of new corms as compared with smaller size corms. They also reported greater inflorescence yield and higher inflorescence quality from large corms as compared with smaller corms. Lepez Oliveras et al. (1984) produced large number of grade one corms (4.8 cm diameter) in Peter Pears and White Goddes through division of corms planted in 50% peat and 50% perlite substrate, while soaking the corms for 24 h in 500 ppm GA_3 solution increased the cormel production. Gromov (1972) also reported that division of the corms markedly increased the growth of the filial corms, the weight of the corms, the

Figure 3. Memon et al. (2009a) used half corms of *gladiolus*.

number and weight of cormels in comparison with those produced from whole corms. If corm production is not the objective then medium sized corms are best to achieve an acceptable flower spike by market standards.

Singh and Dohare (1994) reported maximization of corm and cormel production in three cultivars (Pusa Suhagin, Mayur and Melody) of gadiolus using various improved cultural techniques. They obtained maximum number and weight of corms and cormels per plant in response to manual removal of two central apical buds. However, the reduction in weight and number of corms and cormels was observed in response to division to the half corms and quarter corms. When translated in terms of yield of corms per unit stock, plantation with quarter corms, showed maximum increase in yield over control (no improved cultural technique), followed by that with half corms. Memon et al. (2009a) almost obtained the same results by using half corms. They obtained half corms from whole corms with diameter of 3.6 to 3.8 cm (Figure 3). They used three varieties, namely, Traderhorn, White Friendship and Peter Pears and observed reduced yield of corms in each variety as compared to whole corms but yield of the corms was maximum on the basis of unit stock. On a unit stock basis, they observed increased yield of new corms 64% in Traderhorn, 36% in White Friendship and 37% in Peter Pears as compared to whole corms. They also produced jumbo size (>5.1 cm) corms from half corms as from whole corms.

Size of corm affects the vegetative, floral and corm yield attributes in *Gladiolus*. Smaller sizes of the corms are poor yielder, and larger sized corms add in cost of cultivation (Singh, 1992). Therefore, it is essential to find out optimum size of corms for obtaining the best results. Generally, it is advisable to have medium sized (> 2.5 to ≤ 3.8 cm) corms than small sized corms (> 1.3 to ≤ 2.5). Growers usually prefer small to medium sized corms for commercial cultivation of *Gladiolus*. The performance of large and medium corms was better with respect to corm and cormel production as compared with smaller ones (Mohanty et al., 1994). Similarly, other studies (Singh, 1996; Syamal et al., 1987; Kalasareddi et al., 1998) reported that large corms were superior in terms of number of shoots per corm, plant height, spike length, number of spikes, number of florets per spike and the diameter of corms produced. According to Hong et al. (1989), the number of daughter corms and flowering ability increased with increasing corm size up to 4 to 5 cm diameter, but there was no further increase for corms >5 cm diameter. In another study, Misra et al. (1985) studied the effect of 9 different corm sizes (from Jumbo to 0.6 cm in diameter) on flowering and corm production. They reported that the number of florets did not vary significantly up to 3.5 cm corm diameter. Number and weight of corms and cormels increased with the increase in corm size. These results are supported by the findings of Mukhopadhyay and Yadav (1984) who also reported more flowers, corm and cormel production from larger corms of 4.6 to 5.0 cm diameter. However, contradictory results were reported by Singh (1992) who reported production of more number of corms and cormels from large sized corms (6 to 8 cm diameter) than 5 cm.

Farid Uddin et al. (2002) studied the effect of corm size and depth of planting on the growth and flowering of *Gladiolus* cv. *Friendship* using the combination of four corm sizes (15, 10, 5 and 3 g) and three planting depths (10.0, 7.5 and 5.0 cm). Corm size had significant influence on all the parameters studied. Large corm (15 g) took

shortest time to complete 80% emergence (15.89 days) and flower initiation (60.44 days). Maximum plant height (97.56 cm), number of leaves (62.33), and length of flower stalk (26.07 cm) was observed from large sized corm planted at 5.0 cm depth and the lowest from very small corm (3 g) planted at 10 cm depth. Further, they observed that the plants planted with large sized corms showed the highest lodging but differed significantly with planting depth. The lodging was high in shallow planting (5 cm) than the deep planting (10 cm). Memon et al. (2009b) planted corms of three different sizes, namely, small (diameter 2.2 to 2.4 cm), medium (diameter 2.7 to 3.0 cm) and large (diameter 3.2 to 3.5 cm) from three different varieties of *Gladiolus*. They observed that large sized corms significantly increased the leaf breadth, length of flowering spike, and number of florets per spike over those produced from small and medium sized ones, whereas plant height was greatly decreased in response to large sized corms. Regarding corm production, large sized corms produced significantly higher weight of corms per plant, cormels per plant and combined total weight of corms and cormels per plant in all the three varieties of *Gladiolus*. However, variety Peter Pears produced the best results. The yield of new corms per plant was significantly increased in response to large sized corms both in White Friendship and Peter Pears, whereas, Traderhorn had no effect of corm size for number of corms per plant. Cormel production also depicted significant results in response to large sized corms in all the three varieties of *Gladiolus*. The results of Memon et al. (2009b) are in accordance with the results of Noor-ul-Amin et al. (2013). They planted cormels of white Friendship of three different sizes (>1.5 cm and < 2 cm, >1.0 cm and < 1.5 cm and >0.5 cm and < 1 cm) and observed the effect of various cormel sizes on the growth and development of gladiolus corms. They reported maximum percentage of sprouting (70.40) and survival (77.46) from large sized cormels. The greater number of leaves per plant (6.77), leaf area (61.14 cm^2), plant height (61.25 cm), diameter of corms (3.18 cm), number of cormels per plant (4.74) and corms weight (9.616) were recorded from large sized cormels. Kareem et al. (2013) also reported that large sized corms (3 to 3.5 cm) produced the best results in terms of vegetative growth and reproductive characteristics. More number of cormels per plant was also observed from large sized corms as compared to medium (2 to 2.5 cm) and small (1 to 1.5 cm) sized corms.

Clipping of leaves and flower spike

Gladiolus normally produces 6 to 7 leaves per plant, and depending on the variety, it may have 6 to 9 leaves (Misra, 1994). Misra (1994) reported critical leaf number per plant for proper corm and cormel growth is 4, and that the retention of 3 leaves per plant is sufficient for better corm growth if the spike is removed. The results of this study indicate that the removal of the flower spike and leaves (1 to 3) promote the development of corms and cormels in *Gladiolus* var. *Ratna's* Butterfly. This is because removal of few leaves conserves the plant's energy and metabolites that ultimately enhance the production of corms and cormels. However, if flower production is not the objective, the energy required for flower production may also be diverted towards corm and cormel development by removing the spike as well (Roberts and Milbrath, 1943; Halevy and Monselise, 1961; Mukhopadhyay and Das, 1977; Misra and Singh, 1979). Chowdhury et al. (1999) clipped off all seven leaves started from three to seven with or without flower spike. Better and significant results were found regarding corm diameter and weight of corms and cormel $plant^{-1}$ in response to removal of four leaves along with flower spike. Memon et al. (2009a) conducted a field experiment on the use of various improved techniques using three different varieties of *Gladiolus*. They used improved techniques included simple half corms (SHC) and half corms treated with activated charcoal (HCAC), clipping of three leaves (LR), and clipping of three leaves along with flower spike (LFsR). Whole corms (WC) were used as control. They observed the best response from the LFsR for number of cormels, number of corms and collective total weight of corms and cormels in each variety of the *Gladiolus*. On the basis of varietal comparison, White Friendship had more number of cormels (86.63) as compared to whole corms (71.57) (Figure 4). However, more collective total weight of corms and cormels was observed from Peter Pears (161.75 g) in response to LFsR as compared to WC (138.87 g) (Figure 5). Contradictory results were found by Ahmad and Siddique (2005). They found the best results in response to removal of only flower spike. They removed flower spike with one leaf, two and 3 leaves subsequently keeping control with no removal of spike and leaf. Removal of spike without leaf produced the highest number of corms (2.58), more weight (72.68 g), maximum diameter (6.19 cm) and volume (80.80 cm^3). Mukhopadhyay and Das (1977) also showed removal of spikes at early stages resulted in the increase of corm weight, whereas flower spikes removed along with two leaves had an adverse effect. Singh (1992) reported that corm yield increases by 60% when the flower spikes are removed as they appear, compared with plants on which the flower spikes are left to develop.

***IN VITRO* PROPAGATION OF CORMELS**

Mass propagation of cormels through modern technologies such as tissue culture techniques have been adopted at commercial level. Advanced countries are using highly sophisticated modern technologies for the commercial production of desired varieties in order to

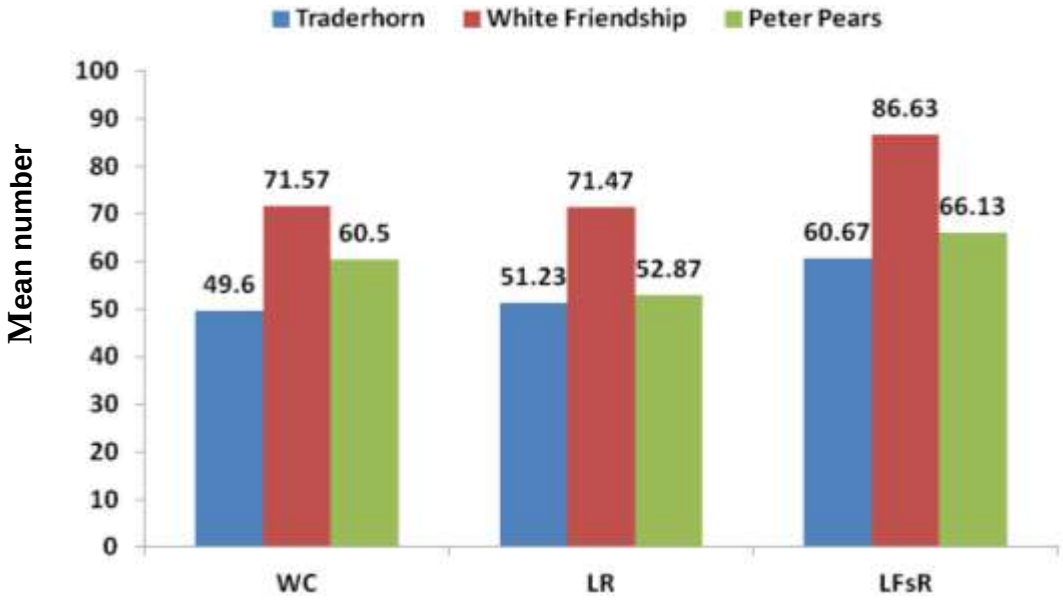

Figure 4. Mean number of cormels as affected by various improved cultural techniques in various varieties of *gladiolus* (WC = Whole corms; LR = clipping of 3 leaves; LFsR = clipping of 3 leaves plus flower spike). Source: Memon et al. (2009a).

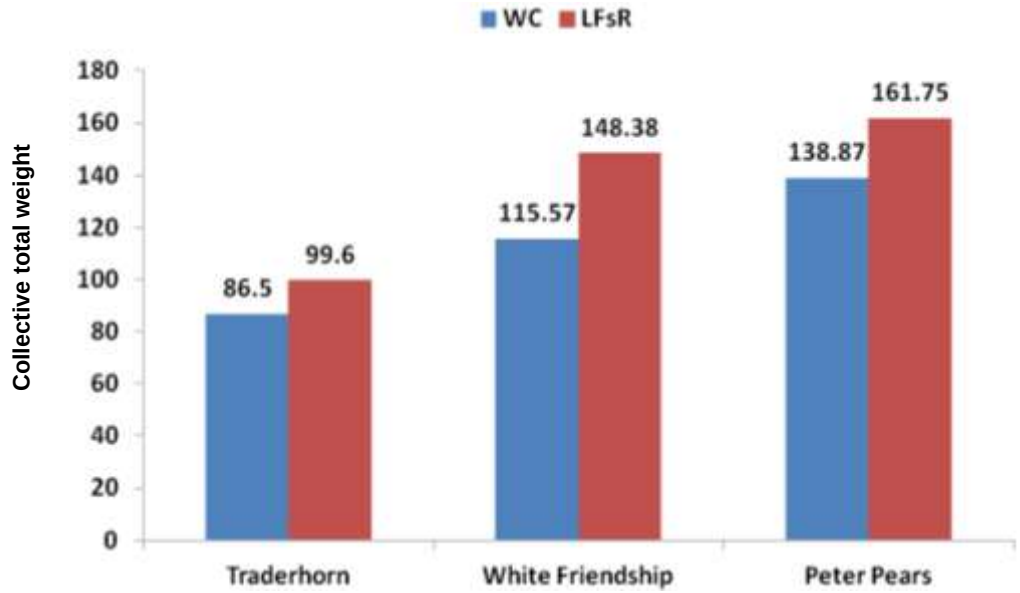

Figure 5. Collective total weight of corms and cormels as affected by clipping of leaves along with flower spike (LFsR) as compared to whole corms (WC) in various varieties of *gladiolus*. Source: Memon et al. (2009a).

compete in the international markets. This technology makes also possible to produce disease free and true to type planting material. *In vitro* techniques are useful for the propagation of corm producing species, because most of the hybrid cultivars of *Gladiolus* have a very low rate of multiplication. 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. A consolidated account of *in vitro* cormel propagation of *Gladiolus* is dealt with in the present

review.

In vitro regeneration of cormels

The ultimate goal of successful *in vitro* propagation of *Gladiolus* is the mass production of cormels (Steinitz et al., 1991; Dantu and Bhojwani, 1995; Sen and Sen, 1995; Al-Juboory et al., 1997; 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). They may also reduce the transplantation difficulties which occurred during acclimatization (Ziv, 1979; Sengupta et al., 1984). Various explants such as nodal buds (Memon et al., 2010; Grewal 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 reported to be used for *in vitro* cormel production in *Gladiolus* with the application of different growth hormones and sucrose in the medium.

GROWING MEDIUM REQUIREMENT FOR CORMEL FORMATION

The chemical composition of the growing medium is the most important factor for successful micropropagation and cormel development. Most of the reports of Gladiolus tissue culture indicated that Murashige and Skoog's (1962) medium supplemented with auxins and cytokinins is ideal for shoot initiation, multiplication and rooting (Lilien-Kipnis and Kochba, 1987; Logan and Zettler, 1985). However, addition of growth retardants and increased sucrose concentration improved cormel development (Ziv, 1989, 1990; Steinitz et al., 1991). Cormels can develop either using IBA or 2iP with different efficiency level, depending on the genotype; it is clear that in the presence of the cytokinin 2 iP either corms or shoots can develop from mother plant but in the presence of IBA the growth of shoots was strongly inhibited as reported by Ruffoni et al. (2012).

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; Memon et al., 2009b). It also has beneficial effect on multiplication of shoots (Kumar et al., 1999; De Bruyn and Ferreira, 1992), somatic embryogenesis (Loiseau et al., 1995) and rooting response of microshoots (Rahman et al., 1992; Romano et al., 1995). The increased growth of tuberous organs needs a relatively high (> 50 g/L) concentration of sucrose in the medium (Mares et al., 1985; Dantu and Bhojwani, 1987; Nagaraju et al., 2002). Higher concentration (6 or 10%) of sucrose favoured the formation of large corms (Dantu and Bhojwani, 1987). Hussain et al. (1995) reported that a high concentration of sucrose (5%) in combination with triadimefon resulted in 11 fold increase in size of in vitro corms in Cv. Friendship.

Most of the reports reported use of sucrose along with a rooting hormone such as indole butyric acid (IBA) or naphthalene acetic acid (NAA). 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%) by 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 *Gladiolus* culture weight, cormel number and weight in (Nagaraju et al., 2002). Other works (Ziv, 1979; Steinitz and Yahel, 1982; Sutter, 1986) reported that sucrose was totally utilized for corm filling as indicated by weight. Nagaraju et al. (2002) further reported that the plants grown in the presence of 12% sucrose in MS basal medium exhibited elongated leaves but small cormels. This suggests that sucrose is limiting growth in general and that the supply of carbohydrates from the leaves is not enough for cormel growth. According to Ziv (1979), the growth of these longer leaves was not related to the synthesis of more food by photosynthesis for the development of cormels. This might be due to the poor photosynthetic rate of *in vitro* cultures under low irradiance. Sinha and Roy (2002) produced three categories of corms, namely, small (5 to 10 mm), medium (10 to 15 mm) and large (16 to 22 mm) from rooted shoots cultured in half strength of MS supplemented with indole butyric acid (2 mg/L) and sucrose (6%). Memon et al. (2009b) obtained three different sizes of cormel production, namely, large (2.8 to 3.2 mm), medium (2.1 to 2.6 mm) and small (0.8 to 1.2) from rooted shoots cultured in MS medium supplemented with higher levels of sucrose (7%) but lower levels of IBA (1 mg/L) in variety White Friendship. Memon et al. (2014) observed the highest number of cromels (12.06) on MS medium supplemented with sucrose 5% plus IBA at 1 mg L^{-1} by using cormel slices of gladiolus (Figure 6) Jala (2013) cultured in vitro propagated propagules on MS medium supplemented with NAA 0.1 to 0.5 mg L^{-1} instead of IBA with sucrose (3%) got the highest number of cromels (5.8) per explant and fresh weight (144 mg per explant). De-Bruyn and Ferreira (1992) reported sucrose at 6 to 9% for *in vitro* cormel production. They also replaced sucrose by mannitol but could not find any beneficial effects on cormel production. Goo and Kim (1994) reported *in vitro* cormel formation from the shoot base of *Gladiolus* cv. *Topaz* was the 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)

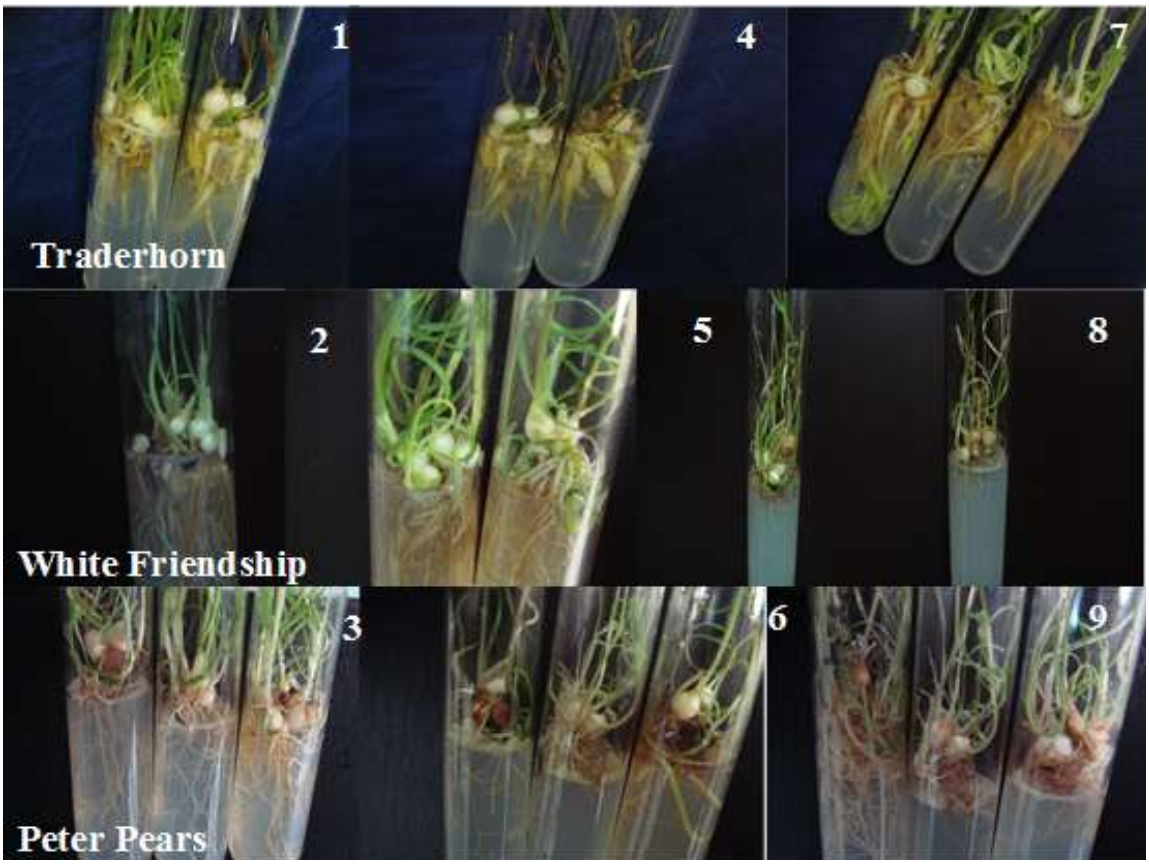

Figure 6. Cormel production from different explant sources. 1-3: Shoot tip of cormel on MS basal medium supplemented with IBA (1 mg L^{-1}) and sucrose (7%); 4-6: Middle and 7-9: Bottom slice of cormel on MS basal medium supplemented with IBA (1 mg L^{-1}) and sucrose (5%). Source: Memon (2010).

observed cormel formation on medium containing high sucrose concentration (> 6% and up to 12%).

Use of growth retardants for cormel formation

Growth retardants such as chloromequat (Kim and Han, 1993), paclobutrazol (Courduroux, 1967; 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 to 10 mm in diameter. Paclobutrazol with sucrose was also observed to be beneficial for *in vitro* cormel formation as reported by Nagarju et al. (2002). They observed large size cormels from the medium had paclobutrazol (10 mg/L) and sucrose (120 g/L) in MS medium. This report clears here that paclobutrazol (a retardant) with sucrose increased growth of the cormels by decreasing the growth of the leaves and stem. Reduction in stem elongation in several ornamental species was also reported by Coulston and Shearing (1985) due to the anti-gibberellin activity of paclobutrazol (Rademacher et al., 1984; Graebe, 1987) and promoted corm formation (Steinitz and Lilien-Kipnis, 1989; Ziv, 1989) when grown in media enriched with sucrose. Steinitz et al. (1991) reported that paclobutrazol using 10 mg/L, sucrose 60 g/L supplemented with BAP 3.0 mg/L promoted corm formation in liquid media.

Role of cytokinins in cormel formation

Very few reports were reported 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 cytokinin role in the regulation of *Gladiolus* corm formation. Emek and Erdag (2007) reported corm formation on MS basal media contained Benzyl amino purine (BAP) at 0.1 mg/L. Kinetin induces cormel formation on excised stolon tips (Ginzburg and Ziv, 1973). BAP adversely affects corm

formation at the shoot base (Steinitz and Lilien-Kipnis, 1989). Ginzburg and Ziv (1973) used four plant hormones, namely, 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. However, Kumar et al. (2002) observed corm formation on MS medium even without addition of growth regulators.

Acclimatization of *in vitro* propagules

Acclimatization of *in vitro* propagules to the *ex vitro* environment is a critical step for successful propagation. In *Gladiolus*, successful acclimatization can be possible by taking *in vitro* regenerants at three different stages: (i) When *in vitro* regenerated plantlets have optimum shoot/root ratio but no cormel formation; (ii) After cormel formation but before dormancy of the cormels; (iii) When cormels goes under dormant period and plant shoot dries up. Generally, the first option is in more practice 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. 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 (Pospisilova et al., 1992, 1997; Buddendorf-Joosten and Woltering, 1994; Desjardins, 1995) make difficult for the plantlets to survive *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 (1979) transferred *in vitro* raised propagules on half-strength MS medium supplemented with a reduced sucrose concentration (1.5%), 0.4 mg/L thiamine, 0.5 mg/L 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 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 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).

To reduce the losses which 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. However, 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 to 3 mm diameter) showed low survival as compared to large one (4 to 10 mm diameter) (Naik and Nayak, 2005). Paek and Murthy (2002) reported that 100% survival of *in vitro* rooted bulblets had diameter of more than 10 mm. Cormels usually undergo dormancy and thus do not sprout upon planting. A cold treatment is followed to break the dormancy of the cormels before plantation of cormels (De Hertogh et al., 1974; Stimart and Ascher, 1982). *Gladiolus* requires cold treatment for a period of four weeks at a temperature range of 2 to 5°C as reported by Hussey (1977). He also reported that dormancy can also be broken when *in vitro* produced cormels are subcultured on a medium containing BA. A period of 4 to 8 weeks at 0 to 5°C was required to break dormancy in bulblets (Bacchetta et al., 2003). Paek and Murthy (2002) employed cold treatment for 5 weeks at 5°C in *Fritillaria thunbergii*.

Role of corm size in acclimatization

Corm size also plays major role in the acclimatization of the bulbous plants as poor survival rate was observed within five to seven days from bulblets having smaller than 4 mm diameter whereas with larger bulblets more survival rate was obtained (Hannweg et al., 1996). Paek and Murthy (2002) also planted *in vitro* regenerated bulblets of *F. thunbergii* of different sizes in equal ratio of peat moss, vermiculite and perlite. They recorded survival rate of 17.6% after five weeks from bulblets having less than 5 mm diameter whereas 86 and 100% was observed from bulblets having 6 to 10 mm and more than 10 mm diameter, respectively. Naik and Nayak (2005) reported that bulblets of small size (2 to 3 mm diameter) had survival rate of 40 to 50%, whereas the larger bulblets (4 to 10 mm diameter) had a 70 to 80% survival rate.

Conclusions

It is concluded from various works done by scientists that corm and comel production can be multiplied successfully from large sized corms as compared to small sized corms. However, commercial producers may be able to use large sized corms for producing both marketable flower spikes as well as corm and cormel production. Regeneration of plantlets and *in vitro* production of cormels was successfully achieved through direct and indirect mode of regeneration. However, production of *in vitro* cormels through direct regeneration procedures seems to be promising for commercial production of corms and the production quality in the future.

Conflict of interests

The authors have not declared any conflict of interests.

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