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IN VITRO CORMEL PRODUCTION OF GLADIOLUS

N. Memon[1], M.Qasim[2] M.J. Jaskani[2] and R. Ahmad[3]
[1]Department of Horticulture, Sindh Agriculture University Tandojam, Sindh, Pakistan
[2]Institute of Horticultural Sciences, University of Agriculture Faisalabad-38040, Pakistan
[3]Departmetn of Crop Physiology, University of Agriculture Faisalabad-38040, Pakistan
* Corresponding author- norimemon@gmail.com

An efficient *in vitro* protocol for mass production of cormels of gladiolus cv. *White friendship* was developed by using different explant types and media supplemented with various growth regulators. The explants viz. nodal cultures from different stages of flower spike, whole cormels of various size, cormel sprouts taken at different time intervals and cormel segments of *White friendship* were cultured and arranged in completely randomized design with factorial arrangements. The heading stage of nodal cultures, large sized whole cormels (0.6 g), 12 days old cormel sprouts and top segments of cormels were evaluated the best stage/size from each explant type for efficient shoot regeneration on MS medium supplemented with BAP (4 mg L^{-1}). The best responded stage/size from each explant type was further explored for rooting and cormel production. The better response for rooting was observed from the shoots of cormel sprouts on MS medium having IBA (2 mg L^{-1}) and sucrose (3%) as compared to other explants. The maximum cormel production was also observed from the same explant on MS medium supplemented with IBA (1 mg L^{-1}) and sucrose (7%) followed by IBA+ KIN (1 mg L^{-1} each). The grading of *in vitro* produced cormels exhibited the highest cormel diameter (2.8-3.2 mm) from cormel sprouts as compared to other explants.

INTRODUCTION

Gladiolus being a potential ornamental cut flower is cultivated throughout the world for its attractive spikes. The current gladiolus range is the result of hybridization with the aim of extending vase life, and producing novel colours (Kumar *et al*., 1999). It is commercially propagated by vegetative underground grown corms and cormels (Bose *et al*., 2003). However, one of the major constrains in commercial cultivation is non-availability of a large quantity of propagules (Singh and Dohare, 1994). A large corm produces one daughter corm and few cormels for its multiplication. Besides, most of the hybrid cultivars of gladiolus have a very slow rate of multiplication and it takes many years before the cultivar can be released. Gladioli production also declines due to incidence of Fusarium corm rot caused by *Fusarium oxysporum* f. gladioli, viral and some other diseases which cause to a great extent of commercial losses (Roy *et al*., 2006; Sinha and Roy, 2002). *In vitro* propagation technique is a potential tool used for efficient production and propagation of the elite plant material of many flowering plants such as Lilium (Bacchetta *et al*., 2003; Joshi and Dhar, 2009), tuberose (Sangavai and Chellapandi, 2008), Rose (Iqbal *et al*., 2003; Razavizadeh and Ehsanpour, 2008) Dahlia (Fatima, *et al*., 2007). *In vitro* cormel production is an alternative to the conventional methods for vegetative cormel production. It increases many times the multiplication level (Novak and Petru, 1981; Takayama and Misawa, 1983; Wickremesinhe *et al*., 1994), clonal uniformity (Iqbal *et al.*, 2003) and enables material free from viruses and other diseases to be obtained (Blom-Barnhoorn and Van Aartrijk, 1985; Van Aartrijk *et al*., 1990).

A number of protocols developed for the establishment of *in vitro* propagation of gladiolus (Ziv *et al*., 1970; Lilien-Kipnis and Kochba, 1987, Sinha and Roy, 2002; Prasad and Gupta, 2006; Emek and Erdag, 2007) lacks *in vitro* corm formation studies. Only few authors reported *in vitro* corm formation in very few varieties of gladiolus such as *Pacificia* (Roy *et al*., 2006) *Balady* (Al-juboory *et al*., 1997), *Golden wave* (Sinha and Roy, 2002), *Friendship* (Dantu and Bhojwani, 1995) and *Green Bay* (Sen and Sen, 1995). According to the review of Ascough *et al*. (2009), no storage organ formation (cormel) was reported in *White Friendship.* The present study was therefore conducted to optimize and develop an efficient *in vitro* protocol for the production of cormels in *White Friendship* by observing the response of different explants at different stages/sizes.

MATERIALS AND METHODS

Various explant types viz. nodal cultures from different stages of flower spike, whole cormels of various size, cormel sprouts taken at different time intervals and

cormel segments were used to evaluate the best stage/size from each explant types for efficient regeneration of propagules. Nodal cultures from actively growing flower spikes and cormels from harvested stock of corms were collected from field plants grown in Experimental Floriculture Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The explant types and stage/size of each explant is mentioned in Table 1. The cormel sprouts were obtained by placing the whole cormels (0.6 g) on medium in apolar position. The nodal cultues were washed thoroughly with distilled water 2-3 times and surface sterilized by dipping the plant material into 70% (v/v) ethanol for 6-8 minutes. Then it was transferred to a solution of 1% sodium hypochlorite containing 2-3 drops of Tween-20 for 10 minutes followed by 3-4 washings with sterile distilled water.

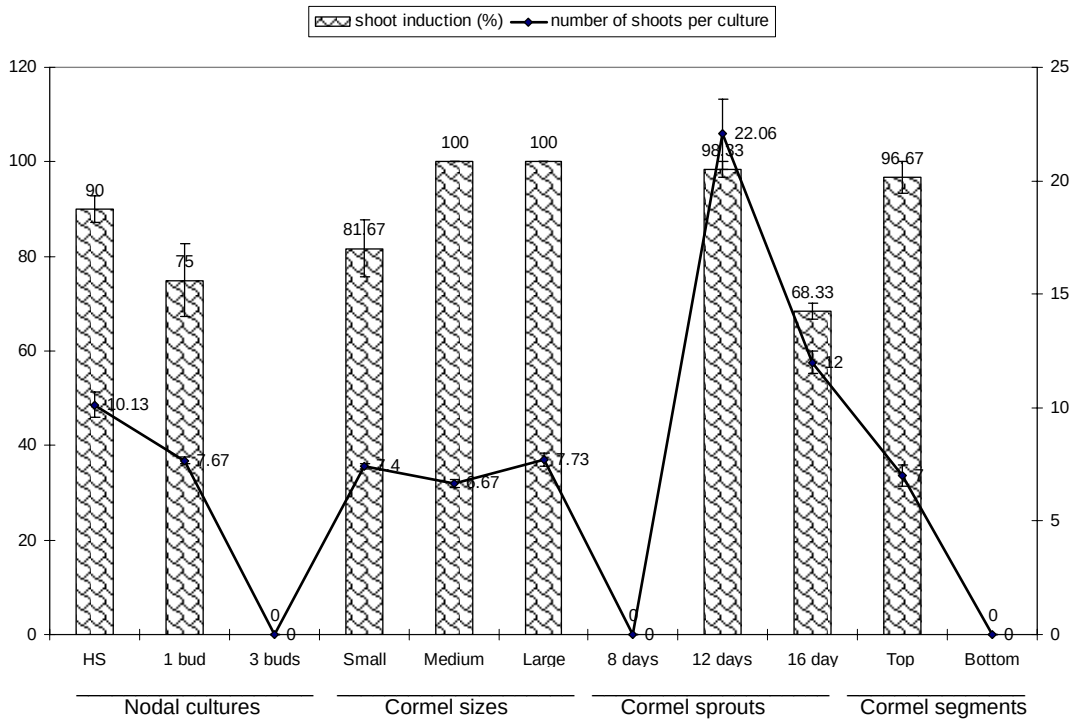
In case of cormels, the outer scale of cormels was removed with surgical blade. The descaled cormels were soaked in tap water for 30 minutes to remove any sticky material present on the cormels following 4-5 washings with distilled water. The cormels were then treated with 70% (v/v) ethanol for 15 minutes, 3-4 minutes in sodium hypochlorite, 1 minute in 1% $HgCl_2$ followed by 5-6 rinses in sterile distilled water under a laminar airflow cabinet. MS containing standard salts and vitamins, 3% sucrose and 0.8% agar was used and pH of the each medium was adjusted separately to 5.7 prior to addition of Agar. Medium was sterilized in autoclave at temperature of 121^0C and pressure 15 psi for 20 minutes. The medium was placed in growth room for one week to check any medial contamination before use for culture of explant. The explants were incubated in a culture room where the temperature was maintained at 25-27ºC under continuous photoperiod of 16h light and 8h dark. The basal MS medium supplemented with BAP (4 mg L^{-1}) was used in order to evaluate the best stage/size from each explant type for efficient shoot regeneration. The cluster of shoots with 1-3 shoots of 3 to 5 cm in length from regenerated cultures were separated from the base of the each explant and cultured on MS basal medium supplemented with IBA (1 and 2 mg L^{-1}) and various sucrose (3, 5 and 7%) levels. The whole cluster of rooted plantlets having multiple number of shoots were taken out from glass tubes and divided in such a way that each cluster having 2-3 shoots and few initiated roots. Trimming of the roots in few cultures was also followed where it was thought necessary for shifting of the rooted plantlets to the cormel induction media. The basal MS medium with constant level of IBA had different levels of sucrose (3, 5 and 7%) or cytokinins (BAP or KIN @ 1 mg L^{-1}) was used as cormel induction media. The experiments were laid out in Completely Randomized Design (CRD) using three replications per treatment. Means were compared using LSD test (Steel *et al*. , 1997).

RESULTS

Various explants viz. nodal cultures from different stages of flower spike, whole cormels of various size, cormel sprouts taken at different time intervals and cormel segments (top and bottom) were used to evaluate critical stage/size in each explant for efficient shoot regeneration and proliferation of multiple shoots. The MS basal medium supplemented with BAP (4 mg L^{-1}) was used as a growth medium for all explants. The response for shoot regeneration varied with type of the explant and stages/sizes in each explant (Fig. 1).

Table 1. Explants used for *in vitro* production of cormels in gladiolus

Sr. No.	Explant source	Stage/size of explant
1	Nodal cultures obtained from different stages of flower spike	a. Heading stage- 1 cm b. one bud opened stage- 1cm c. 3 buds opened stage -1 cm
2.	Whole cormels of various size	d. Small (0.2 g) e. Medium (0.4 g) f. Large (0.6 g)
3.	Cormel sprouts*	g. Small (8 days old) h. Medium (12 days old) i. Large (16 days old)
4	Cormel segment sections (transverse)	j. Top (3-4 mm thick) k. Bottom (3-4 mm thick)



HS= Heading stage; 1 bud= 1 bud opened stage, 3 buds = 3 bud opened stage

Fig 1. Shoot induction (%) and number of shoots per culture obtained from different explant types on MS medium supplemented with BAP (4 mg L^{-1}).

Out of three stages of nodal cultures, 3 buds opened stage didn't exhibit any response for shoot regeneration. Shoot initiation started in the nodal cultures after two weeks of inoculation and all cultures were shifted to same fresh medium after 25 days of culture. The stages of nodal culture had significant difference with each other producing the highest mean shoot induction from heading stage (90%) as compared to one bud opened stage (75%). More number of shoots (10.13) was also recorded from same stage (Fig. 1)**.** Cormels of three different sizes were also cultured on same shoot regeneration medium with growing point upward (polar inoculation). Sprouting of the cormels was observed within 4-5 days of inoculation in most of the cultures. However, shoot induction (%) increased with increase in the weight of the cormels from small to medium, whereas medium and large size cormels produced the same results for shoot regeneration (100%). The maximum number of shoots was observed from large size cormels.

Cormel sprouts at different time intervals were obtained by culturing cormels of uniform size (0.6 g) in apolar inoculation. The cormels sprouted downward into medium within four days of inoculation. These sprouts (single from each cormel) gradually underwent swelling at the base along with elongation and were of light green colour. These swelled cormel sprouts (with cormel base) were taken out at an interval of 4 days and graded as small (8 days old), medium (12 days old) and large (16 days old) cormel sprouts. The sprouts were re-cultured on the same medium with growing point upward (polar inoculation) and physiological base downward. Within a week, all these swelled sprouts of 12 and 16 days old burst into number of multiple shoots, whereas, no response was recorded from 8 days old sprouts. Top and bottom segments of cormels (0.6 g) were also explored for efficient regeneration. Top segment section of cormel induced almost shoots in 96.67% cultures within 2-3 days. However, bottom segment of cormel took more time (35-42 days) and induced shoots in only 35% cultures. The upper cut surface of the bottom sections of cormel became sequentially dark and dead in most of the cultures.

From above results, it is clear that the heading stage of nodal cultures, large sized whole cormels, medium cormel sprouts (12 days old sprouts) and top segment of cormels were evaluated the best stage/size from

each explant source for efficient shoot regeneration on MS medium supplemented with BAP (4 mg L^{-1}). The best responded stage/size from each explant types source was further explored for rooting and cormel production.

Cluster of shoots containing 2-3 shoots from each explant source was separated along with the base of the original explant and cultured on MS media supplemented with different plant growth regulator (PGR) combinations. The size of all shoots in a cluster was not the same; however, it was tried to maintain the uniformity while culturing on different rooting media. Few shoots from each explant types were initially tested for rooting on full and half strength MS medium free of any PGR and checked frequently after every four days. No root initiation was observed even after 20-25 days of inoculation on any medium. In contrast, root initiation was observed on various MS media supplemented with IBA (1 or 2 mg L^{-1}) and sucrose (3, 5 and 7%) within two weeks of inoculation. However, quick response (8 days) to rooting was observed on MS media supplemented with IBA (1 or 2 mg L^{-1}) and sucrose (5%). Induction of new shoot buds was also observed from each explant base; however, profuse shoot bud initials were recorded from cormel sprouts on most of the PGR combinations. Nodal cultures comparatively took more time (18-23 days) to root initiation than all other explants (8-14 days). Both explants and PGR combinations had independently and interactively significant effects on root induction. MS media supplemented with IBA (1 or 2 mg L^{-1}) and sucrose (3% or 5%) statistically exhibited similar results for mean root formation (Table 2). Addition of sucrose at 7% in MS medium supplemented with IBA (1 or 2 mg L^{-1}) led to the lowest mean root induction in each explant type. Comparison of explant means showed the lowest root induction from nodal cultures (63.61%) and the highest from cormel sprouts (93.33%). Each explant except nodal cultures produced optimum number of roots on MS medium supplemented with IBA (2 mg L^{-1}) and sucrose (3%) with mean value of 19.80. The increase in sucrose from 3 to 7% in MS medium with IBA (2 mg L^{-1}) reduced the mean number of roots per culture. The lowest mean number of roots (9.17) was observed on MS medium supplemented with IBA (1 mg L^{-1}) and sucrose (7%). Cormel sprouts got the mean highest number of roots (17.31) as compared to rest of the explants.

Table 2. Root regeneration from different explant types as affected by various levels of IBA and oucrase

MS+ IBA (mg L^{-1}) + sucrose (%)	Nodal cultures	Whole cormels	Cormel sprouts	Cormel segments	Mean
	Root induction (%)				
MS (Cont.)	-	-	-	-	-
MS + 1 + 3	63.33 efg	100.00 a	100.00 a	100.00 a	90.83 A
MS + 1 + 5	70.00 defg	90.00 abc	96.67 ab	80.00 bcde	84.17 A
MS + 1+ 7	46.67 h	60.00 fgh	78.33 cde	73.33 cdefg	64.58 B
MS + 2 + 3	78.33 cde	80.00 bcde	100.00 a	90.00 abc	87.08 A
MS + 2 + 5	76.67 cdef	75.00 cdef	100.00 a	100.00 a	87.92 A
MS + 2+ 7	46.67 h	56.67 gh	85.00 abcd	66.67 efg	63.75 B
Mean	63.61 D	76.94 C	93.33 A	85.00 B	
	Number of roots per culture				
MS (Cont.)	-	-	-	-	-
MS + 1 + 3	9.20 jkl	16.47 def	14.47 fg	8.07 jkl	12.05 C
MS + 1 + 5	10.60 hijk	8.87 jkl	17.40 de	10.53 hijk	11.85 C
MS + 1+ 7	6.80 l	7.60 kl	14.27 fg	8.00 jkl	9.17 D
MS + 2 + 3	10.20 ijk	25.87 a	22.67 b	20.47 bc	19.80 A
MS + 2 + 5	12.47 ghi	17.13 def	16.73 def	15.27 efg	15.40 B
MS + 2+ 7	10.00 ijk	11.00 hij	18.33 cd	13.40 gh	13.18 C
Mean	9.88 D	14.49 B	17.31 A	12.62 C	

MS- Murashige and Skoog (1962); IBA –Indole butyric acid

PRODUCTION OF CORMELS

After two weeks of root initiation (not fully developed roots), the whole cluster of rooted plantlets were taken out from culture tubes and equally divided into two halves in such a way that each had both shoots and roots. The divided clusters were cultured on cormel induction media. New root formation and further development of already existed roots was observed in each explant type during the 1st week of culture following cormel induction within 4-7 weeks. Induction of new shoot primordia was also observed. Explant types and PGR combinations had significant differences for cormel induction and number of cormels produced per culture (Table 3). Among explants, whole cormels (84.67%) was evaluated the best explant in terms of mean cormel induction and it was statistically similar with cormel sprouts (77.33%). MS medium supplemented with IBA + KIN (1 mg L^{-1} each) yielded higher mean cormel induction (95%). The cormel induction in nodal cultures delayed and gradually occurred within 5-7 weeks on all PGR combinations except MS medium supplemented with IBA+BAP (1 mg L^{-1} each) that did not produce cormels. Based on PGR combinations, the maximum number of cormels were recorded from each explant type on MS medium containing IBA (1 mg L^{-1}) and sucrose (7%) with mean value of 14.10. The cormels sprout (14.28) was found the best explant for mean more number of cormel production.

GRADING OF *IN VITRO* PRODUCED CORMELS

The dried stock of *in vitro* produced cormels was harvested after 3 weeks of cormel formation. Different sizes of cormels produced from different explant types and grading was made on the basis of their cormel diameter. Cormels were randomly taken from cultures of each explant and the comparison of explants showed that cormel sprouts produced more percentage of A (55%) grade cormels as compared to rest of the explants (Table 4). Nodal cultures and top segment of cormels produced similar percentage of B (40%) grade cormels. Likewise higher percentage of B grade cormels was recorded from large sized whole cormels 50%.

Table 3. Cormel production from different explant types as affected by various combinations of plant growth regulators and sucrose.

PGR combinations (mgL^{-1})	Nodal cultures	Whole cormels	Cormel sprouts	Cormel segments	Mean
	Cormel induction (%)				
MS+1IBA+3% sucrose	63.33 def	86.67 abc	53.33 efg	50.00 efg	63.33 C
MS+1IBA+5% sucrose	70.00 cde	96.67 ab	86.67 abc	76.67 bcd	82.50 B
MS+1IBA+7% sucrose	76.67 bcd	100.00 a	100.00 a	76.67 bcd	88.33AB
MS+1IBA+1BAP	0.00 h	40.00 g	46.67 fg	43.33 fg	32.50 D
MS+1IBA+1KIN	90.00 abc	100.00 a	100.00 a	90.00 abc	95.00 A
Mean	60.00 B	84.67 A	77.33 A	67.33 B	
		Number of cormels per culture			
MS+1IBA+3% sucrose	5.20 ij	6.00 ghij	9.93 cdefgh	4.80 ij	6.48 C
MS+1IBA+5% sucrose	5.93 hij	9.73 cdefgh	13.27 bc	9.20 defgh	9.53 B
MS+1IBA+7% sucrose	8.13 efghi	12.40 bcd	22.47 a	13.40 bc	14.10 A
MS+1IBA+1BAP	0.00 k	4.00 j	10.00 cdefg	7.73 efghij	5.43 C
MS+1IBA+1KIN	7.00 fghij	11.60 cde	15.73 b	10.27 cdef	11.15 B
Mean	5.25 C	8.75 B	14.28 A	9.08 B	

MS- Murashige and Skoog (1962); IBA – Indole butyric acid; BAP – Benzyl aminopurine, KIN-Kinetin

Table 4. Percentage of various cormel sizes produced from different explant types.

Grade	Nodal cultures	Whole cormels	Cormel sprouts	Cormel segments
A	25	40	55	30
B	40	50	30	40
C	35	10	15	30

A= 2.8-3.2 mm, B = 2.1-2.6 mm, C = 0.8-1.2 mm

DISCUSSION

In vitro propagation techniques by using different explant types and media supplemented with various growth regulators were explored in order to develop an efficient protocol for mass production of cormels in *White Friendship*. The cormels are storage organs and can be stored and planted easily as seed in the soil (Ziv and Lillien-Kipinis, 1990; Estrada *et al*., 1986) as compared to *in vitro* propagated plantlets that cause survival difficulties during acclimatization (Ziv, 1979; Steinitz and Yahel, 1982; Sengupta *et al*., 1984). The cormels are also easier to handle and reduce labour cost (Slabbert and Niederwieser, 1999).

Generally multiplication is achieved through excessive shoot proliferation and transfer of rooted plantlets to the soils. However, in gladiolus delivery can be made by producing cormels *in vitro*. In both the cases, shoot regeneration is the basic and major step for subsequent regeneration. In the present study, a number of explant types and each at different stages/sizes were evaluated for efficient shoot regeneration and selected the best one from each explant types for further regeneration and production of cormels. The past work reported on shoot regeneration only from uniform stage/size of any explant such as nodal buds (Grewal *et al*., 1995; Ahmad *et al*., 2000), whole cormels (Kumar *et al*., 1999; Aftab *et al*., 2008) and shoot tip of cormel (Goo *et al*., 2003). Only few reports were recorded on the use of cormel segments viz. top, middle and bottom slice of cormel (Babu and Chawla, 2000) in cultivar *American Beauty*. Various explant types from individual plant behave differently even on same nutrient medium as each explant have different totipotency. In the same way different stages/sizes of any explant type have also different regenerative capacity. In present study, heading stage of flower spike showed more potential for efficient shoot regeneration as compared to other stages of nodal cultures. Ahmad *et al*. (2000) got more number of multiple shoots from nodal buds (sleeping stage) on MS medium containing 9 µM KIN in *White Prosperity*. The nodal cultures took at 3 buds opened stage didn't exhibit any response as most of this stage cultures were of hardy. The white milky streaks present on inner phloem rather than succulent (watery) as produced from heading stage. This hardy nature of the nodal cultures might be the maturity condition of the flower spike that hindered the regeneration. Pierik (1987) stated that parts from juvenile plants regenerate more readily than parts from adult plants. This confirms the point that explant maturity had major role for efficient shoot regeneration and regenerative capacity vary in mature and juvenile tissues. In contrast to nodal cultures, all sizes of whole cormels exhibited better sprouting of shoots. These results are in accordance with the results of Kumar *et al*. (1999) who observed 100% sprouting in intact cormels of *Her Majesty, Aldebaran* and *Bright Eye* even on basal MS medium. This is because whole cormels basically are storage tissue and storage organs (bulbs, tubers, corms) have reserve food for readily regeneration. So in this sense sprouting for shoot regeneration is much dependent on storage tissue of the cormel rather than addition of cytokinin. However, number of shoots greatly increased with the addition of cytokinins. Aftab *et al*. (2008) reported more number of shoots (16) per culture vessel from cormels on MS supplemented with BAP (1 mg L^{-1}). However, this report did not mention the variety and size of cormels used for shoot regeneration; hence it is difficult to justify the results. Priyakumari and Sheela (2005) recorded the highest number of shoots (4) from axillary buds on MS medium supplemented with BAP+NAA (4+0.5 mg L^{-1}) in *Gladiolus grandiflorus* cv. Peach Blossom.

No report found on the use of cormel sprouts produced through apolar inoculation of whole cormels. The shoot regeneration from cormel sprouts proved very applicable method for efficient regeneration of multiple shoots. The cormels sprouted downward into media become swelled and of light green color. This may be due to chlorophyll deficiency due to submerged condition of explant and better regeneration of shoots may be result of an improved oxygen supply along with other factors. Accumulation of endogenous hormones might also have part in this regard. Orlikowska *et al*. (2000) observed more number of shoots from shoots inoculated vertically or horizontally in an inverted position with shoot tip of down. Peirik and Steegmans (1975) reported that regeneration of roots and shoots generated more easily and more rapidly with apolar inoculation. The present results indicate that cormel sprout of small size was not better source for shoot regeneration as the smallest sprout had poor regenerative capacity as compared to medium and large sized sprout of the cormel. Accumulation of endogenous hormones might also have part in this regard.

Top segment of cormel had better potential for efficient shoot regeneration as compared to bottom segment of cormel on MS supplemented with BAP (4 mg L^{-1}). Babu and Chawla (2000) also recorded better shoot induction (89%) from top slice of cormel with an average of 2.4 shoots per explant in response to MS medium supplemented with KIN (18.6 µM). Top

segment section of cormel induced early shoot induction (within 2-3 days) as compared to bottom section of cormel (35-42 days). The physiological base of the top segment of the cormel was placed on medium and had more absorption area for nutrient uptake. This could be a reason due to which the cultures showed more and healthy shoots. The bottom section of cormels showed mortality where the physiological base of the bottom section was on the nutrient medium and the cut surface on upper side. The large cut surface might be the reason of death of explants due to oxidative stress (Halliwell and Gutteridge, 1996) as there might be the chance to produce free radicals that cause activation of peroxidases, catalase and SOD (Lehsem, 1988; Olmos *et al*., 1994). Emek and Erdag (2007) reported also no regeneration from transverse slices of cormel.

Efficient methods for developing roots are equally important for better cormel formation. It was observed that all of the explants produced roots and IBA was considered as an important auxin for root formation. In the present study, more root induction was observed from multiple number of PGR combinations. However, more number of roots per culture was greatly affected by increasing levels of IBA (2 mg L^{-1}) and sucrose (3%). Priyakumari and Sheela (2005) produced the earliest and longest roots on IBA (2 mg L^{-1}), whereas the highest number of roots (24) was recorded in "*Peach blossom"* on MS medium containing NAA (1 mg L^{-1}). Sinha and Roy (2002) observed high rooting (100%) in shoots produced from the callus of cormel sprouts by using IBA (2 mg L^{-1}) with sucrose (6%). Same results were also reported by Aftab *et al.* (2008). Kumar *et al*. (1999) recorded no or very poor response for root initiation on MS medium containing IBA or NAA. On the other side they reported that sucrose concentration had positive effect on the rooting response and quality of roots formed in "*Her Majesty*" and "*Aldebaran*" varieties. However, they could not find results for root initiation from same sucrose concentration in "Bright Eye". Rooting was also reported on low levels of IBA as reported by Begum and Haddiuzaman (1995) obtained rooting on 0.5 mg L^{-1}. Hussain *et al*. (1994) produced extensive root growth from *in vitro* shoots in variety *White Friendship* in response to MS medium supplemented with IBA (2 mg L^{-1}).

The cormel induction and number of cormels was greatly affected by increasing levels of sucrose (Dantu and Bhojwani, 1987; Mares *et al*., 1985) which is considered to be stored as starch in the storage tissue of the bulbous plants (Van Aartrijk and Blom-Barnhoorn, 1980). Higher sucrose concentrations have been reported for bulblet formation in many bulbous plants such as tulip (Rice *et al*., 1983; Taeb and Al-derson, 1990), narcissus (Squires *et al*., 1991), hyacinth (Bach, 1992), *Lachenalia* (Slabbert and Niederwieser,1999). In gladiolus cormel formation on higher levels of sucrose was reported by Roy *et al*. (2006) in "*Pacifica*"; Sinha and Roy (2002) in *Golden Wave*; Kumar *et al*. (1999) in "*Her Majesty*", "*Aldebaran*", and "*Bright Eye*" and Steinitz *et al*. (1991) in "*Kinneret*". Sinha and Roy (2002) produced more number of cormels (16.5 per shoot) of different sizes in "*Golden Wave*" on half strength MS medium supplemented with IBA (2 mg L^{-1}) and 6% sucrose. In this study more number of cormels of different sizes were recorded on MS medium supplemented with IBA (1 mg L^{-1}) and sucrose (7%) followed by KIN medium. Cytokinins are also effective in production in *Gladiolus grandiflorus* (Ginzburg and Ziv, 1973; Hussey, 1977). Ginzburg and Ziv (1973) reported that KIN promoted cromel formation on excised stolon tips. In the present project the use of KIN (1 mg L^{-1}) for cormel formation was also found successful instead of using BAP. Paclobutrazol (a growth retardant) with sucrose is also reported to promote cormel formation in gladiolus (Nagaraju *et al*., 2002; Steinitz *et al*., 1991).

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