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CORMEL FORMATION IN GLADIOLUS THROUGH TISSUE CULTURE

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An efficient tissue culture protocol was developed to regenerate the cormels of commercially grown varieties of Gladiolus. Callus was initiated in the cultures after three to five weeks from middle and bottom slices of the cormels in *White Friendship* and *Peter pears*. The maximum mean callus induction of 72.50% was observed on MS medium supplemented with NAA at 4 mg L^{-1} while best proliferation (0.80 g) was recorded on basal MS medium supplemented with NAA at 0.5 mg L^{-1}. On the basis of cormel slices, best results were observed from bottom slice of the cormel producing 0.506 g of fresh callus growth in variety *White Friendhsip*. The efficient shoot regeneration from proliferated calli was observed increased in the presence of BAP from 2 to 4 mg L^{-1} and exhibited the highest mean percentage of shoot regeneration (92.92%) and number of shoots (18.25). Middle slice of the cormel had more potential for mean shoot induction (70.95%) while more number of shoots (12.98) was observed from bottom slice of the cormel in variety *Peter Pears*. Root formation was reduced on 7% sucrose as compared to 3-5 % sucrose supplemented with IBA. The *in vitro* propagules initiated the development of cormels producing the highest mean number of cormels (12.06) on MS medium supplemented with 5% sucrose plus IBA (1 mg L^{-1}). Middle and Bottom slice of the cormels exhibited statistically similar and more potential results for cormel induction and number of cormels.

Keywords: Gladiolus, cormel, callogenesis, *in vitro*, cut flower, micropropagation

INTRODUCTION

Gladiolus is one of the potential cut flower grown throughout the world for its attractive spike of different hues. Seed is not used for its commercial cultivation but seed propagation is only used to evolve new and improved varieties by plant breeding technology (Singh, 1992). Presently a wide range of genetic variation in Gladiolus is the result of hybridization. At commercial level, gladiolus is generally propagated by corms and cormels (Bose *et al*., 2003; Hartman *et al*., 1990; Ziv and Lilien-Kipnis, 1990). One of the major constrains in commercial cultivation is non-availability of a large quantity of propagules (Singh and Dohare, 1994). Being a cormous plant, it is principally propagated by the natural multiplication of new corms and cormels (Hartman *et al*., 1990; Ziv and Lilien-kipnis, 1990). However, owing to their low rate of multiplication and to a high percentage of spoilage of corms during storage, there is an insufficient supply of planting material (Singh and Dohare 1994). A mother corm normally produces one new daughter corm each season along with several cormels. These cormels require three to four seasons to attain sufficient size for flowering. Physiological dormancy of the corms and cormels, which usually lasts for 4–5 months and the incidence corm rot during storage are other problems (Priyakumari and Sheela, 2005; Memon *et al*., 2009). Mass propagation of gladiolus is also severely damaged by soil-borne fungi. Owing to the unavailability of corms in sufficient quantity, corm cost is also increased. For commercial cultivation, conventional methods of propagation are insufficient to meet the demand for planting material (Sajjad *et al*., 2014).

In vitro techniques are applicable for the propagation of corm producing species. This is an alternative to the conventional methods for vegetative propagation that attracts much attention. 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).

A number of protocols have been developed for *in vitro* regeneration of plantlets in gladiolus (Ziv *et al*., 1970; Lilien-Kipnis and Kochba, 1987, Sinha and Roy, 2002; Prasad and Gupta, 2006; Emek and Erdag, 2007; Memon *et al*., 2012, 2014). However, literature is rather scanty on *in vitro* cormel formation. The literature on *in vitro* cormel formation was reported in very few varieties of gladiolus such as *Bright Eye* and *Her Majesty* (Kumar *et al*., 2011) *White flowered variety Pacificia* (Roy *et al*., 2006), *Balady* (Al-juboory *et al*., 1997), *Golden wave* (Sinha and Roy, 2002), *Kinneret* (Steinitz *et al.,* 1991), *Friendship* (Dantu and Bhojwani, 1995) and *Green Bay* (Sen and Sen, 1995). Although Memon *et al*. (2010) reported cormel formation in three varieties of gladiolus through direct mode of

regeneration. Present project was mainly designed to establish a protocol for *in vitro* cormel formation using different slices of the cormels of three varieties of gladiolus. Cormel slices were cultured on MS (Murashige and Skoog, 1962) media contained different plant growth regulators (PGR).

MATERIAL AND METHODS

Cormels of three commercial grown varieties viz. *Traderhorn*, *White Friendship* and *Peter Pears* were obtained after harvesting of gladiolus corms and cormels during the year 2006. Cormels of 0.6 g in weight were dehusked with surgical blade followed by soaking in tap water for 30 minutes to remove any sticky material present on the cormels. Later, cormels were washed 3-4 times with distilled water. Then cormels were treated with 70% ethanol for 15 minutes and after that 3-4 minutes in 5% sodium hypochlorite, and 1 minute in 1% $HgCl_2$ followed by 5-6 times rinsed in sterile distilled water in a laminar airflow cabinet. The transverse cormel slices viz. top, medium and bottom approximately 1-2 mm thick were used as explant for callus initiation. Each cormel slice was placed with a cut surface in contact with callus initiation medium. The concentrations of callus initiation medium are mentioned in Table 1. The variety *Traderhorn* did not exhibit any response for callus initiation from any cormel slice and is not presented in data tables. Callus initiation was observed from middle and bottom slice of cormel in *White Friendship* and *Peter Pears* after 3 weeks of culture. Some cultures initiated callus after 5 weeks of culture.

The initiated calli of two months old were aseptically removed from the glass tubes on sterilized Petri dishes inside the laminar air flow cabinet and divided into chunks of approximately equal size and re-cultured on basal MS medium having different but low concentrations of growth hormones for further proliferation of callus (Table 1). The contamination was observed among callus subcultures when calli was measured for taking chunks of equal and accurate size. That's why study was made on approximation. After proliferation, fresh growth of callus observed on the medium containing different combinations of Plant growth regulator (PGR). Five random samples of calli were taken and weighed individually and then mean value was recorded for data interpretation. The fresh calli (one month old after proliferation) was subdivided into equal chunks and cultured on shoot regeneration media (Table 1).

The initiation of buds from calli was observed within 9-12 days. The cluster of shoots about 4-5 weeks old containing 3-4 shoots with 2-3 cm height were separated from the base and cultured on half strength MS basal medium supplemented with different levels of IBA sucrose. The induction of roots was observed within 2-3 weeks of culture. After 5 weeks of root induction, rooted plantlets having multiple numbers 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. This was done as in some culture the roots were in more spreading way and they might cause contamination during the insertion of the explant in test tubes. The media used for rooting and cormel induction is presented in Table 1.

Table 1. Growing media used at different concentrations for callus initiation and organogenesis

Media for callus initiation
MS medium (without PGR)
MS medium + BAP 0.5 mg L^{-1}
MS medium + KIN 0.5 mg L^{-1}
MS medium + 2,4-D 0.5 mg L^{-1}
MS medium + NAA 0.5 mg L^{-1}
Media for callus proliferation
MS medium (without PGR)
MS medium + BAP 0.5 mg L^{-1}
MS medium + KIN 0.5 mg L^{-1}
MS medium + 2,4-D 0.5 mg L^{-1}
MS medium + NAA 0.5 mg L^{-1}
Shoot induction and proliferation media
MS medium (without PGR)
MS medium + BAP 2 mg L^{-1}
MS medium + BAP 4 mg L^{-1}
MS medium + Kinetin 2 mg L^{-1}
MS medium + Kinetin 4 mg L^{-1}
MS medium + BAP 2 mg L^{-1} + Kinetin 2 mg L^{-1}
MS medium + BAP 4 mg L^{-1} + Kinetin 4 mg L^{-1}
MS medium + BAP 2 mg L^{-1} + Kinetin 2 mg L^{-1} + NAA 0.5 mg L^{-1}
Media for root induction and regeneration
Basal MS medium (without PGR)
Half strength MS medium + IBA 1mg L^{-1} + sucrose 3%
Half strength MS medium + IBA 1mg L^{-1} + sucrose 5%
Half strength MS medium + IBA 1mg L^{-1} + sucrose 7%
Half strength MS medium + IBA 2mg L^{-1} + sucrose 3%
Half strength MS medium + IBA 2mg L^{-1} + sucrose 5%
Half strength MS medium + IBA 2mg L^{-1} + sucrose 7%
Media for induction of cormels
MS medium + IBA 1 mg L^{-1} + sucrose 3%
MS medium + IBA 1 mg L^{-1} + sucrose 5%
MS medium + IBA 1 mg L^{-1} + sucrose 7%
MS medium + IBA 1 mg L^{-1} + BAP 1 mg L^{-1}
MS medium + IBA 1 mg L^{-1} + KIN 1 mg L^{-1}

The culture conditions including temperature, light, sterilization and humidity was properly maintained. The laminar air flow cabinet of the culture room was sterilized with 95% ethanol followed by sterilization of the whole room by exposing it to the ultra violet radiations for 30 minutes. The medium was placed in culture room before

ultra violet radiation light was on. The laminar air flow cabinet was again sterilized with 95% ethanol. The forceps, blades and glass wares used in the culture room were autoclaved. All the experiments were maintained on solidified basal medium. For solidification of media 0.8% agar was used. The pH of each medium with every concentration was adjusted separately to 5.7 with 0.1N HCl or 0.1N NaOH prior to addition of agar. The agar mixed in distilled water and kept on heat for proper jelling of the medium. When it boiled properly, mixed in medium. The medium was immediately poured in glass tubes of 18 x 150 mm up to 10 ml in each tube. All tubes were placed in racks and covered with transparent polyethylene papers to avoid contamination. Medium was also autoclaved at 121°C at a 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, humidity at 85% and either under continuous a photoperiod of 16 h light and 8 h dark. The light intensity was fixed at 2500 lux by using white fluorescent tubes in the growth room. Each experimental set consisted of three replicates. Means were calculated by taking an average of 3 replicates.

RESULTS

The observed results for callus initiation and organogenesis is presented in the present segment and step wise exhibited as Fig. 1. Callus was initiated after three weeks of cultures in two varieties of gladiolus viz. *White Friendship* and *Peter Pears*. This was observed in the cultures where explant was taken from middle and bottom parts of cormels. The variety *Traderhorn* didn't exhibit any response for callus initiation that's why data is only presented for two responsive varieties. The highest mean callus induction of 72.50% was observed on MS medium supplemented with NAA at 4 mg L^{-1} (Table 2). Callus initiation was also observed from MS medium supplemented with the lowest levels of NAA i.e. 2 mg L^{-1}. No response for callus initiation was observed when 2,4-D was used at the concentration of 2 to 4 mg L^{-1}. However the lowest concentration of 2,4-D (2 mg L^{-1}) along with BAP (1 mg L^{-1}) exhibited response for mean callus initiation (58.33%).

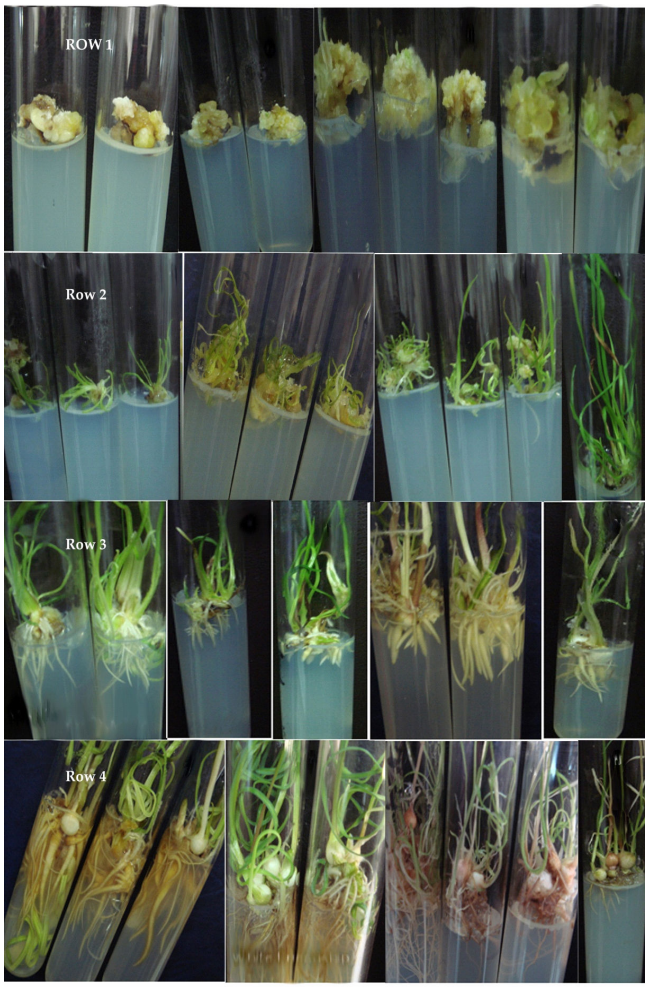

Figure 1. Cormel induction from cormel slices via callogenesis. 1st row- callus induction from cormel slices; 2nd row- shoot induction from calli cultures, 3rd row- root induction from regenerated shoots, 4th row- cormel induction from the vitro propagules.

Table 2. Initiation of callus (%) from middle and bottom slice of the cormels.

MS + different concentrations of PGR (mg L^{-1})	White Friendship		Peter Pears		Mean
	Middle slice	Bottom slice	Middle slice	Bottom slice	
	Callus induction (%)				
MS (control)	--	--	--	--	--
MS+2 NAA	61.67	78.33	55.00	68.33	65.83 B
MS+4 NAA	75.00	70.00	73.33	71.67	72.50 A
MS+2 2,4-D	--	--	--	--	--
MS+4 2,4-D	--	--	--	--	--
MS+2 2,4-D + 1 BAP	68.33	50.00	58.33	56.67	58.33 C
Mean	28.44	28.56	25.00	29.67	

Calli chunks of approximately equal size from the best PGR combinations (4 and 2 mg L^{-1} NAA) were sub-cultured on callus proliferation media presented in Table 1. Callus was proliferated on most of the PGR combinations; however, a wide range of variation occurred on the basis of fresh weight and texture of calli. Significant differences were recorded for fresh callus growth among varieties, PGR combinations and their interactions (Table 3). Table 3 indicates that most of the PGR combinations produced more than 0.5 g of mean fresh callus weight from cormel slices except MS medium supplemented with KIN (0.5 mg L^{-1}) which produced 0.430 g callus which was embryogenic in texture. These results are followed by the results of 0.08 g callus obtained from medium had NAA at 0.5 mg $L^{-1.}$ On the basis of cormel slices, bottom slice produced the highest mean fresh callus growth of 0.506 g in *White Friendship.*

The fresh calli was subdivided into equal clumps and cultured on shoot regeneration media. Green shoot buds were induced on calli and time for bud initiation ranged from 9-12 days from cormel slices (Fig. 1). It is evident from the analysis of variance that both varieties and PGR combinations had independently and interactively significant differences for shoot induction and number of shoots per culture (Table 4). The mean shoot induction and number of shoots from callus of cormel slices increased as the concentration of BAP increased from 2 to 4 mg L^{-1} and exhibited the highest mean percentage of shoot regeneration (92.92%) and number of shoots (18.25). On the basis of cormel slices and varieties, the highest shoot regeneration (70.95%) was observed from bottom slice of cormel in *White Friendship* followed by the results (66.91%) obtained from bottom slice (Table 4). However more mean number of shoots was observed from middle slice in both *White Friendship* (12.49) and *Peter Pears* (12.98).

Cluster of shoots containing 3-4 shoots with 2-3 cm height were separated from the base and cultured on MS basal

Table 3. Fresh growth of the callus obtained from middle and bottom slice of the cormels.

MS + different concentrations of PGR (mg L^{-1})	White Friendship		Peter Pears		Mean	Callus texture
	Middle slice	Bottom slice	Middle slice	Bottom slice		
	Fresh callus growth (g)					
MS (control)	0.539 def	0.801 a	0.686 bc	0.708 ab	0.683 A	Compact
MS+0.5 BAP	0.649 bcd	0.622bcde	0.580 cde	0.519 efg	0.593 B	Compact
MS+0.5 KIN	0.401 hi	0.510 fgh	0.419 ghi	0.391 i	0.430 C	Embryogenic
MS+0.5 2,4-D	0.703 ab	0.519 efg	0.443 fghi	0.571 cde	0.559 B	Friable
MS+0.5 NAA	0.077 j	0.079 j	0.119 j	0.044 j	0.080 D	Hairy and cottony
Mean	0.474 AB	0.506 A	0.450 B	0.447 B		

Table 4. Shoot regeneration (%) and number of shoots produced from calli of cormel slices

MS + different concentrations of PGR (mg L^{-1})	White Friendship		Peter Pears		Mean
	Middle slice	Bottom slice	Middle slice	Bottom slice	
	Shoot induction (%)				
MS (control)	-	-	-	-	
MS + 2BAP	100.00 a	80.00 cd	78.33 d	81.67 cd	85.00 B
MS + 4BAP	100.00 a	90.00 abc	81.67 cd	100.00 a	92.92 A
MS + 2KIN	15.00 i	25.00 i	0.00 j	0.00 j	10.00 F
MS + 4KIN	51.67 h	61.67 gh	0.00 j	0.00 j	28.33 E
MS + 2BAP+2KIN	90.00 abc	75.00 de	75.00 de	85.00 bcd	81.25 BC
MS+ 4BAP + 4KIN	66.67 efg	61.67 gh	81.67 cd	63.33 fg	68.33 D
MS+2BAP + 2KIN+ 0.5 NAA	73.33 def	75.00 de	73.33 def	93.33 ab	78.75 C
Mean	70.95 A	66.91 B	55.71 D	60.48 C	
	Number of shoots/culture				
MS (cont.)	-	-	-	-	
MS + 2BAP	10.13 ijk	17.13 b-e	17.53 bcde	18.60 bc	15.85 B
MS + 4BAP	19.13 b	18.07 bcd	13.40 fghi	22.40 a	18.25 A
MS + 2KIN	2.73 m	2.73 m	0.00 m	0.00 m	1.37 F
MS + 4KIN	6.47 l	9.80 ijkl	0.00 m	0.00 m	4.07 E
MS + 2BAP+2KIN	10.53 ij	11.87 g-j	10.67 hij	16.40 b-f	12.37 C
MS+ 4BAP + 4KIN	14.13 efgh	15.07 c-g	15.07 cdefg	18.93 b	15.80 B
MS+2BAP + 2KIN+ 0.5 NAA	6.87 kl	12.73 ghi	8.33 jkl	14.53 d-g	10.62 D
Mean	10.00 B	12.49 A	9.29 B	12.98 A	

medium supplemented with different levels of IBA (1 and 2 mg L^{-1}) and sucrose (3%, 5% and 7%). No root growth was observed on control media (without PGR). New shoot growth was also observed on all PGR combinations.

Sucrose treatments exhibited significant effect for root induction and the number of roots from shoots of cormel slices in *White Friendship* and *Peter Pears* (Table 5). The treatments receiving 3% sucrose were the slowest and took approximately more than 3 weeks for root induction but produced the highest mean root induction (99.17%) and the number of roots (15.93). Increasing the level of sucrose to 7% hastened root induction by 5-7 days but decreased percentage of root induction (59.17%) and the number of roots (10.03). Explant either middle or bottom from both the varieties did not produce any significant influence on any parameter except the number of roots in *Peter Pears* which were significantly higher for middle slice (14.31) using IBA at 2 mg L^{-1} (Table 5). These results are at par with the results (14.22) obtained from the lowest levels of IBA (1 mg L^{-1}).

There was no significant effect of explant on cormel induction and number of cormels produced per culture (Table 6). However, the interaction of varieties, cormel slices and PGR combination exhibited highly significant differences only for number of cormels. Varieties had highly significant differences for cormel induction and number of regenerated cormels. Table 5 exhibited the highest mean cormel induction (62.33%) in *White Friendship* followed by 52% in *Peter Pears.* MS medium supplemented with IBA (1 mg L^{-1}) with sucrose (5%) showed more cormel induction of 75.00% and number of cormels (12.06). The maximum mean cormels (7.80) produced in *White Friendship* as compared to *Peter Pears* (6.80). The bottom slice of cormel depicted more number of cormels (7.99) as compared to middle slice of cormel (7.60) in *White Friendship.* The reversed was true for *Peter Pears* (Table 6). The maximum number of cormels (13.93) produced from middle slice of cormel on MS medium supplemented with IBA (1 mg L^{-1}) with sucrose (5%) in *Peter Pears*.

Table 5. Root induction (%) and number of roots regenerated from callus cultures of cormel slices

Sucrose (%)	White Friendship				Mean	Peter Pears				Mean
	Middle slice		Bottom slice			Middle slice		Bottom slice		
	Root induction (%) IBA (mg L^{-1})									
	1	2	1	2		1	2	1	2	
3	100.00	100.00	100.00	100.00	100.00 A	100.00	100.00	100.00	96.67	99.17 A
5	83.33	73.33	83.33	83.33	80.83 B	86.67	86.67	73.33	83.33	82.50 B
7	63.33	66.67	80.00	76.67	71.67 B	60.00	53.33	56.67	66.67	59.17 C
Mean	82.22	80.00	87.78	86.67		82.22	80.00	76.67	82.22	
	Number of roots/culture									
3	12.40 cde	21.20 a	16.40 a-d	17.27 abc	16.82 A	18.00 a	15.53 ab	12.60 bcd	17.60 a	15.93 A
5	18.00 ab	12.60 cde	17.20 abc	14.93 bcd	15.68 A	13.40 bc	16.00 ab	10.60 cde	15.93 ab	13.98 B
7	8.60 e	11.53 de	8.67 e	13.00 b-e	10.45 B	11.27 cde	11.40 cde	9.33 de	8.13 e	10.03 C
Mean	13.00	15.11	14.09	15.07		14.22 A	14.31 A	10.84 B	13.89 A	

Table 6. Cormel induction and number of cormels produced from callus cultures of cormel slices.

MS + different concentrations of PGR (mg L^{-1}) + sucrose	White Friendship		Peter Pears		Mean
	Middle slice	Bottom slice	Middle slice	Bottom slice	
	Cormel induction (%)				
MS + 1 IBA + 3% sucrose	66.67	46.67	36.67	30.00	45.00 B
MS + 1 IBA + 5 % sucrose	90.00	73.33	60.00	76.67	75.00 A
MS + 1 IBA + 7 % sucrose	76.67	70.00	73.33	60.00	70.00 A
MS + 1 IBA + 1 BAP	20.00	30.00	0.00	36.67	21.67 C
MS + 1 IBA + 1 KIN	80.00	70.00	76.67	70.00	74.17 A
Mean	66.67 A	58.00 AB	49.33 B	54.67 B	
	Number of cormels/culture				
MS + 1 IBA + 3 % sucrose	5.53 gh	5.67 gh	2.13 ij	3.20 hi	4.13 C
MS + 1 IBA + 5 % sucrose	8.47 ef	13.30 a	13.93 a	12.53 ab	12.06 A
MS + 1 IBA + 7 % sucrose	9.33 c-f	8.80 ef	11.80 abc	6.87 fg	9.20 B
MS + 1 IBA + 1 BAP	3.07 i	2.13 ij	0.00 j	2.80 i	2.00 D
MS + 1 IBA + 1 KIN	11.60 a-d	10.07 b-e	9.20 def	5.60 gh	9.12 B
Mean	7.60 A	7.99 A	7.41 A	6.20 B	

DISCUSSION

Generally 2,4-D and NAA are considered best auxins for callus initiation (Boonvanno and Kanchanpoom, 2000). Our results confirmed that the presence of NAA in the medium is must for callus induction and produced regenerable callus from cormel slices. Our results are in harmony with Aftab *et al*. (2008) who reported callus initiation in cormel slices on MS medium supplemented with NAA (3 or 4 mg L^{-1}). Kamo (1994) reported a greater number of regenerants from the callus cultured on medium having NAA than from callus cultured on medium containing 2,4-D. On the basis of cormel slices, top slice of cormel couldn't initiate callus in any variety. Initiation of shoot tip was observed from top slice of the cormel instead of callus induction. This might be due to presence of growing point (meristem) within the top slice of cormel which sprouted into shoot tip. Besides, physiological base of the top slice of the cormel had more absorption area for nutrient uptake as compared to middle and bottom slices. In variety *Traderhorn,* middle and bottom slices of the cormel turned brown and caused death of the plants. This might be due to release of phenolic compounds in *Traderhorn* as compared to *White Friendship* and *Peter Pears.* Emek and Erdag (2007) reported no callus induction on any PGR combination from any transverse cormel slices due to the death of the explants. 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). In contrast, callus formation was recorded from longitudinal sections of cormels at higher levels of NAA (8.5 mg L^{-1}) as reported by Emek and Erdag (2007). The results of the present study indicate that middle and bottom slice of cormel had statistically similar potential for callus induction in *White Friendship* and *Peter Pears.* Remotti and Loffler (1995) observed more potential for callus regeneration from central slice of cormel compared to apical and basal slices in variety "*Peter Pears*".

The auxin 2,4-D induced callus in cormel slices when used along with BAP (0.5 mg L^{-1}). Aftab*et al*. (2008) also recorded callus initiation from cormel slices on MS medium having BAP (1 mg L^{-1}) in combination with 2,4-D (2 mg L^{-1}). Kumar *et al*. (1999) reported callus induction in cormel segments on MS medium supplemented with BAP (1.1 mg L^{-1}) and 2,4-D (1.1 to 2.2 mg L^{-1}) in *Her Majesty* and *Aldebaran*. The callus induction with 2,4-D alone is reported by Torabi-Giglou and Hajieghrari (2008) who examined callus in cormel segments of variety *Gladiolus grandiflorus* cv. "*Pink*" on MS medium supplemented with 2,4-D (1 mg L^{-1}) and sucrose (3%). Remotti and Loffler (1995) observed callus formation in cormel slices of *Peter Pears* by using 2,4-D (9 mM). Sinha and Roy (2002) produced high frequency callus from transverse slices of cormel sprouts *of Gladiolus primulinus* cv. Golden Wave in response to NAA (4 mg L^{-1}), BAP (2.0 mg L^{-1}) or 2,4-D (1 mg L^{-1}).

Best calli produced on MS medium supplemented with NAA was used for regeneration of shoots. Shoot regeneration was observed from each PGR combination except control (MS medium). Control exhibited high frequency of callus proliferation but shoots were not regenerated. This might be due to the absence of cytokinins (BAP or KIN) in the medium as the addition of cytokinins in the medium encourages bud/shoot regeneration as reported by Sinha and Roy (2002) Torabi-Giglou and Hajieghrari (2008) and Pragya *et al*. (2012). Budiarto (2009) recommended BA concentrations of 2–3 mg L^{-1} for potential shoot induction in gladiolus. In the present study, maximum number of shoots were observed on medium containing BAP (4 mg L^{-1}). Our results are in agreement with the results reported by Sinha and Roy (2002). They used different concentrations of BAP or KIN alone or in combination and observed the highest number of shoots (28) from slices of cormel sprouts in variety *"Golden Wave"* on MS medium supplemented with BAP (2 mg L^{-1}). Similar results are reported by Dantu and Bhojwani (1987) in variety "*Her Majesty*". They reported BA as effective cytokinin for shoot multiplication. Boonvanno and Kanchanapoom (2000) observed multiple shoots from callus initiated from axillary buds on MS medium containing 1 mg L^{-1} BA. Shaheenuzzaman *et al*. (2011) recorded the highest percentage of shoot regeneration (91.66%) on MS basal medium supplemented with BAP (3 mg L^{-1}) and Kinetin (0.5 mg L^{-1}.

In the present study root initiation was reduced on 7% sucrose as compared to 3-5 % sucrose in the media. However, IBA levels (1 or 2 mg L^{-1}) did not differ regarding root initiation in both the varieties. Begum and Haddiuzaman (1995) obtained rooting even on low levels of IBA (0.5 mg L^{-1}). 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 (0.348M) had positive effect on the rooting response and quality of roots formed in "*Her Majesty*" and "*Aldebaran*" varieties. However they did not obtain root initiation from same sucrose concentration in "Bright Eye".

Various reports states that cormel formation, size and number of cormels produced under *in vitro* conditions is mostly dependent on increasing levels of sucrose in the tissue culture media as reported by various workers. In the present study, number of cormels increased with increasing levels of sucrose (3 to 5%) having IBA 1 mg L^{-1}. Sinha and Roy (2002) obtained maximum cormels from rooted shoots on medium having sucrose 6% and IBA 2 mg L^{-1} in *Gladiolus primulinus*. Kumar *et al.* (1999) obtained maximum cormel formation on high sucrose levels ranging from 6 to 12%. Kumar (2011) reported higher number of cormlets with increased concentration of sucrose up to 232

mM. Dantu and Bhojwani (1995) showed that the percentage of cormel formation increased by using 6% sucrose in the medium but further increase in sucrose concentration decreased the percentage of cormel formation. Similar findings were reported by Azadi and Khosh-khui (2007) in lily.

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