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COMPARISON OF VARIOUS EXPLANTS ON THE BASIS OF EFFICIENT SHOOT REGENERATION IN GLADIOLUS

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Abstract

Different stages/sizes of the same explants have different regenerative capacity even cultured on the same nutrient medium. In present study different stages/sizes of various explants including nodal cultures from different stages of flower spike, whole flower buds only at sleeping stage, whole cormels of different sizes, cormel sprouts of different sizes and cormel slices of the cormel were explored and optimized for efficient shoot regeneration in three commercial grown varieties of the gladiolus. Different plant growth regulators including benzyl aminopurine (BAP), kinetin (KIN) and naphthalene acetic acid (NAA) alone or in combination with each other were used for each explant in order to explore the possibility of increasing rate of shoot induction and regeneration. Out of five explants, cormel sprouts of medium size (12 days old) was evaluated the best explant in terms of mean shoot induction (77.50%) and number of shoots (11.60) in *White friendship.* On the basis of interaction of varieties and plant growth regulators, the highest results for shoot induction (98.33%) and number of shoots (22.07) were observed from same cormel sprout on MS medium containing BAP 4 mg L^{-1}. The heading stage of nodal cultures (7.67), medium size of cormels and cormel sprouts (11.60) each and top slice of cormels (3.65) were considered the best stages/sizes from each explant for efficient number of shoots.

Introduction

Different explants from individual plants behave differently even on the same nutrient medium as each explant has a different totipotency. In the same way different growth stages/sizes have different regenerative capacity for each explant. Plant parts taken from juvenile plants regenerate more readily than parts from adult plants. In the same way plant parts containing a large amount of food reserves such as tubers, bulbls, cormels etc. generally regenerate more readily *In vitro* than those containing fewer reserves. However, regeneration and multiplication from any explant under *In vitro* conditions is usually based on shoot induction and subsequent shoot regeneration. The rate of shoot induction may vary from explant to explant and other multiple factors including media, culture conditions etc. Efficient multiple shoot regeneration has a key role in subsequent steps of *In vitro* regeneration. The previous work reported regarding shoot regeneration in gladiolus only from uniform growth stage/size of each explant source viz. nodal/flower buds (Ziv, 1989; Grewal *et al*., 1995; Ahmad *et al*., 2000; Memon *et al*., 2010), whole cormels (Kumar *et al*., 1999; Aftab *et al*., 2008; Memon *et al*., 2010), shoot tip of cormel (Goo *et al*., 2003) and shoots derived from longitudinal corms (Nhut *et al*., 2004). Only cormel slices viz. top, middle and bottom were used by Babu & Chawla (2000) in cultivar *American Beauty*. In present study, a number of different explants and each at different growth stages/sizes were evaluated on the basis of efficient shoot regeneration and selected the best one from each explant for further regeneration of multiple shoots. Different plant growth regulators at different concentrations were tried for each of the explant in order to explore the possibility of increasing rate of shoot induction and regeneration.

Material and Methods

A number of laboratory experiments were conducted to develop a valuable protocol for efficient shoot regeneration by observing the response of different explants under different combinations and concentrations of plant growth regulators. However, culture conditions for regeneration were maintained uniform for each of the explant.

Corms of three commercially grown varieties of gladiolus viz. *Traderhon* (red with white throat), *White Friendship* (white) and *Peter Pears* (Peach with red throat) with uniform size of 12/14 cm were obtained from Sunny Seeds Distributor of SAKATA Seed Corporation, Japan. The corms were planted at Floriculture Research Area, University of Agriculture, Faisalabad during the year 2006-08 and *In vitro* experiments were carried out in the Plant Tissue Culture Cell, University of Agriculture Faisalabad, Pakistan. The explants were obtained at various growth stages/sizes of the flowering and cormel formation of the gladiolus. The detail of each explant material used for regeneration is presented in Table 1. The explants nodal part of inflorescence stem and flower buds were washed 2-3 times with distilled water and surface sterilized by dipping the plant material into 70% (v/v) ethanol for 6-8 minutes. Then the explants material 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 under a laminar airflow cabinet. 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 explants were then treated with 70% 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.

Table 1. Various explants used for *In vitro* shoot regeneration.

S #	Plant material	Growth stage/size of explant
1.	Nodal cultures obtained from different stages of flower spike	a. Heading stage- 1 cm b. one floret opened stage-1cm c. 3 florets opened stage-1cm
2.	Whole flower buds	d. (sleeping stage)-1.5 cm in length
3.	Whole cormels of different sizes	e. Small (0.2 g) f. Medium (0.4 g) g. Large (0.6 g)
4.	Cormel sprouts	h. Small (8 days old) i. Medium (12 days old) j. Large (16 days old)
5.	Cormel sections/slices (0.6 g)	k. Top (approx. 3-4 mm thick) l. Bottom (approx. 3-4 mm thick)

Cormel sprouts were obtained by apolar inoculation of whole cormels on MS medium supplemented with BAP 4 (mg L^{-1})

Table 2. Media used for shoot induction and multiple shoot regeneration from various explant sources.

PGR combinations (mg L^{-1})	Media
T_0	Basal MS medium (without PGR)
T_1	Basal MS medium + BAP 2 (mg L^{-1})
T_2	Basal MS medium + BAP 4 (mg L^{-1})
T_3	Basal MS medium + Kinetin 2 (mg L^{-1})
T_4	Basal MS medium + Kinetin 4 (mg L^{-1})
T_5	Basal MS medium + BAP 2 mg + Kinetin 2 (mg L^{-1})
T_6	Basal MS medium + BAP 4 mg + Kinetin 4 (mg L^{-1})
T_7	Basal MS medium +BAP 2 mg + Kinetin 2 mg + NAA 0.5 (mg L^{-1})

All the experiments were maintained on solidified basal medium. MS (Murashige & Skoog, 1962) inorganic salts, organic supplements, and vitamins were used as basal medium. The required concentrations of plant growth regulators were added in basal MS medium. The medium formulation and composition used for regeneration is presented in Table 2. For solidification of media 0.8% agar was used. The pH of each medium with every concentration was adjusted separately to 5.7 with 0.1 N HCl or 0.1 NaOH prior to addition of Agar.. Medium was 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 25-27°C, 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. The experiments were laid out in Completely Randomized Design (CRD) with factorial arrangements along with three replications. Twenty cultures were maintained in each treatment. The data were subjected to statistical analysis of variance by using Statistics software and treatment means were compared according to DMR test at 5% level of probability (Steel *et al*., 1997).

Results

Selection of better stage/size from each explants: The response for shoot induction and regeneration varied with the type of the explant, combination and the concentration of PGR and varietal differences. Control cultures (without PGR) exhibited better response for shoot induction from most of the explants compared to the KIN treatments. Whole flower buds didn't give any response on any medium and were excluded from data interpretation. The results for efficient shoot regeneration from each explant are presented under the following headings.

Shoot regeneration through nodal cultures: Nodal cultures were obtained from three different stages (heading, 1 bud opened and 3 buds opened stage) of flower spike. Out of three stages, 3 buds opened stage didn't exhibit any response for shoot initiation on any medium and in any of the variety. However, no response was also recorded in *Traderhorn* at any stage of the flower spike; that's why it was excluded from data interpretation. The response for shoot initiation in *White Friendship* and *Peter Pears* was significantly influenced by both nodal stage and PGR combinations rather than the variety cultured (Table 3, Fig. 1). MS media having BAP (2 or 4 mg L^{-1}) alone or BAP with KIN and NAA (2 + 2 + 0.5 (mg L^{-1}) exhibited better results for shoot regeneration (Table 3). No shoot induction was recorded from rest of the PGR combinations. Shoot initiation started after two weeks of inoculation and all cultures were shifted to same fresh media after 25 days of the culture. The nodal cultures initiated shoot primordia with the time period of 16.33-38.92 days. The earlier mean shoot induction (17.50%) was observed on MS medium supplemented with BAP and KIN (2 mg L^{-1}) each in16.33 days. After two weeks of shoot initiation same heading stage of the explant produced significant differences over one bud opened stage as mentioned in Table 4.

Table 3. Days to shoot initiation and response for shoot initiation (%) from nodal cultures as influenced by different stages of the flower spike and PGR combinations.

PGR combinations (mg L^{-1})	*White Friendship*		*Peter Pears*		Mean
	Heading	1bud opened	Heading	1 bud opened	
	Days to shoot initiation				
MS (cont)	-	-	-	-	-
MS+2BAP	37.00	39.00	39.67	40.00	38.92 A
MS+4BAP	30.33	28.67	35.00	42.67	34.17 B
MS+2KIN	-	-	-	-	-
MS+4KIN	-	-	-	-	-
MS+2BAP+2KIN	33.33	0.00	32.00	0.00	16.33 D
MS+4BAP+4KIN	-	-	-	-	-
MS+2BAP+2KIN +0.5 NAA	26.33	26.00	29.33	34.67	29.08 C
Mean	**31.75**	**23.42**	**34.00**	**29.33**	
	Shoot induction (%)				
MS (cont)	-	-	-	-	-
MS+2BAP	38.33	50.00	40.00	43.33	42.92 A
MS+4BAP	51.67	36.67	50.00	38.33	44.17 A
MS+2KIN	-	-	-	-	-
MS+4KIN	-	-	-	-	-
MS+2BAP+2KIN	35.00	0.00	35.00	0.00	17.50 B
MS+4BAP+4KIN	-	-	-	-	-
MS+2BAP+2KIN +0.5 NAA	36.67	40.00	35.00	50.00	40.42 A
Mean	**40.42**	**31.67**	**40.00**	**32.92**	

Table 4. Total shoot induction (%) and number of shoots per culture obtained from nodal cultures as influenced by different stages of the flower spike and PGR combinations

PGR combinations (mg L^{-1})	*White Friendship*		*Peter Pears*		
	Heading	1 bud opened	Heading	1bud opened	
	Total shoot induction after 2 weeks of shoot initiation				
MS (cont)	-	-	-	-	-
MS+2BAP	86.67	65.00	88.33	70.00	77.50 A
MS+4BAP	90.00	75.00	86.67	75.00	81.67 A
MS+2KIN	-	-	-	-	-
MS+4KIN	-	-	-	-	-
MS+2BAP+2KIN	50.00	0.00	46.67	0.00	24.17 C
MS+4BAP+4KIN	-	-	-	-	-
MS+2BAP+2KIN +0.5 NAA	71.67	61.67	75.00	70.00	69.58 B
Mean	**74.58 A**	**50.42 C**	**74.17 A**	**53.75 B**	
	Number of shoots per culture				
MS (cont)	-	-	-	-	-
MS+2BAP	8.000 b	6.13 fg	7.00 bcdef	6.80 cdefg	6.98 B
MS+4BAP	10.13 a	7.67 bc	7.80 bc	7.40 bcd	8.25 A
MS+2KIN	-	-	-	-	-
MS+4KIN	-	-	-	-	-
MS+2BAP+2KIN	5.93 g	0.00 i	4.93 h	0.00 i	2.72 C
MS+4BAP+4KIN	-	-	-	-	-
MS+2BAP+2KIN +0.5 NAA	6.60 defg	6.07 fg	7.27 bcde	6.27 efg	6.55 B
Mean	**7.67 A**	**4.97 C**	**6.75 B**	**5.12 C**	

All factors viz nodal stages, PGR combinations and varieties significantly influenced the number of shoots per culture and that the interactions between any two factors were highly significant. This indicated that the response to PGR treatments depended upon the nodal stage and variety. The maximum number of shoots (10.13) obtained from heading stage on MS medium supplemented with BAP (4 (mg L^{-1}) in *White Friendship* (Table 4). The heading stage produced more mean number of shoots (7.21) compared to one bud opened stage (5.04).

Shoot regeneration through whole cormels of different sizes: Non-dormant cormels of three different sizes were cultured on shoot regeneration media with growing point

upward (polar inoculation) exhibited significant effect of the cormel sizes and PGR combinations in each variety. However, the interaction was highly significant in *Traderhorn* and *White Friendship*. Sprouting of the cormels was observed within 4-5 days of inoculation with better shoot induction from number of multiple PGR combinations in each of the variety (Table 5, Fig. 1). The highest mean shoot induction of 98.33%, 96.11% and 95% was observed on MS medium supplemented with BAP+KIN+NAA (2+2+0.5 (mg L^{-1}) in *Traderhorn*, *White Friendship* and *Peter Pears* respectively. On mean basis of cormel size, shoot induction (%) increased with increase in the weight of the cormels produced the highest (91.87%) in *Traderhorn* followed by *White Friendship* (88.96%) and *Peter Pears* (88.96%) in response to large sized cormels (Table 5).

The cormels of different sizes had non-significant effect for number of shoots per culture in *Traderhorn* and *White friendship* (Table 6). In all three varieties, more number of shoots was recorded on MS medium supplemented with higher level of BAP (4 (mg L^{-1}) in response to large sized cormels produced 8.87, 7.73 and 7.07 shoots in *Traderhorn*, *White Friendship* and *Peter Pears*, respectively. The lowest response for number of shoots per culture was observed in control (without PGR) by all cormel size in each variety.

Shoot regeneration through cormel sprouts of various sizes: Non-dormant cormels of uniform size (0.6 g) with growing point downward (apolar inoculation) and physiological base upward were cultured on shoot induction media for shooting. The cormels sprouted downward into media within four days of inoculation on each PGR combination. 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, medium and large cormel sprouts. The sprouts were re-cultured on the same media with growing point upward (polar inoculation) and physiological base downward. Within a week, all these cormel sprouts of large and medium size burst into number of multiple shoots (Fig. 1). However, there was no effect of any PGR combination on cormel sprouts of small size in any variety and that's why it was excluded from statistical analysis.

All the factors viz. different sizes of cromel sprouts, varieties and PGR combinations exhibited highly significant differences for both shoot induction and number of shoots per culture (Table 7). Successful shoot induction response was found from medium and large sprout of the cormel in *White Friendship* and *Peter Pears*. Whereas, *Traderhorn* showed no response from large cormel sprouts. On mean basis, statistically better response was found in each variety from medium sprout of the cormel compared to large one showed 100%, 52% and 45% increase in shooting in *Traderhorn*, *White Friendship* and *Peter Pears*, respectively. BAP at 2 or 4 (mg L^{-1}) showed the best results for mean shoot induction (65.0% and 64.4%, respectively).

Table 5. Shoot induction (%) from whole cormels of different size by using different PGR combinations.

PGR combinations (mg L^{-1})	Small (0.2 g)	Medium (0.4 g)	Large (0.6g)	Mean
	Traderhorn			
MS (cont)	68.33 cde	98.33 a	100.00 a	**88.89 BC**
MS+2BAP	91.67 ab	100.00 a	90.00 ab	**93.89 AB**
MS+4BAP	90.00 ab	96.67 a	100.00 a	**95.56 AB**
MS+2KIN	65.00 de	81.67 bc	88.33 ab	**78.33 D**
MS+4KIN	55.00 e	65.00 de	80.00 bc	**66.67 E**
MS+2BAP+2KIN	71.67 cd	80.00 bc	96.67 a	**82.78 CD**
MS+4BAP+4KIN	60.00 de	70.00 cd	81.67 bc	**70.56 E**
MS+2BAP+2KIN +0.5 NAA	96.67 a	100.00 a	98.33 a	**98.33 A**
Mean	**74.79 C**	**86.46 B**	**91.87 A**	
	White friendship			
MS (cont)	66.67 hi	85.00 bcdef	100.00 a	**83.89 C**
MS+2BAP	80.00 defgh	88.33 abcde	95.00 abc	**87.78 BC**
MS+4BAP	81.67 cdefg	100.00 a	100.00 a	**93.89 AB**
MS+2KIN	70.00 ghi	85.00 bcdef	90.00abcde	**81.67 CD**
MS+4KIN	81.67 cdefg	63.33 i	63.33 i	**69.44 E**
MS+2BAP+2KIN	65.00 i	70.00 ghi	90.00abcde	**75.00 DE**
MS+4BAP+4KIN	80.00 defgh	71.67 fghi	76.67 efghi	**76.11 DE**
MS+2BAP+2KIN +0.5 NAA	91.67 abcd	100.00 a	96.67 ab	**96.11 A**
Mean	**77.08 C**	**82.92 B**	**88.96 A**	
	Peter pears			
MS (cont)	80.00	100.00	96.67	**92.22 A**
MS+2BAP	88.33	96.67	100.00	**95.00 A**
MS+4BAP	81.67	100.00	100.00	**93.89 A**
MS+2KIN	70.00	78.33	86.67	**78.33 BC**
MS+4KIN	61.67	71.67	80.00	**71.11 C**
MS+2BAP+2KIN	85.00	81.67	78.33	**81.67 B**
MS+4BAP+4KIN	75.00	85.00	73.33	**77.78 BC**
MS+2BAP+2KIN +0.5 NAA	88.33	100.00	96.67	**95.00 A**
Mean	**78.75 B**	**89.17 A**	**88.96 A**	

Table 6. Number of shoots regenerated from whole cormels of different size using different PGR combinations

PGR combinations (mg L^{-1})	Small (0.2 g)	Medium (0.4 g)	Large (0.6g)	Mean
	Traderhorn			
MS (cont)	1.00 i	1.00 i	1.00 i	1.00 F
MS+2BAP	6.60 cd	4.67 e	4.60 ef	5.29 B
MS+4BAP	7.40 bc	8.20 ab	8.87 a	8.16 A
MS+2KIN	2.20 h	3.60 fg	3.60 fg	3.13 E
MS+4KIN	4.13 efg	4.00 efg	3.53 g	3.89 D
MS+2BAP+2KIN	3.87 efg	4.13 efg	3.60 fg	3.87 D
MS+4BAP+4KIN	4.0 efg	3.93 efg	3.40 g	3.78 D
MS+2BAP+2KIN +0.5 NAA	3.53 g	4.00 efg	6.20 d	4.58 C
Mean	**4.09**	**4.19**	**4.35**	
	White friendship			
MS (cont)	1.00 k	1.00 k	1.00 k	1.00 F
MS+2BAP	4.20 de	5.13 c	5.20 c	4.84 B
MS+4BAP	7.40 ab	6.67 b	7.73 a	7.27 A
MS+2KIN	3.40 efg	1.60 jk	1.67 jk	2.22 E
MS+4KIN	3.40 efg	2.00 ij	2.13 ij	2.51 DE
MS+2BAP+2KIN	3.27 fgh	4.13 def	4.00 def	3.80 C
MS+4BAP+4KIN	3.40 efg	2.67 ghi	2.47 hij	2.84 D
MS+2BAP+2KIN +0.5 NAA	4.20 de	5.13 c	4.40 cd	4.58 B
Mean	**3.78**	**3.54**	**3.57**	
	Peter pears			
MS (cont)	1.27 no	1.00 o	1.33 mno	**1.20 E**
MS+2BAP	5.27 bc	4.40 cdef	5.00 bcd	**4.89 B**
MS+4BAP	4.40 cdef	5.40 b	7.07 a	**5.62 A**
MS+2KIN	2.40 jkl	2.27 klm	2.00 lmn	**2.22 D**
MS+4KIN	1.73 lmno	2.07 lmn	2.40 jkl	**2.07 D**
MS+2BAP+2KIN	3.40 ghi	3.27 hij	4.13 defgh	**3.60 C**
MS+4BAP+4KIN	4.80 bcde	3.13 ijk	4.27 defg	**4.07 C**
MS+2BAP+2KIN +0.5 NAA	4.53 bcde	3.47 fghi	3.93 efghi	**3.98 C**
Mean	**3.47 A**	**3.12 B**	**3.77 A**	

Table 7. Shoot induction (%) from cormel sprouts of different size using different PGR combinations.

PGR combinations (mg L^{-1})	Traderhorn		White friendship		Peter pears		Mean
	Medium	Large	Medium	Large	Medium	Large	
MS (cont)	45.00 klmn	0.0 p	65.00 ghi	28.33 o	65.00 ghi	30.00 o	38.89 D
MS+2BAP	83.33 bcde	0.0 p	95.00 ab	73.33 defg	80.00 cdef	58.33 hijk	65.00 A
MS+4BAP	95.00 ab	0.0 p	98.33 a	68.33 fgh	73.33 defg	51.67 ijkl	64.44 A
MS+2KIN	70.00 efgh	0.0 p	70.00 efgh	45.0 klmn	66.67 fgh	43.33 lmn	49.17 C
MS+4KIN	68.33 fgh	0.0 p	48.33 jklmn	30.00 o	36.67 mno	35.00 no	36.39 D
MS+2BAP+2KIN	73.33 defg	0.0 p	98.33 a	61.67 ghij	68.33 fgh	50.00 jklm	58.61 B
MS+4BAP+4KIN	90.00 abc	0.0 p	71.67 defgh	51.67 ijkl	70.00 efgh	43.33 lmn	54.44 B
MS+2BAP+2KIN +0.5 NAA	66.67 fgh	0.0 p	73.33 defg	48.3 jklmn	85.0 abcd	65.00 ghi	56.39 B
Mean	**73.96 A**	**0.0 D**	**77.50 A**	**50.83 C**	**68.13 B**	**47.08 C**	

The swelled cormel sprouts of medium size also regenerated more number of shoots in each variety (7.16, 11.60 and 9.47 in *Traderhorn*, *White Friendship* and *Peter Pears*, respectively) (Table 8). MS medium supplemented with BAP (4 (mg L^{-1}) revealed the highest mean number of shoots (12.19) per culture followed by 9.03 shoots on MS medium supplemented with BAP+KIN (2 mg L^{-1} each).

Shoot regeneration from cormel sections: Top and bottom sections of cormels (0.6 g) were cultured on shoot induction media to evaluate the best section of cormel in each variety for efficient shoot regeneration. Top section of cormel induced almost shoots in 90% cultures within 2-3 days on each combination of PGR including control (without PGR) (Table 9, Fig. 1). However, bottom section of cormel took more time (35-42 days) and induced shoots in only 35% cultures (data not presented). The upper cut surface of the bottom sections of cormel became sequentially dark and dead in most of the cultures.

Varieties had significant differences produced the highest shoot induction (96.25%) in *Traderhorn* followed by *White Friendship* (92.5%) from top section of the cormel (Table 9). Like shoot induction *Traderhorn* and *White Friendship* produced statistically similar number of shoots (Table 10). MS medium supplemented with BAP (4mg L^{-1}) induced mean more number of shoots (6.16) followed by BAP at 2 mg L^{-1} (5.76) with statistically non-significant differences (Table 10).

Table 8. Number of shoots per culture obtained from cormel sprouts of different size using different PGR combinations.

PGR combinations (mg L^{-1})	Traderhorn		White friendship		Peter pears		Mean
	Medium	Large	Medium	Large	Medium	Large	
MS (cont)	3.67	0.00	5.53	2.47	4.00	2.00	2.94 F
MS+2BAP	7.33	0.00	12.00	9.00	9.60	7.80	7.62 CD
MS+4BAP	12.00	0.00	22.07	12.00	16.07	11.00	12.19 A
MS+2KIN	4.33	0.00	9.47	3.00	7.53	5.60	4.99 E
MS+4KIN	5.27	0.00	5.00	3.67	5.80	3.47	3.87 EF
MS+2BAP+2KIN	8.20	0.00	14.73	11.00	11.53	8.73	9.03 B
MS+4BAP+4KIN	7.33	0.00	10.00	8.00	8.87	7.33	6.92 D
MS+2BAP+2KIN +0.5 NAA	9.27	0.00	14.00	8.00	12.33	7.67	8.54 BC
Mean	**7.17 C**	**0.00D**	**11.60 A**	**7.14 C**	**9.47 B**	**6.70 C**	

Table 9. Shoot induction (%) from top section of cromel as affected by different PGR combinations.

PGR combinations	Traderhorn	White friendship	Peter pears	Mean
MS (cont)	100.00	85.00	93.33	92.78
MS+2BAP	95.00	98.33	86.67	93.33
MS+4BAP	100.00	96.67	91.67	96.11
MS+2KIN	91.67	88.33	86.67	88.89
MS+4KIN	100.00	90.00	90.00	93.33
MS+2BAP+2KIN	90.00	90.00	96.67	92.22
MS+4BAP+4KIN	93.33	91.67	91.67	92.22
MS+2BAP+2KIN +0.5 NAA	100.00	100.00	91.67	97.22
Mean	**96.25 A**	**92.5 AB**	**91.04 B**	

Table 10. Number of shoots produced per culture from top section of cormel as affected by different PGR combinations.

PGR combinations	Traderhorn	White friendship	Peter pears	Mean
MS (cont)	1.47	1.07	1.13	1.22 D
MS+2BAP	5.80	6.80	4.67	5.76 A
MS+4BAP	6.13	7.00	5.33	6.16 A
MS+2KIN	1.93	1.33	1.67	1.64 CD
MS+4KIN	1.27	1.00	1.13	1.13 D
MS+2BAP+2KIN	4.20	3.87	3.47	3.84 B
MS+4BAP+4KIN	3.13	2.40	1.67	2.40 C
MS+2BAP+2KIN +0.5 NAA	5.27	4.20	4.20	4.56 B
Mean	**3.65 A**	**3.46 A**	**2.91 B**	

Choice of plant material and proliferation media: The best responded stage/size from each explant was selected on the basis of efficient shoot induction and cultured on proliferation media to produce multiple number of shoots. Among different stages/sizes of each explant, heading stage from the nodal cultures, whole cormels of large size, medium size cormel sprouts and top section of the cormels were evaluated the best responded stage/size of each explant for efficient shoot regeneration.

The cluster of induced shoots was equally divided into number of sections having three number of shoots and cultured on the best responded PGR combinations viz. BAP (2 and 4 mg L^{-1} alone or in combination with NAA. Each section/piece of the shoots had some original basal part of the explant.

Multiple shoots from different explant sources: The multiple shoot formation was significantly influenced by various explant source in each variety of the gladiolus. The different explant sources behaved differently for multiple number of shoots. Early multiple shoot induction (10 days) was observed in all explants except nodal cultures on MS media supplemented with BAP+KIN+NAA (2+2+0.5 mg L^{-1}) or BAP+NAA (2+0.5 mg L^{-1}). The cormel sprout was recorded the best explant in terms of multiple shoot production in each variety with 12.05, 15.24 and 13.48 shoots in *Traderhorn*, *White Friendship* and *Peter Pears*, respectively (Table 11). Same explant also produced more number of shoots on control (without PGR) compared to rest of the explants. MS medium supplemented with BAP and NAA (2+0.5 mg L^{-1}) regenerated the highest mean number of multiple shoots in *Traderhorn* (14.39), *White Friendship* (14.62) and *Peter Pears* (14.83).

Table 11. Number of multiple shoots produced from different explants sources in response to different PGR combinations.

PGR combinations (mg L^{-1})	Nodal cultures	Whole cormel	Cormel sprouts	Top cormel sections	Mean
	Traderhorn				
MS (cont.)	-	1.00 e	6.40 d	0.00 e	2.47 D
2BAP	-	8.13 cd	15.20 a	12.00 b	11.78 B
4BAP	-	7.07 d	11.87 b	10.40 bc	9.78 C
2BAP+2KIN+0.5NAA	-	2.33 e	9.20 bcd	0.00 e	3.84 D
2BAP+0.5NAA	-	10.58 bc	17.60 a	15.00 a	14.39 A
Mean	**-**	**5.82 C**	**12.05 A**	**7.48 B**	
	White friendship				
MS (cont.)	0.00 l	1.00 kl	6.20 hij	0.67 kl	1.97 C
2BAP	14.67 cde	14.13 de	17.53 bc	6.93 hi	13.32 A
4BAP	16.20 bcd	10.87 fg	18.87 b	10.93 fg	14.22 A
2BAP+2KIN+0.5NAA	6.80 hi	3.67 ijk	10.60 fg	3.20 jkl	6.07 B
2BAP+0.5NAA	14.40 cde	12.67 ef	23.00 a	8.40 gh	14.62 A
Mean	**10.41 B**	**8.47 C**	**15.24 A**	**6.03 D**	
	Peter pears				
MS (cont.)	0.00 g	1.00 g	4.07 f	0.00 g	1.27 D
2BAP	12.00 c	10.00 de	17.87 b	8.40 de	12.07 B
4BAP	12.00 c	8.40 de	15.00 b	7.03 ef	10.61 B
2BAP+2KIN+0.5NAA	0.00 g	4.60 f	9.20 cde	0.00 g	3.45 C
2BAP+0.5NAA	15.73 b	11.73 c	21.27 a	10.60 cd	14.83 A
Mean	**7.95 B**	**7.15 B**	**13.48 A**	**5.21 C**	

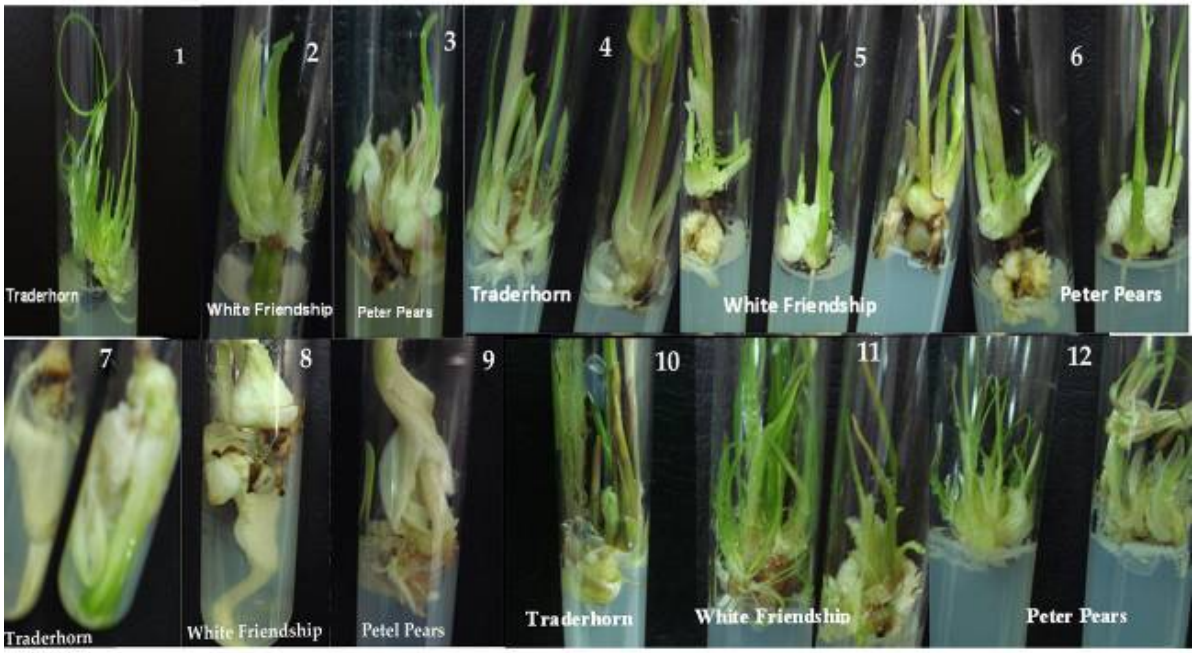

Fig. 1. Shoot induction from various explant sources. 1-3 nodal cultures; 4-6 whole cormels, 7-9 cormel sprouts, 10-12 cormel segments.

Discussion

Various stages/sizes of any explant might have different regenerative capacity and this regenerative capacity is much depended upon the type, concentration and combination of plant growth regulators. In present study we tested number of explants for efficient shoot regeneration by using different combinations of plant growth regulators. Among plant growth regulators, BAP was found potent cytokinin for efficient number of shoots in gladiolus. These results are also supported by the published work done on various ornamental plants including *Lilium candidum* (Khawar *et al*., 2005) *Clerodendrum incisum* (Goyal *et al*., 2010); *Ochradenus arabicus* (Nadeem *et al*., 2012); Dendranthema morifolium (Waseem *et al*., 2011).

In case of nodal cultures, the heading stage of the flower spike of *White Friendship* and *Peter Pears* had more potential than the explant taken at one bud opened stage for efficient shoot regeneration on BAP (2 or 4 mg L^{-1}). Ahmad *et al.,* (2000) also obtained more number of shoots from nodal buds of sleeping or heading stage of the flower spike in cultivar *White Prosperity* compared to cormel tips or auxiliary buds by using 4 mg L^{-1} BA and 7.3 (mg L^{-1}) IBA in the MS medium. Grewal *et al*., (1995) reported more multiple shoot primordia from nodal buds in response to higher level of BAP. They obtained a single shoot per explant on MS medium supplemented with BAP (1 mg L^{-1}) in cultivars viz. *Mayur, Sylvia, Spic* and *Span*, whereas those cultured on MS medium supplemented with BAP (5 mg L^{-1}) produced 14-20 shoot primordia within 4 weeks.

In our study no shoot regeneration was observed from nodal cultures of variety *Traderhorn*. This might be due to mature nature of explants as juvenile explants have high regeneration capability than adult explants (Pierik, 1987). All three varieties were planted on the same day but *Traderhorn* maturity was observed earlier compared to the *White Friendship* and *Peter* Pears. It was also noted that the nodal explants of this variety (Traderhorn) were hard, less succulent and had white milky color streaks on inner phloem opposed to *White friendship* and *Peter pears*. The nodal cultures taken at 3 buds opened stage also didn't exhibit any response in any variety and confirming the point that explant maturity had major role for efficient shoot regeneration and regenerative capacity varies in mature and juvenile tissues. *Traderhorn* cultures also showed phenolic exudation which was managed by sub-culturing on fresh media.

In contrast to nodal explants, whole cormel explants of various sizes induced shoots on all PGR combinations including control (MS medium without PGR). 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* on basal MS medium. This is because whole cormels basically are storage tissue and storage organs (bulbs, tubers, corms) have reserve food for readily shoot induction. So in this sense sprouting for shoot induction is much dependent on the size of storage tissue of the cormel rather than PGR combinations. However, the effect of PGR combinations was significantly visible for number of shoots per culture. The number of shoots increased with increasing levels of BAP (2 or 4 mg L^{-1}) which produced the highest 8.87 number of shoots from large sized cormels (0.6 g) in *Traderhorn* on MS medium supplemented with BAP (4 mg L^{-1}). However, these results are not in harmony with Aftab *et al*., (2008) who used quite a low concentration of BAP (1 mg L^{-1}) for producing more number of shoots (16) per culture vessel from cormels. This report did not mention variety and size of the cormels used for shoot regeneration; hence it is difficult to justify the results here. The differences in results might be due to cormel size or varietal differences as the effect of concentration and combination of PGR varied with variety and explant size.

Cormel sprouts of various sizes produced through apolarly oriented cormels found efficient regarding efficient shoot induction and multiple shoot regeneration. The literature on the use of cormel sprouts produced from apolarly orientated cormels (upside down, physiological base out of the medium and top in the medium) for regeneration of shoots is scanty. Generally, cormels are cultured with the basal plate in contact with the growing medium (polar inoculation-straight up, with the physiological base in the medium) whereas, in the present study cormels orientated apolarly found it very applicable and easy method for regeneration of multiple shoots. Pierik & Steegmans (1975) reported that apolar inoculation of the explants regenerate shoots more easily and more rapidly as compared to polar inoculation. This better regeneration of shoots may be a result of an improved oxygen supply, there may be other factors which may play a role (Pierik & Steegmans, 1975). The cormels sprouted downward into media swelled up and had light green color. Light green colour of cormel sprouts might be due to chlorophyll deficiency. The apolarly orientated explants have substances accumulated at the basal end which cannot diffuse into the agar, since it is not in contact with the medium. Hence presence of endogenous hormones might also have part in this regard. Apolar inoculation is of great importance in all bulbous plants as regeneration of organs takes place at the base of bulb scales. They further reported that better adventitious bulb formation takes place with apolar orientated explants than with polar explants. Jacobs *et al.,* (1992) reported the effect of explant orientation (polar & apolar) on bulblet formation by culturing twin-scale explants in *Nerine bowdenii*. They observed better results from apolarly orientated explants for average weight per bulblet and total fresh weight of bulblets per growing explants than polarly orientated explants. *Orlikowska et al*., (2000) observed more number of shoots from shoots inoculated vertically in an inverted position with shoot tip down.

Transverse sections of cormels (top and bottom) of 0.6 g were cultured and top section of cormel had better potential for efficient shoot regeneration on BAP (4 mg L^{-1}) than bottom section of the cormel. This might be due to the presence of growing point (meristematic tissues) in the cormel. Besides, the physiological base of top section of the cormel after cutting was placed on medium and had more absorption area for nutrient uptake. This could be a reason due to which the cultures showed healthy shoots. Babu & Chawla (2000) also recorded better shoot induction (89%) from top slice of cormel (dia. 1.0 to 1.5 cm) with an average of 2.4 shoots per explant in response to MS medium supplemented with KIN (4 mg L^{-1}) rather than BAP (Garshasbi *et al*., 2012; Asim, 2012). Regarding bottom section of cormels, most of the cultures exhibited 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 & 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 & Erdag (2007) reported also no regeneration from transverse slices of cormel.

Conclusion

A number of explants were tried to develop a potential protocol for efficient shoot induction and multiple shoot regeneration in different varieties of the gladiolus. The heading stage of nodal cultures, large sized cormel, medium cormel sprouts and top section of the cormels were evaluated the best stage/size from each explant source for efficient shoot regeneration on MS medium supplemented with BAP (2 or 4 (mg L^{-1})). Out of these explants, cormel sprout was evaluated the best explant for cheap and easy way of multiple shoot regeneration. This would be the best explant for *In vitro* propagation of elite varieties.

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