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Effect of Plant Growth Regulators on *In Vitro* Morphogenesis in Gladiolus (*Gladiolus hybridus* Hort.) from Cultured Corm Slice

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Abstract In present research, corm slice explant of gladiolus (*Gladiolus hybridus*) was cultured on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in different combinations. Regarding *in vitro* response, induction medium MS2B.5D (MS + 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} 2,4-D + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) proved well for callus initiation. MS medium supplemented with BA in range of 2.0-3.0 mgl^{-1} in combination with 0.5 mgl^{-1} NAA, 30.0 gl^{-1} sucrose and 7.5 gl^{-1} agar exhibited higher shoot proliferating efficiency, number of shoot (s) per explant and shoot of higher length. In respect to *in vitro* rooting response, higher root proliferating efficiency was documented on rooting medium MS.5IB.5Kn (MS + 0.5 mgl^{-1} IBA + 0.5 mgl^{-1} Kinetin + 15.0 gl^{-1} sucrose + 7.5 gl^{-1}agar), while number of root (s) with higher length were recovered on rooting medium MS.5IB (MS+0.5 mgl^{-1} IBA+15.0 gl^{-1} sucrose +7.5 gl^{-1} agar). Phenotypically normal plantlets were acquired and subsequently transferred to pots and hardened in Environmental Growth Cabinet and Net House during initial tiring period and transferred to field successfully.

Keywords: *Gladiolus hybridus*, corm slice, direct organogenesis, indirect organogenesis, plantlet regeneration.

Abbreviations: B_5:Gamborg's medium; MS: Murashige and Skoog,s medium; Wh: White's medium; BA: 6-benzylaminopurine; TDZ: Thidiazuron; Zea: Zeatin; Kn-Kinetin; NAA: α-Naphthalene acetic acid; 2, 4-D: 2, 4-dichlorophenoxyacetic acid; 2, 4, 5-T: 2, 4, 5 trichlorophenoxyacetic acid; GA_3: Gibbrellic acid and IBA: Indole-3-butyric acid.

Introduction

Gladiolus (*Gladiolus hybridus* Hort.) belongs to the family Iridaceae is one of the most important bulbous commercial ornamental plant grown for cut flowers. Due to its magnificent inflorescence with a variety of colours makes it attractive for use in herbaceous borders, beddings, pots and for cut flowers. It

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has a great economic value and wide market in the country. It propagates either by seed, corm formation or by cormel differentiation. Although, seed is an effective means of gladiolus propagation but seed-raised plants may not produce true type population (Hussain*et al.*, 2001). Additionally, it takes four seasons for blooming. The propagation by corm and cormel formation is another conventional method of multiplication but it may transmit several viral, fungal and bacterial diseases i.e. *Fusarium*corm rot, *Botrytis* blight, bacterial leaf spot *etc*., thus causing a heavy loss. These low propagation rates hinder the introduction of new varieties or virus-free plants. However, in gladiolus there is a clear scope for further refinement in *in vitro* culture methodology to acquire a higher number of shoots to complement traditional nursery methods (Hussain*et al.*, 2001). Plant tissue culture offers a potential to deliver large quantities of disease-free, true type healthy stock within a short span of time (Hussain*et al.*, 2001). The method guaranteed the identical reproduction of the parents tested and selected, and prevented genotypic alteration which would occur after the generative multiplication.

In gladiolus, an array of explants have been used efficiently to produce regenerable cultures *via* somatic embryogenesis and/or organogenesis are auxiliary bud (Hussain*et al*., 1994; Dantu and Bhojwani, 1995; Begum and Haddiuzaman, 1995; Grewal*et al*., 1995; Kamo,1995; Sen and Sen, 1995; Boonvanno and Kanchanapoom, 2000; Priyakumari and Sheela, 2005), inflorescence stalk (Longan and Zettler, 1985; Kamo*et al*.,1990; Kumar *et al*.,1999; Ziv and Lilien Kipnis, 2000), apical meristem (Longan and Zettler, 1985), Basal leaves and leave discs (Kamo 1994; Remotti, 1995; Babu and Chawla, 2000; Hussain*et al*., 2001; Kumar *et al*., 2002; Emek and Erdag, 2007), nodal segments and shoot tips (Dantu and Bhojwani, 1995; Grewel*et al*., 1995; Ahmad *et al*., 2000; Hussain*et al*., 2001), Cormel stem tip (Simonsen and Hildebrant, 1971; Kumar *et al*., 1999; Goo *et al*., 2003; Aftab*et al*., 2008), cormel sprouts (Ziv,1979; Longan and Zettler, 1985; Lilien-Kipnis and Kochba, 1987; Nagaraju and Parthasarathy, 1995; Remotti, 1995; Kumar *et al*.,1999; Hussain*et al*., 2001; Pathania*et al*., 2001 ; Sinha and Roy, 2002; Aftab *et al*., 2008), Stolon (Ginzburg and Ziv, 1973), Shoot bud (Dantu and Bhojwani, 1987), corm slice (Ziv, 1979; Bajaj *et al*.,1983; Longan and Zettler, 1985; Lilien-Kipnis and Kochba, 1987; Kamo, 1994; Bose *et al*., 2003), cormel segment (Kumar *et al*., 1999), Cormel section (Lehsem, 1988) and cell suspension cultures (Bajaj *et al*., 1983; Kamo,1994) with varying degree of success.

Moreover, no enormous work has been done so far in Madhya Pradesh on these aspects with popular varieties of the species. In view of this, an effort was made to search out the most responding explant, computing the optimal

magnitude of plant growth regulators to be added in culture medium, other physical factors exhibiting higher *in vitro* morphogenesis with popular cultivar of Madhya Pradesh and adjoining regions by implying corm slice culture.

Materials and methods

Three different basal media *viz*: MS (Murashige and Skoog, 1962), Wh (White, 1963) and B_5 (Gamborg*et al*., 1968) were investigated to detect better *in vitro* response. During the preliminary investigation, MS basal medium was found more acquiescent than Wh and B_5 media (data not presented); hence, for afterward experimentations corm slice explant of Candyman cultivar was cultured on different fortifications of basal MS medium. Experimental material was collected from KNK, College of Horticulture, Mandsaur (M. P.). All initial culture media were made using readymade basal medium (HiMidia^{TM}). Apart from MS basal micro and macro salts, vitamins and agar powder three different sets of plant growth regulators were tested. In first set, four different auxins (alone), *namely*: 2,4-D, NAA , 2,4,5-T and IBA (Table 1) , in second set, four diverse cytokinins (sole) *viz*. BAP, Kn, TDZ and zeatin (Table 2) and in third set: varying concentrations of 2,4-D and NAA in combinations with BAP and Kn (Table 3) were added to fortify MS media for achieving the best morphogenic response. Apart from MS basal macro and micro salts, vitamins, all initial culture media were supplemented with 30.0 gl^{-1} sucrose and the final volume was made to 1000 ml and pH was adjusted to 5.8 ± 0.1 with 1N KOH solution. After adjusting the pH, agar powder @ 7.5 g l^{-1} was added to the media as a semi-solidifying agent. Warm culture media, still in liquid state were poured into baby food bottles (50-60 ml / bottle) and culture tubes (15-20 ml/ tube) followed by autoclaving at 121^{o}C under 15 psi pressures for 20-25 minutes. Readymade basal media, plant growth regulators and other ingredients were procured from Hi-media Laboratories, Mumbai, India.

Table 1. Effects of different auxins (alone) in varying concentrations on *in vitro* response of corm slice cultures in gladiolus.

Culture media	Plant growth regulators mgl^{-1}				Callus induction (%)	Shoot Proliferating explant (%)	No. of shoots/explant	Mean shoot length (in cm)
	2,4-D	NAA	2,4,5-T	IBA				
MS.1D	0.1	-	-	-	33.33^{h}(35.2)	34.35^{g}(35.8)	2.97^{d}(9.88)	**2.87^{d}(9.7)**
MS.5D	0.5	-	-	-	34.17^{gh}(35.)	38.23^{f}(38.1)	3.67^{cd}(11.04)	**2.68^{d}(9.4)**
MSD	1.0	-	-	-	36.66^{f}(37.2)	41.15^{e}(39.8)	2.99^{d}(9.93)	**2.67^{d}(9.4)**
MS2D	2.0	-	-	-	46.13^{c}(42.7)	41.59^{e}(40.1)	4.98^{ab}(12.89)	**3.77^{c}(11.)**
MS3D	3.0	-	-	-	59.10^{a}(50.2)	38.65^{f}(38.4)	3.26^{d}(10.40)	**3.90^{c}(11.)**
MS4D	4.0	-	-	-	41.73^{d}(40.2)	35.71^{g}(36.6)	3.90^{c}(11.38)	**2.63^{d}(9.3)**
MS5D	5.0	-	-	-	34.71^{g}(36.0)	35.71^{g}(36.6)	3.70^{c}(11.09)	**2.68^{d}(9.4)**
MS.1N	-	0.1	-	-	34.27^{g}(35.8)	42.40^{de}(40.6)	4.21^{c}(11.73)	**3.81^{c}(11.)**
MS.5N	-	0.5	-	-	36.12^{f}(36.9)	43.38^{cd}(41.1)	4.77^{b}(12.61)	**4.46^{bc}(12.)**
MSN	-	1.0	-	-	38.40^{e}(38.2)	51.50^{a}(45.8)	5.80^{a}(13.92)	**5.36^{ab}(13.)**
MS2N	-	2.0	-	-	41.50^{i}(40.0)	52.18^{a}(46.2)	5.60^{a}(13.68)	**5.88^{a}(14.)**
MS3N	-	3.0	-	-	49.31^{b}(44.5)	48.15^{b}(43.9)	4.29^{bc}(11.95)	**3.36^{cd}(10.)**
MS4N	-	4.0	-	-	36.71^{ef}(37.2)	43.88^{c}(41.4)	5.41^{a}(13.44)	**3.32^{d}(10.)**
MS5N	-	5.0	-	-	35.31^{fg}(36.4)	42.91^{d}(40.9)	3.84^{c}(11.26)	**3.26^{d}(10.)**
MS.1T	-	-	0.1	-	27.21^{l}(31.4)	23.51^{k}(28.9)	1.32^{f}(6.59)	**1.34^{e}(6.6)**
MS.5T	-	-	0.5	-	32.05^{i}(34.4)	23.49^{k}(28.9)	1.50^{f}(7.03)	**3.17^{d}(10.)**
MST	-	-	1.0	-	32.34h^{i}(34.)	31.23^{i}(33.9)	2.67^{de}(9.35)	**2.58^{d}(9.2)**
MS2T	-	-	2.0	-	34.70^{g}(36.0)	38.21^{f}(38.1)	2.82^{d}(9.66)	**3.20^{d}(10.)**
MS3T	-	-	3.0	-	32.12^{i}(34.5)	32.67^{h}(34.8)	2.79^{d}(9.61)	**2.53^{d}(9.1)**
MS4T	-	-	4.0	-	34.21g(35.7)	27.70^{j}(31.7)	2.90^{d}(9.71)	**2.60^{d}(9.2)**
MS5T	-	-	5.0	-	23.43^{m}(28.9)	23.98^{k}(29.3)	1.43^{f}(6.86)	**1.39^{e}(6.7)**
MS.1IB	-	-	-	0.1	23.36^{m}(28.8)	23.51^{k}(28.9)	1.50^{f}(7.03)	**1.46^{e}(6.9)**
MS.5IB	-	-	-	0.5	27.30^{l}(31.4)	31.71^{i}(34.2)	2.50^{e}(9.09)	**1.51^{e}(7.0)**
MSIB	-	-	-	1.0	29.31^{k}(32.7)	30.64^{i}(33.6)	2.90^{d}(9.71)	**1.60^{e}(7.2)**
MS2IB	-	-	-	2.0	29.65^{jk}(32.9)	32.14^{h}(34.5)	2.95^{d}(9.87)	**1.68^{e}(7.4)**
MS3IB	-	-	-	3.0	31.10^{ij}(33.8)	31.75^{i}(34.2)	1.68^{ef}(7.44)	**1.95^{e}(8.0)**
MS4IB	-	-	-	4.0	30.89^{j}(33.7)	31.76^{hi}(34.2)	2.34^{e}(8.80)	**2.30^{de}(8.7)**
MS5IB	-	-	-	5.0	28.00^{kl}(31.3)	28.71^{j}(32.3)	1.62^{f}(7.31)	**2.23^{e}(8.5)**
Mean					**34.75(36.02)**	**35.74(36.58)**	**3.22(10.12)**	**2.86(9.55)**
CD(0.05)					**1.84**	**1.37**	**1.00**	**1.14**

- Figures in parenthesis are transformed values (Arc-sine transformation).
- Values within column followed by different letters are significantly different at 5% probability level.

Table 2. Effects of different cytokinins (alone) in varying concentrations on *in vitro* response of corm slice cultures in gladiolus.

Culture media	Plant growth regulators mgl^{-1}				Callus induction (%)	Shoot Proliferating explant (%)	No. of shoots/explant	Mean shoot length(in cm)
	BA	Kn	TDZ	Zea				
MS.1B	0.1		-	-	32.82^{e}(34.94)	72.11^{i} (58.10)	2.91^{i}(9.82)	**3.98^{de}(11.50)**
MS.5B	0.5		-	-	34.72^{d}(36.09)	90.96^{c}(72.48)	5.82^{fg}(13.94)	**4.47^{d}(12.09)**
MSB	1.0		-	-	36.23^{c}(36.99)	89.73^{c}(71.30)	5.46^{g}(13.51)	**4.67^{d}(12.40)**
MS2B	2.0		-	-	38.86^{a}(38.53)	96.34^{b}(78.94)	10.83^{c}(19.21)	**4.55^{d}(12.31)**
MS3B	3.0		-	-	36.24^{bc}(37.0)	98.00^{a}(82.11)	16.95^{a}(24.30)	**12.04^{a}(20.2)**
MS4B	4.0		-	-	35.17^{cd}(36.3)	65.67^{n}(54.11)	10.84^{c}(19.21)	**4.50^{d}(12.24)**
MS5B	5.0		-	-	32.05^{e}(34.47)	61.90^{o}(51.86)	7.25^{e}(15.57)	**3.84^{e}(11.29)**
MS.1Kn	-	0.1	-	-	19.11 ^{j}(25.91)	51.12^{r} (45.62)	5.33^{g}(13.29)	**1.90^{e}(7.92)**
MS.5Kn	-	0.5	-	-	22.15^{i} (28.06)	77.23^{g} (61.47)	2.51^{i}(9.11)	**3.70^{e}(11.09)**
MSKn	-	1.0	-	-	29.28^{fg}(32.75)	87.96^{d}(69.67)	2.76^{i}(9.56)	**3.81^{e}(11.25)**
MS2Kn	-	2.0	-	-	31.16^{ef}(33.92)	75.32^{h}(60.19)	5.14^{g}(13.10)	**3.41^{e}(10.64)**
MS3Kn	-	3.0	-	-	33.12^{de}(35.12)	71.71^{ij}(57.84)	7.27^{de}(15.64)	**3.31^{e}(10.48)**
MS4Kn	-	4.0	-	-	24.41^{h} (29.60)	42.42^{s}(40.62)	6.40^{ef}(14.65)	**3.58e(10.90)**
MS5Kn	-	5.0	-	-	17.21j(24.50)	31.33t(34.02)	3.17i(10.25)	**2.01^{e}(8.08)**
MS.1Td	-	-	0.1	-	12.30^{k} (20.52)	72.00^{i} (58.03)	4.84g^{h}(12.70)	**5.72^{d}(13.35)**
MS.2Td	-	-	0.2	-	29.01g(32.58)	80.94^{f} (64.09)	4.46^{h}(12.14)	**8.50^{c}(16.94)**
MS.3Td	-	-	0.3	-	32.55^{e} (34.77)	81.10^{f} (64.21)	11.44^{c}(19.76)	**8.71^{bc}(17.16)**
MS.4Td	-	-	0.4	-	33.01^{e} (35.05)	85.79^{e}(67.83)	12.13^{b}(20.37)	**10.88^{ab}(19.4)**
MS.5Td	-	-	0.5	-	35.30^{c}(36.44)	81.90^{f} (64.80)	11.48^{bc}(19.77)	**8.28^{c}(16.68)**
MSTd	-	-	1.0	-	35.84^{c} (36.76)	70.38^{jk}(57.00)	7.79^{d}(16.20)	**5.71^{d}(13.82)**
MS2Td	-	-	2.0	-	38.25^{ab}(38.19)	55.33^{q} (48.04)	6.40^{f}(14.65)	**5.98^{d}(14.04)**
MS.1Z	-	-	-	0.1	28.33^{g}(32.15)	51.31^{r} (45.73)	3.73^{hi}(11.10)	**2.81^{e}(9.63)**
MS.2Z	-	-	-	0.2	30.83^{f}(33.71)	58.73^{p}(50.01)	4.22^{h}(11.85)	**3.20^{e}(10.30)**
MS.3Z	-	-	-	0.3	30.17^{f}(33.30)	68.95^{kl}(56.11)	3.95^{h}(11.46)	**3.78^{e}(11.21)**
MS.4Z	-	-	-	0.4	29.96(33.02)	67.38^{m}(55.15)	4.27^{h}(11.92)	**3.28^{e}(10.43)**
MS.5Z	-	-	-	0.5	28.17^{g}(32.04)	68.27^{lm}(55.6)	4.05^{h}(11.61)	**2.71^{e}(9.47)**
MS1Z	-	-	-	1.0	28.15^{g}(32.03)	74.38^{h}(59.57)	4.38^{h}(12.08)	**2.51^{e}(9.11)**
MS2Z	-	-	-	2.0	27.27^{g}(31.47)	71.60^{j}(57.77)	3.37^{i}(10.57)	**2.31^{e}(8.74)**
Mean					**27.16^{g}(31.39)**	**71.42(58.66)**	**6.40(14.19)**	**4.79(12.24)**
CD(0.0)					**2.14**	**1.48**	**1.22**	**2.13**

- Figures in parenthesis are transformed values (Arc-sine transformation).
- Values within column followed by different letters are significantly different at 5% probability level.

Table 3. Combined effects of different auxins and cytokinins in varying concentrations on *in vitro* response of corm slice cultures in gladiolus

Culture media	Plant growth regulators mgl^{-1}				Callus induction (%)	Shoot Proliferating explant (%)	No. of shoots/explant	Mean shoot length (in cm)
	BA	Kn	2,4-D	NAA				
MS.1B.5D	0.1	-	0.5	-	41.03^{k}(39.82)	52.33^{p}(46.3)	2.95^{n}(9.85)	**4.85^{k}(12.70)**
MS.5B.5D	0.5	-	0.5	-	46.35^{hi}(42.8)	69.00^{lm}(56.1)	6.35^{k}(14.58)	**4.54^{k}(12.29)**
MSB.5D	1.0	-	0.5	-	61.16^{b}(51.43)	70.38^{l}(57.00)	7.52^{j}(15.90)	**5.32^{k}(13.32)**
MS2B.5D	2.0	-	0.5	-	65.76^{a}(54.16)	77.30^{h}(61.53)	9.24^{h}(17.68)	**5.58^{j}(13.60)**
MS3B.5D	3.0	-	0.5	-	59.91^{bc}(50.7)	74.60^{i}(59.72)	9.18^{h}(17.62)	**8.95^{gh}(17.3)**
MS4B.5D	4.0	-	0.5	-	48.28^{gh}(44.0)	71.95^{jk}(58.00)	8.95^{hi}(17.39)	**11.92^{ef}(20.)**
MS5B.5D	5.0	-	0.5	-	45.32^{ij}(42.29)	71.27^{kl}(57.56)	7.71^{ij}(16.11)	**4.47^{k}(12.13)**
MS.1B.5N	0.1	-	-	0.5	21.50^{q}(27.61)	77.38^{h}(61.58)	6.40^{k}(14.65)	**3.72^{kl}(11.0)**
MS.5B.5N	0.5	-	-	0.5	31.27^{o}(33.98)	84.73^{de}(66.98)	13.21^{e}(21.2)	**7.45^{i}(15.80)**
MSB.5N	1.0	-	-	0.5	52.38^{f}(46.35)	87.14^{c}(68.97)	14.95^{c}(22.7)	**21.95^{a}(27.9)**
MS2B.5N	2.0	-	-	0.5	58.35^{cd}(49.7)	97.44^{a}(80.94)	16.82^{b}(24.2)	**22.92^{a}(28.5)**
MS3B.5N	3.0	-	-	0.5	56.27^{de}(48.5)	98.54^{a}(83.51)	19.35^{a}(26.0)	**23.24^{a}(28.7)**
MS4B.5N	4.0	-	-	0.5	55.35^{e}(48.05)	85.96^{cd}(67.98)	13.24^{de}(21.3)	**15.15^{cd}(22.)**
MS5B.5N	5.0	-	-	0.5	48.75^{g}(44.27)	78.90^{gh}(62.63)	4.24^{mn}(11.86)	**13.50^{de}(21.)**
MS.1Kn.5D	-	0.1	0.5	-	23.66^{pq}(29.0)	53.12^{p}(46.77)	3.95^{n}(11.44)	**5.32^{jk}(13.3)**
MS.5Kn.5D	-	0.5	0.5	-	31.72^{o}(34.26)	61.73^{n}(51.76)	4.82^{lm}(12.66)	**5.55^{j}(13.62)**
MSKn.5D	-	1.0	0.5	-	32.15^{no}(34.5)	67.38^{m}(55.15)	9.92^{gh}(18.35)	**8.72^{h}(17.16)**
MS2Kn.5D	-	2.0	0.5	-	34.45^{mn}(35.9)	66.90^{m}(54.86)	10.84^{g}(19.21)	**10.35^{fg}(18.)**
MS3Kn.5D	-	3.0	0.5	-	43.84^{j}(41.44)	79.96^{g}(63.39)	11.83^{f}(20.11)	**16.55^{c}(23.9)**
MS4Kn.5D	-	4.0	0.5	-	41.17^{k}(39.90)	54.31^{op}(47.45)	13.98^{cd}(21.94)	**11.04^{f}(19.3)**
MS5Kn.5D	-	5.0	0.5	-	41.18^{k}(39.90)	55.10^{o}(47.91)	11.25^{fg}(19.59)	**6.24^{ij}(14.45)**
MS.1Kn.5N	-	0.1	-	0.5	23.98^{p}(29.31)	73.71^{ij}(59.13)	5.84^{l}(13.97)	**2.51^{l}(9.11)**
MS.5Kn.5N	-	0.5	-	0.5	34.65^{m}(36.0)	77.94^{h}(61.96)	7.02^{jk}(15.35)	**2.35^{l}(8.81)**
MSKn.5N	-	1.0	-	0.5	38.17^{l}(38.14)	78.10^{h}(62.08)	12.60^{ef}(20.78)	**12.33^{e}(20.5)**
MS2Kn.5N	-	2.0	-	0.5	36.62^{m}(37.2)	80.67^{fg}(63.90)	12.34^{f}(20.55)	**15.14^{d}(22.8)**
MS3Kn.5N	-	3.0	-	0.5	43.90^{j}(41.48	89.96^{b}(71.52)	10.35^{g}(18.75)	**17.02^{b}(24.3)**
MS4Kn.5N	-	4.0	-	0.5	34.17^{n}(35.75)	82.79^{ef}(65.46)	6.04^{kl}(14.21)	**16.93^{bc}(24.)**
MS5Kn.5N	-	5.0	-	0.5	31.75^{o}(34.28)	44.42^{q}(41.78)	5.97^{l}(14.13)	**7.95^{hi}(16.3)**
Mean					**42.25(40.40)**	**73.68(60.07)**	**9.53(17.58)**	**10.41(18.0)**
CD (0.05)					**2.33**	**2.31**	**1.37**	**1.79**

- Figures in parenthesis are transformed values (Arc-sine transformation).
- Values within column followed by different letters are significantly different at 5% probability level

Table 4. Combined effects of different plant growth regulators on *in vitro* rooting in gladiolus.

Culture media	Plant growth regulators mgl^{-1}				*In vitro* rooting response		
	IBA	**NAA**	**TDZ**	**Kn**	**Root proliferating shootlets (%)**	**No. of root per shootlets**	**Root length (in cm)**
MS.1IB	0.1	-	-	-	55.21^{l}(47.97)	14.33^{f}(22.23)	**6.28^{f}(14.46)**
MS.5IB	0.5	-	-	-	83.74^{b}(66.19)	22.31^{a}(28.17)	**15.37^{a}(23.06)**
MSIB	1.0	-	-	-	83.37^{bc}(65.91)	16.52^{cd}(23.97)	**10.37^{d}(18.78)**
MS2IB	2.0	-	-	-	78.28^{e}(62.20)	16.52^{cd}(23.97)	**11.42^{c}(19.73)**
MS3IB	3.0	-	-	-	83.15^{c}(65.74)	16.21^{d}(23.73)	**9.32^{d}(17.77)**
MS4IB	4.0	-	-	-	82.33^{cd}(65.12)	17.26^{bc}(24.54)	**8.48^{e}(16.92)**
MS5IB	5.0	-	-	-	47.28^{o}(43.42)	8.11^{ij}(16.52)	**7.77^{e}(16.18)**
MS.1N	-	0.1	-	-	46.21^{o}(42.81)	8.32^{i}(16.76)	**7.75^{ef}(16.16)**
MS.5N	-	0.5	-	-	78.12^{e}(62.09)	8.44^{i}(16.88)	**10.27^{d}(18.68)**
MSN	-	1.0	-	-	77.75^{e}(61.83)	12.36^{gh}(20.57)	**9.33^{d}(17.78)**
MS2N	-	2.0	-	-	75.16^{f}(60.08)	7.61^{j}(16.01)	**8.60^{e}(17.04)**
MS3N	-	3.0	-	-	51.28^{mn}(45.72)	6.68^{l}(14.97)	**8.25^{e}(16.69)**
MS4N	-	4.0	-	-	50.90^{n}(45.50)	6.28^{lm}(14.51)	**9.83^{d}(18.26)**
MS5N	-	5.0	-	-	27.28^{p}(31.47)	5.59^{m}(13.67)	**8.61^{e}(17.06)**
MS.1IB.5Td	0.1	-	0.5	-	52.31^{m}(46.31)	7.31^{k}(15.68)	**7.73^{f}(16.14)**
MS.5IB.5Td	0.5	-	0.5	-	81.30^{d}(64.35)	14.23^{f}(22.15)	**10.37^{cd}(18.78)**
MSIB.5Td	1.0	-	0.5	-	78.34^{e}(62.24)	8.89^{i}(17.34)	**9.79^{d}(18.23)**
MS2IB.5Td	2.0	-	0.5	-	76.12^{f}(60.72)	12.78^{g}(20.94)	**8.91^{de}(17.36)**
MS3IB.5Td	3.0	-	0.5	-	58.40^{k}(49.82)	12.12^{h}(20.36)	**8.34^{e}(16.78)**
MS4IB.5Td	4.0	-	0.5	-	62.16^{j}(52.02)	7.38^{jk}(15.76)	**6.56^{f}(14.83)**
MS5IB.5Td	5.0	-	0.5	-	58.31^{k}(49.76)	6.78^{kl}(15.09)	**6.39^{f}(14.64)**
MS.1IB.5Kn	0.1	-	-	0.5	55.90^{l}(48.37)	11.62^{h}(19.92)	**8.39^{e}(16.83)**
MS.5IB.5Kn	0.5	-	-	0.5	88.28^{a}(69.95)	17.33^{b}(24.59)	**12.52^{b}(20.70)**
MSIB.5Kn	1.0	-	-	0.5	72.16^{g}(58.13)	15.32^{e}(23.03)	**10.81^{c}(19.19)**
MS2IB.5Kn	2.0	-	-	0.5	68.38^{h}(55.76)	14.53^{ef}(22.40)	**9.58^{d}(18.00)**
MS3IB.5Kn	3.0	-	-	0.5	65.32^{i}(53.90)	7.28^{k}(15.65)	**8.72^{e}(17.17)**
MS4IB.5Kn	4.0	-	-	0.5	64.28^{i}(53.28)	6.61^{l}(14.89)	**8.48^{e}(16.90)**
MS5IB.5Kn	5.0	-	-	0.5	58.83^{k}(50.07)	5.84^{m}(13.98)	**8.38^{e}(16.79)**
Mean					**66.43(55.03)**	**11.29(19.26)**	**9.17(17.53)**
CD(0.05)					**1.18**	**0.79**	**1.47**

- Figures in parenthesis are transformed values (Arc-sine transformation).
- Values within column followed by different letters are significantly different at 5% probability level.

For culturing, corm slices of gladiolus were collected from field and washed with running tap water for 2 h for the removing of adhering soil particles. The corms of gladiolus have a outer brown protective covering that was removed with surgical blade before surface disinfestations. Then the corm

slice were placed into double distilled water containing 2-3 drops of surfactant Tween 20 for 10-15 min to remove the fine particles. The clean corm slice were then treated with 90% (v/v) ethanol for 30 s. Then 4 mm thick transverse and 4 mm longitudinal slices of corms was subjected to dipping in different concentrations of Bavistin® (BASF, Germany) in combination with two different surface sterilants i.e. $Ca(OCl)_2$ and $HgCl_2$ in different concentrations and combinations for diverse durations with initial vacuum of 100 psi for 5 min (Table 5). Finally corm slice was rinsed 4-5 times with sterilized double distilled water prior to inoculation. Four mm thick slices of corms measuring in diameter were excised with young active buds and cultured flat with side up in baby food bottles containing nutrient medium. All cultures, sealed with Lab film (Parafilm®) and incubated under complete darkness at 25±2°C for a week. Later *in vitro* cultured explants were subjected to photoperiod regime of 16 h light / 8 h dark cycle at an intensity of 2000-lux luminance provided by Photo-synthetically Active Radiation lamps at 25±2 ^{0}C and 60% RH. After 28-35 days of initial culturing, cultures were sub cultured on same medium (initial medium) for regeneration of plantlets. Multiple shoots, obtained from direct organogenesis (auxiliary bud proliferation) were transferred to elongation medium which was MS basal medium supplemented with 1.0 mg l^{-1} GA_3, 20.0g l^{-1} sucrose and 7.5 g l^{-1} agar powder. Cultured baby food bottles /culture tubes were subjected to 25±2^{o}C temperature and photoperiod regimes of 60 μmol m^{-2} s^{-1} luminance provided by cool fluorescent tubes for 16 hr. When root formation was not attained on regeneration medium, plantlets were subsequently transferred to MS rooting medium amended with different concentrations of IBA and NAA alone as well as IBA and NAA in combination with Kn and TDZ, 15.0 g l^{-1} sucrose and 7.5 g l^{-1} agar powder (Table 4). For regeneration, elongation and rooting, reduced level of sucrose was applied on the basis of work conducted by various scientists as well as preliminary experiences of this laboratory.

Table 5. Effect of different surface sterilizing and antifungal agents on recovery of aseptic culture in gladiolus.

Treatments	Concent-ration (%)	Exposure Time (in min)	Aseptic culture (%)	Survival of explants (%)
Ca (OCl) $_2$	10	10	12.69^{n} (20.85)	22.73^{o} (28.46)
Ca (OCl) $_2$	10	15	12.72^{n} (20.88)	31.26^{l} (33.98)
Ca (OCl) $_2$	10	20	33.88^{k} (35.58)	41.26^{j} (39.95)
Ca (OCl) $_2$	15	10	25.21^{m} (30.12)	46.73^{i} (43.11)
Ca (OCl) $_2$	15	15	29.33^{l} (32.77)	53.79^{g} (47.15)
Ca (OCl) $_2$	15	20	35.27^{j} (36.42)	62.05^{e} (51.95)
Ca (OCl) $_2$	20	10	33.88^{k} (35.58)	24.94^{n} (29.95)
Ca (OCl) $_2$	20	15	40.85^{i} (39.71)	32.63^{k} (34.83)
Ca (OCl) $_2$	20	20	44.82^{h} (42.01)	46.33^{i} (42.88)
$HgCl_2$	0.1	5	60.10^{d} (50.80)	67.11^{b} (54.98)
$HgCl_2$	0.1	10	45.99^{g} (42.68)	65.79^{c} (54.18)
$HgCl_2$	0.2	5	45.33^{h} (42.30)	64.00^{d} (53.11)
$HgCl_2$	0.2	10	36.10^{j} (36.91)	58.32^{f} (49.77)
Bavistin+Ca (OCl) $_2$	1+10	20	39.37^{ij} (38.85)	25.12^{n} (30.06)
Bavistin+Ca (OCl) $_2$	1+15	20	48.21^{f} (43.95)	48.17^{h} (43.93)
Bavistin+Ca (OCl) $_2$	1+20	20	50.93^{e} (45.51)	58.04^{f} (49.61)
Bavistin+ $HgCl_2$	1+0.1	10	77.37^{a} (61.57)	72.00^{a} (58.96)
Bavistin+ $HgCl_2$	1+0.2	10	73.05^{b} (58.71)	62.83^{e} (52.41)
Bavistin + $HgCl_2$	2+0.1	10	73.76^{b} (59.16)	34.05^{i} (35.68)
Bavistin+ $HgCl_2$	2+0.2	10	63.67^{c} (52.91)	28.10^{m} (32.00)
Mean	**10**	**10**	**44.12(41.36)**	**47.26(43.30)**
CD(0.05)	**10**	**15**	**1.06**	**0.80**

- **Ca (OCl) $_2$**- Calcium hypochlorite, **$HgCl_2$** - Mercuric chloride.
- Figures in parenthesis are transformed values (Arc-sine transformation).
- Values within column followed by different letters are significantly different at 5% probability level.

The rooted plantlets were carefully washed with running tap water to eradicate the adhering agar and were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were transferred under 30±2^{o}C and 60 ± 5% relative humidity for 20-30 d in an Environmental Growth Cabinet for hardening. Then these regenerants were transferred to the Net House for 30-35 d for acclimatization. Acclimatized plants finally transferred to the field.

The observations were recorded for callus induction, shoot proliferating efficiency, number of shoot (s)/explant and mean shoot length for corm slice cultures and root proliferating efficiency, number of root (s) and mean root length for *in vitro* rhizogenic response. The experiment was laid out in completely randomized design to find out the significance of different culture

medium combination. Each treatment was consisting of two replications. Per replication approximately 100-120 explants were excised and cultured on each media. The arc-sine transformations were made before the analysis of data, since all data were in percentage and were analyzed as per method suggested by Snedecor and Cochran (1967).

Results and Discussion

For controlling of contamination to achieve the goals of tissue culture, different methods with their own advantages and disadvantages have been suggested till now. Applying calcium hypochlorite, mercuric chloride, alcoholic and antibiotic solutions are the most common examples. In our investigation, a combination of 1% bavistin with 0.1% $HgCl_2$ exposed for 10 min showed remarkable aseptic culture establishment (77.37%) with maximum survival percentage (72.00%) so that, this concentration and combination was used for surface sterilization of corm slice explant.

In corm slice culture, plantlets were regenerated *via* direct (Fig.1 A-F) as well as indirect (Fig.1G-I) organogenesis. In direct organogenesis, adventitious structures were developed on explant surface. Adventitious formation started approximately 4-10 d from initial culturing. However, the duration varied from culture to culture and in a few cases adventitious structures formed after 25-30 d. With time, these adventitious structures initiated single shoot (Fig.1B), and elongated (Fig.1C), formed double (Fg.1 D) and multiple shootlets (Fig.1E-F). In indirect approach, plantlets were regenerated from callus mass. The first response of cultured corm slice was similar after 4-7 days and mostly independent from explant and culture media. All explants became swollen and no callus proliferation was evident during first few d.Callus proliferation usually started from the portion in contact with the medium and spread around the corm slice after 7-10 d of culture (Fig.1 G). These calli were of different textures and structures. In indirect organogenesis, single shootlet initiated from the nodules arising on the surface of the callus (Fig.1 H). Shoot formation started approximately 7 d from initial culturing, however, the duration varied from culture to culture and in a few cases shootlets formed after 30 d of initial culturing. Multiple shoots also initiated *via* indirect organogenesis (Fig.1 H-I). Most of the calli, after prolonged culturing on the induction media gave rise to plants. However, subculturing of these shoots/calli on regeneration medium allowed higher plantlet regeneration. In most of the cases complete plants *i.e* shoot with root (Gammo-rhizogenesis) *in vitro* were observed. In a few cases rhizogenesis alone was also seen that has no future. In cases where root formation was not achieved, shootlets were subsequently

transferred in to rooting medium (Fig.1J). Rooted plantlets were elongated after transferring into elongation medium. Complete plantlets were subsequently transferred under 28±2^{o}C and 60±5% Relative Humidity for 20-25 d in a Environmental Growth Cabinet for hardening (Fig.1K). Afterward these regenerants were transferred to the Net House/ Poly House for 25-30 d before transferring in the field. *In vitro* cormlet formation also investigated during course of present investigation (Fig.1 L).

For gladiolus tissue culture experiments, mostly MS basal medium was employed by scientists (Hussey, 1977; Longan and Zettler, 1985; Kamo*et al*., 1990; Dantu and Bhojwani, 1992; Begum and Haddiuzaman, 1995; Dantu and Bhojwani, 1995; Grewal*et al*., 1995; Kamo*et al*., 1995; Nagaraju and Parthasarathy, 1995; Prem*et al*.,1995; Steel *et al*., 1997; Kumar *et al*., 1999; Ahmad *et al*., 2000; Babu and Chawla, 2000; Boonvanno and Kanchanapoom, 2000; Sinha and Roy, 2002; Priyakumari and Sheela, 2005; Emek and Erdag, 2007; Aftab*et al*., 2008; Budiarto, 2009; Memon*et al*., 2010; 2012). As per studies conducted so far, compositions of culture medium do not seem to play major role in *in vitro* response as much as the type and concentration of plant growth regulators. During the present investigation, basal MS medium was used throughout the experiment as this has been found more responsive than other in course of preliminary experiments. conversely for each explant culture, various types and concentrations of plant growth regulators in different combinations were supplemented into basal MS medium. In earlier studies different scientists used various plant growth regulators to modified basal media in gladiolus such as NAA(Hussey, 1977; Ziv,1979; Bajaj *et al*., 1983; Lehsem, 1988; Kamo*et al*., 1990; Kamo, 1994;1995; Stefaniak, 1994; Remotti, 1995; Kasumi *et al*., 1998; Kumar *et al*., 1999; Boonvanno and Kanchanapoom, 2000; Sinha and Roy, 2002; Priyakumari and Sheela, 2005), 2,4-D (Kumar *et al*., 1999; Misra and Singh, 1999; Pathania *et al*., 2001; Priyakumari and Sheela, 2005), IBA(Steinitz *et al*., 1991; Hussain*et al*., 1994; Begum and Haddiuzaman, 1995; Kumar *et al*., 1999; Sinha and Roy, 2002; Priyakumari and Sheela, 2005; Roy *et al*., 2006), BAP (Hussey, 1977, Ziv,1979; Dantu and Bhojwani, 1987; Steinitz and Lilien-Kipnis, 1989; Kamo, 1994;1995; Grewel *et al*., 1995; Nagaraju and Parthasarathy, 1995; Sen and Sen, 1995; Kumar *et al*., 1999; Babu and Chawla, 2000 Hussain *et al*., 2001; Aftab *et al*., 2008; Memon *et al*.,2010),TDZ (Aslam *et al*., 2012), Kn (Ginzburg and Ziv, 1973; Hussey, 1977; Ahmad *et al*., 2000; Babu and Chawla, 2000) and combinations of different auxins with cytokinins (Hussey,1977; Ziv and Lilien-Kipnis, 1990; Hussain *et al*.,1994; Kumar, 1999; Pathania *et al*.,2001; Priyakumari and Sheela, 2005; Prasad and Gupta,2006) with different mrophogenic response. So on the basis of related works conducted by various scientists the world wide

and preliminary experiments conducted at this laboratory,during present investigation, three different sets of culture media were formulated by supplementing different auxins (alone), diverse cytokinins (sole) and auxins NAA and 2,4-D in combination with cytokinins BAP and Kinetin to basal MS media.

The analysis of variance presented in Table 1-5 revealed highly significant ($p<0.05$) differences among the response of different culture media combinations in terms of overall callus induction, shoot proliferating efficiency, number of shoot (s) per explant and mean shoot length for corm slice explant and root proliferating efficiency, number of root (s) and mean root length for *in vitro* rhizogenic response. It indicates the presence of considerable amount of variability amongst different culture media combinations.

For corm slice culture, induction media MS2B.5D/ MSB.5D amended with relatively higher concentration of a cytokinin in combination with a lower concentration of an auxin facilities higher degree of callus induction. Culture media MS3D/ MS3N/MS2D containing a moderate to higher concentration of 2,4–D and NAA also initiated callus in high frequencies than culture medium containing other auxins 2,4,5-T and IBA as well as all cytokinins irrespective of all tested concentration that suggesting that 2,4-D and NAA either sole or in combination with higher concentration of BA is better for callus proliferation from cultured corm slice. Kamo (1994) and Grewel *et al*.(1995) also documented similar findings for diverse explant cultures in gladiolus. In terms of shoot proliferating ability, number of shoot (s) and shoot of higher length, culture media containing auxins (alone) in varying concentrations capitulated lower shoot proliferation efficiency, *i.e.* lesser number of explants proliferated shoots, lesser number of shoot (s) per explant and shoots of minimum length. Shoot proliferation in very lower frequency has been evidenced on medium supplemented with application of 2,4-D as sole, however, medium fotified with NAA responded much better. Earlier studies reported that cytokinin is required in shoot organogenesis (Remotti, 1995; Kumar *et al*., 2002 ; Aslam *et al*., 2012). Hence, in order to achieve the best *in vitro* response, basal medium was also fortified with different types of cytokinins in varying concentrations. Culture media fortified with cytokinin as sole in higher concentrations (BAP and Kn in range of 1.0-4.0 mgl^{-1} and zeatin and TDZ in range of 0.1-0.2 mgl^{-1} retorted well suggesting that a higher concentration of cytokinin is necessitated for this function. Earlier studies also demonstrated that cytokinin induced early bud sprouting in many species including gladiolus (Ginzburg and Ziv, 1973; Hussey, 1977; Ziv,1979; Bajaj *et al*., 1983; Dantu and Bhojwani, 1987; Steinitz and Lilien-Kipnis, 1989; Kamo, 1994;1995; Grewel *et al*., 1995; Nagaraju and Parthasarathy,1995; Sen and Sen, 1995; Kumar *et al*., 1999;

Ahmad *et al*., 2000; Babu and Chawla, 2000; Hussain *et al*., 2001; Aftab *et al*., 2008; Memon*et al*.,2010)**.**However, shoot proliferating efficiency, number of shoot (s) with higher length were augmented when auxins and cytokinins were added into culture medium in combinations. Culture media MS3B.5N/MS2B.5N containing relative higher concentrations of a cytokinin BAP in combination with lower concentration of an auxin NAA, exhibited higher *in vitro* response (more than 97% explants proliferated shoots, ~19.35shoots/explant with shootlet of ~22 cm length) than culture medium containing a higher concentrations of cytokinins as sole (~96% explants proliferated shoots, ~17 shoots/explant with shootletof ~12 cm length) as well as an auxin as alone. This finding is an accordance with (Hussey,1977; Ziv and Lilien-Kipnis, 1990; Hussain *et al*.,1994; Kumar, 1999; Pathania *et al*.,2001; Priyakumari and Sheela, 2005; Prasad and Gupta, 2006) as they suggested that many commercial ornamental plants are being propagated by *in vitro* culture on the culture medium containing auxins and cytokinins in combinations. Furthermore, addition of strong cytokinin (BAP) with NAA promoted better shoot formation as compared to weaker auxin like 2,4,5-T (Aslam *et al*., 2012). Besides, Aslam *et al*. (2012) reported that the supplementation of TDZ in culture medium resulted shoots regeneration in *Echinacea pursuera* L. Lower concentration of TDZ promoted direct shoot regeneration and higher concentrations promoted callus induction in *Hagenia abyssinica* (Bruce). The present study showed similar findings, as TDZ at concentration of 0.4 $mg.l^{-1}$ produced shoots in higher numbers with better shoot proliferating efficiency.However, in this study, at a higher concentration of TDZ (more than 0.5 $mgl-^{1}$), inhibited development and growth of shootlets and promoted callusing.

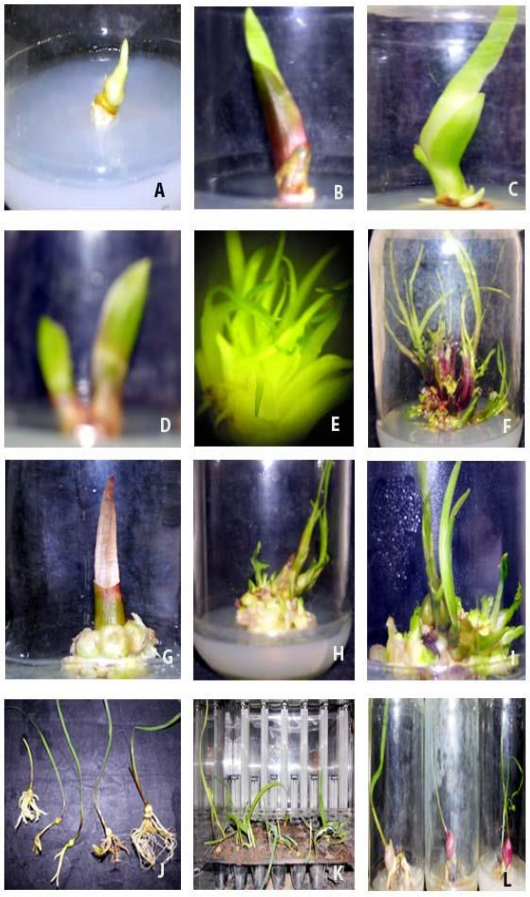

Figure 1. Plant regeneration in *Gladiolus hybridus*: **A.** Cultured corm slice after 3-5 d; **B.** Proliferation of single shoot after 7-10 d; **C.** Elongated single shoot; **D.** Formation of double shoots after 10-14 d; **E.** Proliferation of multiple shoots after 14-21 d; **F**. Formation of multiple shoots after 21-28 d; **G.** Callus formation after 7-10 d from corm slice; **H.** Multiple shoots initiation from callus cultures; **I.** Multiple shoot (s) formation from callus culture; **J.** *In vitro* rooting; **K.** Regnerants transferred in Environmental Growth Cabinet after 40-45 d for hardening and **L**. *In vitro*cormlet formation during long culture phase.

During present research, rhizogenic frequency was found to be higher after transferring shootlets into rooting medium. In general, auxins like IBA (Steinitz *et al*., 1991; Kumar *et al*., 1999; Sinha and Roy, 2002; Priyakumari and Sheela, 2005; Roy *et al*., 2006) as well as NAA (Bajaj *et al*., 1983; Lehsem, 1988; Kamo *et al*., 1990; Kamo, 1994;1995; Sinha and Roy, 2002; Priyakumari and Sheela, 2005) were effective in inducing *in vitro* rooting. Maximum *in vitro* root proliferation was observed on culture media MS.5IB.5Kn. However, roots in higher number (s) with higher length were recorded on rooting medium

MS.5IB. The results clearly indicated that rooting of *in vitro* shoots of gladiolus required moderate concentrations of auxin: IBA at higher nutrient status. Auxins promoted adventitious root development on intact plants as well as excised stems. Of these, IBA was the most effective than any other plant growth regulators in the most of the cases apparently because it is not destroyed by IAA oxidase or other enzymes and therefore persists longer. The above results are in conformity with the earlier findings of Hussain *et al.* (1994).

Usually explant cultures exhibiting higher number of morphogeniccalli regenerated more plantlets, however, the process of morphogenesis was not a predication of higher regeneration frequencies. For *in vitro* cultures, single genotypes or culture medium exhibiting higher morphogenic callus formation may regenerate lesser plants as compared to lower morphogeniccalli. Such deviations occurred as a single morphogeniccalli produced none or many plantlets (up to 19 in cluster). In addition, not all the shootlets developed into complete plants *i.e.* shoots with roots. Sometimes shoot could not develop after initiation, in a few cases they were deformed and not all the shootlets developed roots even after transferring in rooting medium. During present study cormlet formation has also been acquired if regenerated plantlets kept in rooting/elongation medium for long time containing IBA/NAA. Promotion of cormlet formation and root induction was also reported by Sinha and Roy (2002) on MS medium containing up to 2.0 mgl^{-1} NAA. The results of present study displayed that under conditions of these experimentations, *in vitro* response of the corm slice explant culture were under genetic control. As explants cultured on different culture media combinations significant differences were observed for all the culture phases. Trend shown in the present investigation suggested that this explant could be used for micropropagation and advance biotechnological work.

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