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In-vitro Response of Gladiolus (var. American Beauty) towards Plant Growth Regulators Using Cormel Explants

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Gladiolus is one of the most potential bulbous cut flower crops cultivated widely in India due to its attractive and long lasting spikes. The plant is propagated vegetatively through corms and cormels, but due to low rate of multiplication, its cultivation is hindered. Also, it is grown through underground modified stems, they are more often attacked by soil borne diseases such as *Fusarium* corm rot. *In-*

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vitro propagation techniques, assumes significance, especially for securing rapid multiplication of quality planting propagules using dormant cormlet explants and media. The results of the study revealed that the MS growing medium supplemented with 2 mg/l BAP showed improved results in respect of sprouting of explants (54.46 %), days required for sprouting of explants (8.68 DAI) and shoot length at 30 (6.32 cm), 60 (12.40 cm) and 90 (13.21 cm) DAI. The *in vitro* regenerated gladiolus plantlets when placed in the rooting MS medium supplemented with 4 mg/l IBA showed earlier root initiation (8.31 DAI) and higher number of roots/shoot (5.37 DAI) as compared to the rest of the treatment combinations. Hardening medium consisting of garden soil + sand + vermicompost (1:1:1) showed greater survivability (50.98 %) of plantlets. Thus, the study has been initiated an efficient protocol for *in-vitro* propagation of gladiolus through cormlets cultured in MS media containing BAP and IBA as shooting and rooting plant growth regulators and subsequent acclimatization in garden soil + sand+ vermicompost medium.

Keywords: Gladiolus; cormels; growth regulators; in-vitro; quality planting.

1. INTRODUCTION

Gladiolus (*Gladiolus sp.*) is one of the most popular bulbous plants in India due to its magnificent and colourful spikes that have a long vase-life [1]. Major gladiolus growing states of India are Karnataka, Maharastra, Tamil Nadu, Punjab, Haryana, Delhi, Uttar Pradesh, West Bengal, Sikkim and Nagaland (Anil et al. 2013). Earlier it was considered as a crop for temperate regions and its cultivation was restricted to the hilly areas particularly in the North Eastern region, which still continues to supply the planting material to most parts of the country [2]. It is one of the principal flower crops of Terai region of West Bengal. The Terai region possesses unique climatological advantages, which is suitable for gladiolus cultivation for most part of the year (except in rainy season). The commercial method of propagation of gladiolus is through multiplication of new corms and cormels [3]. However, it is limited due to low rate of multiplication to meet the local demand. The commercial production of corms and cormels is also greatly affected by *Fusarium* corm rot and high percentage of spoilage of corms during storage leading to the non-availability of enough planting material [4]. Besides, one mother corm normally produces 1-2 daughter corms and about 25 cormels each season[6]. These cormels require three to four seasons to attain standard size of flowering spike and daughter corms. Still, the lesser the size of cormlets, the lesser will be the chance of survivability after planting in the field. Apart from this, the dormancy of the corms and cormels is another cause of non-availability of planting materials in proper time [5]. Dearth of quality planting material (QPM) at proper time, thus become the potential bottleneck for successful promotion of commercial cultivation of this prospective crop of Terai agro-climatic zone of West Bengal. Therefore, to increase economical production, it is highly desirable to upgrade the conventional methods of propagation in order to increase the rate of production of propagules. Plant biotechnology may pave the ways in upgrading conventional production system for sustainability. Among all the modern biotechnological tools, plant tissue culture may assume to produce large scale disease frees QPM in the stipulated time to complement traditional nursery methods under control environment [6]. Several scientists have reported the direct and complete regeneration of plantlets and *in-vitro* cormel production from different explant sources [7]. In view of the above short coming in gladiolus production the present investigation was conducted to develop an efficient protocol for production of large scale disease-free QPM *in-vitro* using different culture medium and plant bio-regulators and subsequent acclimatization of the plantlets to the natural environment.

2. MATERIALS AND METHODS

The explants used were non-sprouted cormlets of gladiolus (*Gladiolus grandiflorus*), collected from the Department of Floriculture, Medicinal and Aromatic plants, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya. The experiment was conducted at Plant tissue culture laboratory of the Faculty of Horticulture during 2011-12 and 2014-15. The cormlets were dehusked and washed by rubbing gently under running tap water using a nonionic detergent (Tween 20) for 15 minutes, followed by rinsing continuously with distilled water for half an hour. These were then surface sterilized with Carbendazim 50 W.P. (Bavistin) 0.1 % (1g/lit of water) for ½ an hour followed by Plantomycin (Streptomycin Sulphate 9% + Tetracycline Hydrochloride 1%) for ½ an

hour. The surface sterilized cormlets were rinsed thoroughly with sterile distilled water for 5 to 6 times followed by quick dip (5 to 6 sec.) in absolute alcohol. The cormlets were then treated with $HgCl_2$ for 4-5 minutes under laminar air flow.

Murashige and Skoog's [8] (MS) medium was used as basal medium with sucrose (3%), myo-inositol (100 mg w/v) and agar agar (0.8%). Its pH was adjusted between 5.6 and 5.8 with the help of 0.1N NaOH or 0.1N HCl prior to addition of agar agar and medium was autoclaved at a pressure of 105 KPa (121°C) for 15 minutes to make it free from microbes. MS + 16 different combinations of growth regulator consisting of 4 levels of each of BAP and Kinetin (0.5, 1, 1.5, 2 mg/l) alone and each 4 level of BAP and Kinetin in combinations with GA_3(1 mg/l) were used for shoot regeneration purpose. Surface sterilized explants were then placed into the different sterilized nutrient medium (inoculation) under aseptic condition. The cultures were maintained under a controlled environment. Temperature was maintained at 23±2˚C, under diffused light (fluorescent light of 1000-2000 lux) over a light/dark photoperiodic cycle of 16 hours light/8 hours dark with ≥ 90% relative humidity. The microshoots regenerated from the cormlets cultured in the growing medium were transferred to the 8 different combinations of rooting medium for root regeneration comprises of IBA (1, 2, 3, 4 mg/l) alone and in combinations of NAA (1 mg/l) with basal MS medium.

In-vitro developed plantlets were acclimatized using different hardening media *viz*., garden soil, sand, vermiculite and vermicompost in different combinations**.** The plantlets regenerated from cultured cormlets were transplanted into small pots/plastic cup and covered with polythene bags to prevent excessive transpiration. The pots were kept in a culture room for primary hardening. After 3-4 days the polythene bags were gradually removed but the pots were still kept in diffused light in the culture room for another 5-10 days. The plants after 10-15 days were transferred into a greenhouse under shade net cover and gradually the shade level was reduced. After 10-15 days, the acclimatized plants were planted in pots having equal proportion of soil and well rotten FYM.

The experiment was laid out following the Completely Randomized Design (CRD) consisting of 16 number of growing media, 8 number of rooting media and 4 number of hardening media were replicated thrice as for growing and rooting media and five times for hardening media. 15 numbers of explants, cultures and plantlets were considered for each replication respectively. Data observation were made for following parameters *viz*., Sprouting of cormlets (%), Days required for sprouting (days), Length of shoot (cm), Days for root initiation (days), No. of roots/shoot, Survivability % during acclimatization and the data were analysed following Ronald Fisher's statistical hypothesis testing technique through MSTATC (Mathematic and Statistic Compiler) software.

3. RESULTS AND DISCUSSION

Proper surface sterilization may improve the situation markedly, whereas, the proper balance of nutrient and phytohormones in growing media may overcome the problem lies in successful production of disease-free elite plants of true-to-type. Initially [9] reported micro-propagation as a tool for raising of disease free gladiolus plants using inflorescence stalk as explant. With gradual advancement of technique and modification of different nutrient media, the present investigation has been initiated to develop an efficient protocol regarding *in-vitro* propagation of gladiolus, a commercially important ornamental crop of West Bengal.

The results of the investigation established the pathway of plantlet production followed by successful acclimatization through the following phases like selection of explant from field grown crop, surface sterilization of the selected explants, role of nutrients and phytohormones in regeneration from explants, rooting of micro-shoots and subsequent acclimatization of plantlets to outer environment. Nutrient medium is an absolute requirement for successful regeneration whereas surface sterilization, sterile inoculation and culture environment reduces the chance of infection by pathogens [10]. Cormels treated with the suitable combination of growth regulators containing less of auxin and more of cytokinin can directly or indirectly causes the shoot induction, rooting and ultimately the corm production [11]. Here, Gibberellic acid and cytokinin were used either singly or in combination, both of which are well known for their growth promoting activities in plant tissue culture. The explants treated with T_4 and T_{11} showed the highest percent (54.46 %) of sprouting of cormlets (Fig. 1) which was statistically at par with T_5 (53.93 %), T_3 (51.00 %), T_6 (51.00 %), T_{10} (48.47 %), T_2 (48.07 %), T_8 (47.93 %) and the lowest percent (33.00 %) of

spouting was noticed with T_{12} and T_{14}. Also, the appreciated result on minimum (8.68) days for sprouting of cormlets [days after inoculation (DAI)] was obtained with T_4, which was statistically at par with T_1 (8.92 days), T_2 (9.42 days) and T_7 required maximum number of days for sprouting (17.33days) DAI (Table 1). The breaking of dormancy of the cormlets through *in-vitro* culture might be due to the lowered content of inherent ABA and increase in sensitivity to GA_3 and also the maintenance of low level of ABA/GA_3 ratio as a result of hormonal balance and action [12].

Hussey [13] reported that the *in-vitro* produced cormel dormancy can be broken upon sub-cultured on a medium containing BA. Silva et al. [14] found that the regeneration of gladiolus from dormant cormlets suggested the fact regarding breaking of dormancy through hormonal action followed by consequent supply of readily available nutrients suitable to growing tissues from the medium. Induction of shoot bud within 7-8 days[16] and 7.8 days with maximum shooting (96%) [15] was observed in B5 (Gamborg) and MS medium respectively when it was supplemented with 1.0mg/L of BAP. Similar kind of findings was also reported by Budiarto [16] and [17].

The combinations of GA_3 and BAP (T_{11}) enhanced in sprouting as gibberellic acid and BA play an important role in cell elongation and cell division. Also, BA is known for activating cell division which ultimately promoting meristematic activity [18]. Sajjad Y et al. [19] reported the combined effect of BA and GA_3 on an overall accelerating the sprouting response of lily bulbs that was noted due to the cells stimulated to multiply by BA, were also induced to elongate by the activity of GA_3 [19] reported the effectiveness in combined (BAP and GA_3) used of plant growth regulators at low concentration upon germination % and number of sprouted buds of gladiolus and hence composition of suitable nutrient medium is also another important factor in plantlets regeneration.

Further, the study revealed that T_4 also produced the maximum shoot length at 30 (6.32 cm) (Fig. 2), 60 (12.40 cm), and 90 (13.21 cm) DAI. The next best effective result was observed with T_{14} (4.83 cm) and T_7 (9.33 cm) respectively, at 30 and 60 DAI. However, at 90 DAI T_7 (10.87 cm) was statistically at par with T_4. The minimum *in-vitro* shoot length 1.03 cm (T_{16}), 2.62 cm (T_9) and 3.79 cm (T_9) were produced respectively, at 30, 60 and 90 DAI (Table 1). Kumar et al. [20] reported maximum shoot length in MS medium supplemented with 1.0mg/l of BAP. The successful regeneration of gladiolus *in-vitro* with the use of BAP were also reported [21-23].

Table 1. Effect of different concentrations of BAP and Kinetin alone or in combination with GA_3 in MS culture medium on sprouting of cormlets (%), days required for sprouting and *in vitro* shoot length of gladiolus at 30, 60 and 90 DAI

Treatment (T)	Sprouting of cormlets (%)	Days required for sprouting (DAI)	Shoo length at (cm)		
			30 DAI	60 DAI	90 DAI
T_1(MS + 0.5 mg/l BAP)	41.93	8.92	2.73	5.22	6.02
T_2(MS + 1 mg/l BAP)	48.07	9.42	3.73	8.43	9.34
T_3 (MS + 1.5 mg/l BAP)	51.00	13.58	2.88	7.50	8.75
T_4(MS + 2 mg/l BAP)	54.46	8.68	6.32	12.40	13.21
T_5(MS + 0.5 mg/l Kinetin)	53.93	12.50	1.93	6.94	7.29
T_6(MS + 1 mg/l Kinetin)	51.00	13.17	3.58	8.56	8.40
T_7(MS + 1.5 mg/l Kinetin)	36.07	17.33	3.78	9.33	10.87
T_8 (MS + 2 mg/l Kinetin)	47.93	13.67	4.08	8.13	8.53
T_9(MS + 0.5 mg/l BAP + 1 mg/l GA_3)	41.93	15.58	1.62	2.62	3.79
T_{10}(MS + 1 mg/l BAP + 1 mg/l GA_3)	48.47	14.28	1.71	2.97	4.23
T_{11}(MS + 1.5 mg/l BAP + 1 mg/l GA_3)	54.46	13.22	2.73	4.42	5.21
T_{12}(MS + 2 mg/l BAP + 1 mg/l GA_3)	33.00	14.50	1.84	4.33	5.07
T_{13}(MS + 0.5 mg/l Kinetin + GA_3)	39.15	15.18	1.97	4.16	4.92
T_{14}(MS + 1 mg/l Kinetin + 1 mg/l GA_3)	33.00	16.17	4.83	5.77	6.70
T_{15}(MS + 1.5 mg/l Kinetin + 1 mg/l GA_3)	39.00	13.42	1.87	4.87	5.35
T_{16}(MS + 2 mg/l Kinetin + 1 mg/l GA_3)	36.07	13.54	1.03	4.82	5.83
SEm (±)	2.561	0.563	0.467	0.967	1.285
CD (P=0.05)	7.377	1.591	1.319	2.732	3.630

MS medium containing 4mg/l of IBA showed satisfactory *in-vitro* rooting (Fig. 3) of gladiolus as compared to the rooting media containing IBA at lower concentration alone or with a combination of NAA @ 1 mg/l. The sprouted cormlets treated with R_4 took minimum (8.31) days for *in-vitro* root initiation, which was statistically at par with R_8 (8.61 days), R_7 (8.66 days) and R_5 required the maximum days (10.78 days). The minimum time of root induction (7.2 days) with maximum rooting (96%) was observed with 1.0 mg/L of IBA [15]. Similarly, R_4 produced the maximum number of roots/shoot (5.37) at 30 days after inoculation, which was statistically at par with R_6 (5.34) and R_7 developed the minimum number of roots/shoot (3.65) (Table 2). Haouala and Salhi [24] reported that the culture medium containing 1 – 4 mg/l IBA resulted satisfactory (100%) shoot rooting with highest number of roots was obtained at 0.5 – 1 mg/l IBA concentration. The role of IBA in rooting of micro-shoots for *in-vitro* plantlet regeneration of ornamentals is also reported in *Dracaena sanderiana* [25]. In the present experiment successful *in-vitro* rooting of gladiolus took place at a particular concentration of IBA might be due to the nutritional and hormonal balance at that particular concentration resulting higher C:N ratio leading to formation of earlier and higher rooting in *in-vitro* cultured cormlets. This dose specificity of particular plant growth regulator in micropropagation of ornamentals was also reported in *Magnolia sirindhorniae* [26]. The results of the present investigation are in close conformity of the findings of [27,21,28,29] in gladiolus.

The ultimate success of plant tissue culture is acclimatization (Fig. 4) of *in-vitro* regenerated plantlets to the outer environment. Out of four different hardening medium (Table 3) tried on regenerated gladiolus plantlets, the hardening medium (H_3) containing equal proportion of garden soil, sand and vermicompost (1:1:1) resulted highest survivability percentage (50.98 %) whereas the medium (H_1) containing garden soil and sand (1:1) was noticed with lowest survivability (36.39 %). During hardening a thin film of water was maintained on the plant surface initially, that facilitated the plants to remain cool and compensated the water loss through transpiration. The process of acclimatization was done in polyhouse under agro-shade net. After 12 days intensity of water application and magnitude of shade were reduced. The plant growth was noticed after a month. A similar hardening procedure was used in grape plants [30] and in Carnation [17]. Also [31] reported the acclimatization of the lilium *in-vitro* rooted plantlets in the medium composed of sand, soil and vermicompost @ 1:1:1 and found 90% survival of plantlets under *ex vitro* conditions on soil [32].

Table 2. Effect of different concentrations of IBA and NAA alone or in combination on days to root initiation and number of roots/ shoots at 30 DAI of gladiolus *in vitro*

Treatment (R)	Days for root initiation	No. of roots/shoot at 30 DAI
R_1(MS + 1 mg/l IBA)	10.475	4.045
R_2(MS + 2 mg/l IBA)	10.670	4.475
R_3(MS + 3mg/l IBA)	8.551	4.423
R_4(MS + 4mg/l IBA)	8.312	5.375
R_5(MS + 1 mg/l IBA + 1 mg/l NAA)	10.786	4.100
R_6(MS + 2mg/l IBA + 1 mg/l NAA)	10.562	5.348
R_7(MS + 3mg/l IBA + 1mg/l NAA)	8.667	3.650
R_8(MS + 4mg/l IBA + 1 mg/l NAA)	8.611	4.300
SEm (±)	0.380	0.247
CD (P=0.05)	1.085	0.716

Table 3. Effect of different compositions of hardening medium on survivability rate (%) of in-vitro regenerated plantlets of gladiolus during acclimatization

Treatment (H)	Survival % during acclimatization
H_1- Garden soil + sand (1:1)	36.39
H_2- Garden soil + Vermiculite (1:1)	42.36
H_3- Garden soil + sand+ vermicompost (1:1:1)	50.98
H_4- Garden soil + Vermiculite + vermicompost (1:1:1)	45.27
SEm (±)	0.259
CD (P=0.05)	0.777

Fig. 1. *In-vitro* sprouting of dormant cormlet

Fig. 2. Shooting of cormlet explants

Fig. 3. Rooting of cormlet explants

Fig. 4. Acclimatization of plantlets

Figs. 1-4. Regeneration of Gladiolus plantlet

4. SUMMARY AND CONCLUSION

The experiment on micropropagation of gladiolus from dormant cormlets explant where the growing medium containing MS supplemented with 2 mg/l BAP Showed improved result in respect of % of sprouting (54.46 %), days required for sprouting of explant (8.68 days after inoculation) and shoot length at 30 (6.32 cm), 60 (12.40 cm) and 90 (13.21 cm) days after inoculation (DAI). The regenerated gladiolus in-vitro when placed in the rooting medium containing MS supplemented with 4 mg/l IBA showed earlier root initiation (8.31 DAI) and higher number of roots/shoot (5.37) as compared to the others. In-vitro regenerated plantlets of gladiolus when tried to acclimatization to outer environment utilizing T3 [Garden soil + sand+ vermicompost (1:1:1)] as hardening media showed greater % of survivability (50.98 %). Hence the growing media containing MS + 2 mg/l BAP (T4) and rooting media containing MS + 4 mg/l IBA (T4) may be recommended for micropropagation of

gladiolus from dormant cormlets explant followed by subsequent acclimatization under T3 [Garden soil + sand+ vermicompost (1:1:1)] media in the Terai region of West Bengal.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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