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Shoot multiplication of gerbera callus grown from flower buds under different growth regulators composition

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ABSTRACT

An experiment was conducted in at Advanced Seed Research and Biotech Center (ASRBC), ACI Limited; Dhaka, Bangladesh during the period of September to October, 2014 to evaluate different growth regulator composition towards shooting from callus produced using the flower bud of selected mother plant of gerbera. Callus grown from flower buds were subjected to six growth regulator composition viz.T_0: 0 mg/l BAP + 0 mg /l NAA, T_1: 1 mg/l BAP + 0.01 mg /l NAA, T_2:2 mg/l BAP + 0.01 mg /l NAA, T_3: 3 mg/l BAP + 0.01 mg /l NAA, T_4:4 mg/l BAP + 0.01 mg /l NAA, T_5:5 mg/l BAP + 0.01 mg /l NAA. Number of responsive explants (34.3), Percent (%) of responsive explants (85.8%), Number of days taken for shoot initiation (16 days), Number of shoots per clump (8.0), Number of leaves per shoot (7.70), Shoot length (25.4 mm) were found best in T_3 whereas the lowest was observed from T_1 and T_5. Thus, combination of 3 mg/l BAP + 0.01 mg /l NAA can be considered as the best composition of growth regulators for shoot multiplication of gerbera callus grown from flower buds.

Key Words: *Shoot multiplication, BAP, NAA and Protocol development*

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I. Introduction

Gerbera jamesonii Bolus one among the forty species within its genus (Das and Singh, 1989) is a flower of high commercial significance being one of the leading cut flower and ranking among the top tens (Parthasarathy and Nagaraju, 1995). Color variation has made this flowering plant attractive for use garden decoration, such as herbaceous border, bedding and pots and for cut flowers as it has a long vase life (Chung et al. 2005; Chauhan, 2005). Gerbera is generally propagated by vegetative means in Bangladesh as it maintains genetic purity (Peper et al. 1971) usually through clump division although it can also be propagated by cuttings (Schiva, 1975). Commercial production using these propagation methods is quite troublesome and slow to maintain commercial practicability. Also the quality of the

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flowers produced cannot be ensured. As such a tissue culture protocol is needed to sustain as well as ensure the quality seedling of gerbera for the farmers in Bangladesh to develop this commercially promising flower. Tissue culture protocol for propagation of ornamental plants studied by several authors (Siddique et al. 2006; Siddique et al. 2007; Sultana et al. 2011). However, in a previous experiment by Parvin et al. 2017, as a first step towards a sustainable protocol development, it was found that flower bud of gerbera serves as the best explant for callus induction. The following experiment was the continuation of the process to find out the best growth regulator composition for shoot multiplication of calluses grown from flower bud.

II. Materials and Methods

The experiment was carried out at Advanced Seed Research and Biotech Center (ASRBC), ACI during the period from September to October 2014 to evaluate different growth regulator concentration towards shooting from callus produced using the flower bud of selected mother plant of gerbera. It has been already found from a previous experiment that flower bud is the most promising explant for Gerbera in producing callus (Parvin et al. 2017). The present experiment is going to be the next step in development of the standard tissue culture protocol of gerbera for Bangladesh. A gerbera cultivar producing double flower with magenta ray floret and black yellow disc floret was selected as mother plant. Immature inflorescences of 0.5 to 0.7 cm in diameter were collected from the plants. After sterilization, the buds were cut into two halves. The explant washed under running tap water with few drops of Tween twenty for 15 – 20 minutes. Then they were immersed in 70% ethanol for 1 minute and washed thoroughly with distilled water. They were later immersed in 0.1 per cent $HgCl_2$ for 7 minutes and finally washed with double glass distilled water for 3-4 times in laminar air flow cabinet to remove any traces of $HgCl_2$. Each explant was inoculated separately on solid medium and temperature of the culture room was maintained within 25± 1ºC by an air conditioner and 16 hour photoperiod was maintained along with light intensity of 3000 lux for proper growth and development of culture. The developing cultures are then subjected to the following growth regulator concentrations to screen out the best one for shoot multiplication.

Growth regulators:

T_0: 0 mg/l BAP + 0 mg /l NAA	T_1: 1 mg/l BAP + 0.01 mg /l NAA
T_2: 2 mg/l BAP + 0.01 mg /l NAA	T_3: 3 mg/l BAP + 0.01 mg /l NAA
T_4: 4 mg/l BAP + 0.01 mg /l NAA	T_5: 5 mg/l BAP + 0.01 mg /l NAA

Data on number of responsive explants, % of responsive explants, number of days taken for shoot initiation, number of shoots per clump, number of leaves per shoot and shoot length (mm) were collected and were statistically analyzed using MSTAT-C computer package program. Mean for every treatments were calculated and analysis of variance for each one of characters was performed by F–test (Variance Ratio). Difference between treatments was assessed by Least Significant Difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

III. Results and Discussion

Number of responsive explants: The number of explants responded recorded after four weeks of inoculation. The data (Table 1) showed that the highest number of explants responded was observed in T_3, 3 mg/l BAP + 0.01 mg /l NAA ; (34.3) while the least number of explants responded in T_1, 1mg/l BAP + 0.01 mg /l NAA and T_5, 5 mg/l BAP + 0.01 mg /l NAA; (29). T_0, 0 mg/l BAP + 0 mg /l NAA; shows no response to shoot initiation.

Percent of responsive explants: The percent of explants responded recorded after four weeks of inoculation. The data (Table 01) showed that the highest number of explants responded was observed in T_3, 3 mg/l BAP + 0.01 mg /l NAA ; (85.8%), whereas the least number of explants responded in T_1, 1mg/l BAP + 0.01 mg /l NAA and T_5, 5 mg/l BAP + 0.01 mg /l NAA; (72.5 %). T_0, 0 mg/l BAP + 0 mg /l NAA; shows no response to shoot initiation. The findings show similarity to Pierik et al. (1982).

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Number of days taken for shoot initiation: The data on effect of concentrations of BAP and NAA on shoot multiplication recorded after four weeks of culture are presented below. Significant differences were noticed for days taken for initiation of shoots among the treatments (Table 01). T_3, 3 mg/l BAP + 0.01 mg/l NAA; showed the earliest shoot initiation (16 days) and T_1, 1mg/l BAP + 0.01 mg/l NAA; was late (20.7 days). T_0, 0 mg/l BAP + 0 mg /l NAA; shows no shooting. The concentrations of BAP exhibited significant differences on days taken for initiation of shoot. Various combinations of auxins and cytokinins have been tried to achieve multiple shoot induction in Gerbera (Murashige et al. 1974; Barbosa et al. 1993). This result is contrary to the results of Pierik et al. (1975) who reported that addition of high concentration of BA is very essential for regeneration of shoots from capitulum and optimum concentration is 10 mg/l. However, Laliberte et al. (1985) reported that 'Mardi Grass' capitulum explants responded best to the MS medium supplemented with 1 mg/l BAP and 0.1 mg/l IAA. The vigour and number of shoots decreased when the concentration of BAP was raised to 2-3 mg/l. Arello et al. (1991) also found same with 2 mg/l BA and 0.5 mg/l IAA. Nga et al. (2005) also obtained best results in MS medium supplemented with 1 mg/l BA plus 0.3 mg/l kinetin and 0.2 mg/l IAA.

Number of shoots per clump: Significant variation was observed after four weeks in number of shoots per clump where maximum number of shoots produced was recorded by T_3, 3 mg/l BAP + 0.01 mg/l NAA; (8.0) and minimum by T_5, 5 mg/l BAP + 0.01 mg/l NAA; (2.0) (Table 01). The concentrations of BAP exhibited significant differences with respect to number of shoots production. Laliberte et al. (1985) obtained maximum number of shoots per explant on a medium containing 2 mg/l BAP and 0.1 mg/l IAA from 'Pastourelle' variety. The results are in conformity with that of Shailaja (2002) who obtained highest multiplication rates in gerbera with 3 mg/l BAP (14.95) as compared to 1 and 2 mg/l BAP. Maximum number of shoots was recorded at 2 mg/l BAP as compared to 1 mg/l BAP by Parthasarathy et al. (1996). Jerzy and Lubomskii (1991) also obtained the optimum BAP concentrations were 3 and 5 mg/l.

Number of leaves per shoot: The number of shoots produced per explant was recorded after eight weeks of inoculation which exhibited significant variation. T_3, 3 mg/l BAP + 0.01 mg/l NAA; produced the highest number of leaves per shoots (7.70) and T_5, 5 mg/l BAP + 0.01 mg/l NAA; produced the least (4.70) (Table 01).

Shoot length (mm): The length of shoot in different treatments after four weeks of inoculation differed significantly (Table 01 and Plate 01). The maximum shoot length was observed in T_3, 3 mg/l BAP + 0.01 mg /l NAA; (25.4 mm) and minimum was in T_1 (20.84 mm).

Table 01. Effect of different growth regulators combinations on shooting of Gerbera[y]

Treatments[x]	No. of responsive clumps	% of responsive clumps	Days taken for shoot induction	No. of shoots/ clumps	No. of leaves/ shoot	Shoot length (mm)
T_0	0.0 d	0.0 d	0.0 d	0.0 e	0.0 c	0.0 d
T_1	29.0 c	72.5 c	20.7 a	2.7 cd	5.3 b	20.3 c
T_2	31.3 b	78.3 b	20.0 a	3.3 c	5.3 b	21.0 bc
T_3	34.3 a	85.8 a	16.0 c	8.0 a	7.7 a	25.4 a
T_4	30.3 bc	75.8 bc	17.7 b	5.3 b	6.0 b	21.3 b
T_5	29.0 c	72.5 c	20.0 a	2.0 d	4.7 b	20.4 c
CV (%)	*4.2*	*4.2*	*5.5*	*14.2*	*17.7*	*2.6*
LSD 0.05	*1.9*	*4.7*	*1.5*	*0.9*	*1.5*	*0.8*

[x] T_0, 0 mg/l BAP + 0 mg /l NAA; T_1, 1mg/l BAP + 0.01 mg /l NAA ; T_2, 2 mg/l BAP + 0.01 mg /l NAA; T_3, 3 mg/l BAP + 0.01 mg /l NAA ;T_4 ,4 mg/l BAP + 0.01 mg /l NAA; and T_5, 5 mg/l BAP + 0.01 mg /l NAA;

[y] In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.05 level of probability

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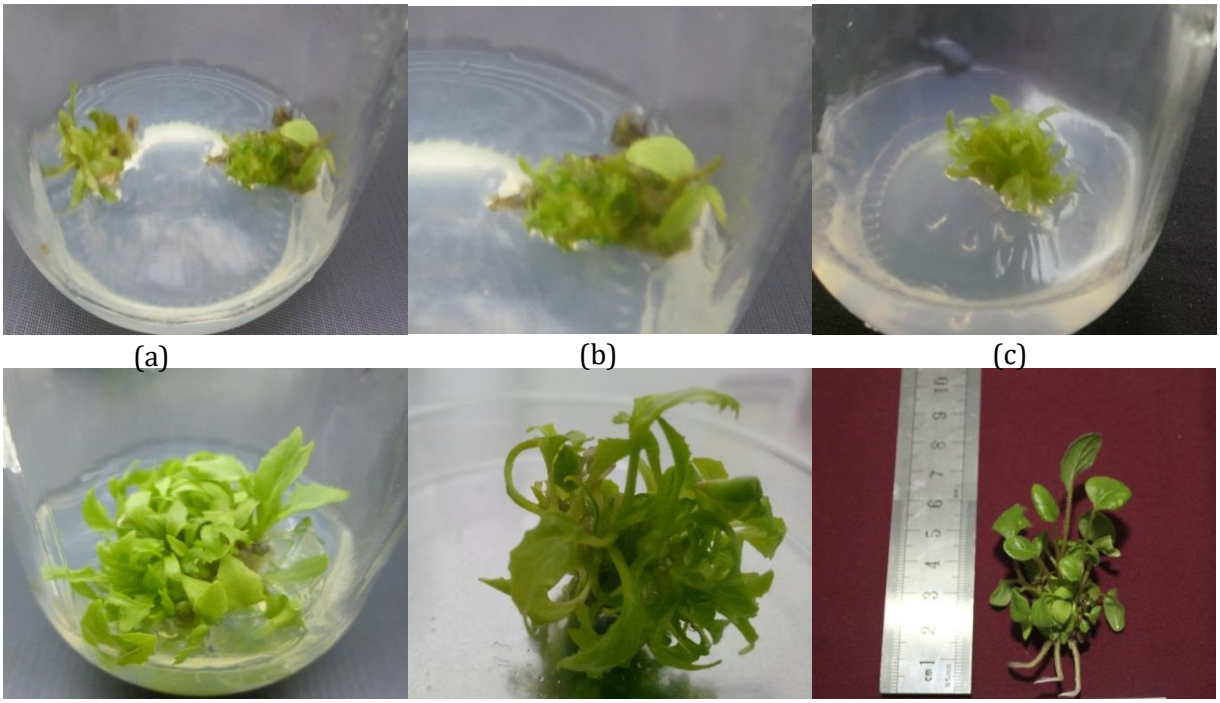

(a) (b) (c)

(d) (e) (f)

Plate 1. Gerbera shoot growth; (a, b) shooting from clumps (c, d, e) shoot multiplication, (f) highest shoot growth in number and length.

IV. Conclusion

The findings of the present experiment shows that the combination of 3 mg/l BAP + 0.01 mg /l NAA performs best in case of shoot multiplication from the proliferating clumps of gerbera callus grown from flower buds. Rooting performance of these will be the next endeavor in formulating the protocol.

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