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Combine effect of BA and IAA on shoot and root induction potentiality in chrysanthemum (*Chrysanthemum morifolium*)

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ABSTRACT

The present investigation was carried out to study the in vitro regeneration in chrysanthemum. MS medium supplemented with different concentrations and combinations of BA and IAA for regeneration and multiple shoot formations. Nodal segments were used as explants. The shoot induction percentage was highest (76%) in the treatment BA 2.0 mg/l+ IAA 1.0 mg/l. The same combination regenerated the highest number of shoot (3.40) with minimum (7.60) days. The maximum days (15.80) was noticed in a simple MS medium. The highest number of leaf (11.60) was observed in the treatment of 3.0 mg/l BA+ 1.0 mg/l IAA. The treatment BA 2.0 mg/l + IAA 1.0 mg/l produced the highest number of main root (4.20) while it was lowest in the control treatment. A robust and healthy plantlet was regenerated within 28 days of subculture in the treatment BA 2.0 mg/l + IAA 1.0 mg/l. The regenerated plantlet was acclimatized in natural pot and soil conditions. An approximate 75% plantlet was established in both systems. Finally, a convenient protocol technique has been established for in vitro regeneration of chrysanthemum which can be used for large scale plantlet production in chrysanthemum.

***Key Words**: Phytohormone, Regeneration, Chrysanthemum, BA, IAA, Shoot induction and Root Induction*

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I. Introduction

Chrysanthemum (*Chrysanthemum morifolium*) commonly known as Gul-e-Daudi or Autumn Queen belongs to the family Compositae (Arora, 1990). Chrysanthemum is important for its outstanding aesthetic beauty. Chrysanthemum accounts for 35 percent of the total cut flower production. Chrysanthemum is the world's second most economically important floricultural crop following the rose (Teixeira da silva, 2003). Nowadays, the dried capitulum of *C. morifolium*, Chrysanthemi Flos, is a most valuable medicinal material in China, Japan, Korean and other countries (Lai et al., 2007; Jin et al.,

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2012) which is used for scattering cold, cleaning heat and toxin and brightening eyes [Chinese Pharmacopoeia Committee (Ed.), 2010].

Normally, it can be propagated vegetatively either through root suckers or terminal cuttings but this conventional process of cutting is very slow (Nhut et al., 2005). Secondly, cuttings obtained repeatedly from mother plants may be subjected to any virus infection and degeneration, thereby increasing production costs (Hahn et al., 1998). High frequency regeneration of plants from the in vitro cultured tissue is a prerequisite for the successful application of tissue culture techniques for crop improvement (Akter, 2001). Chrysanthemum in vitro culture was extremely useful for producing a huge number of explants in a short time as stated by (Dao et al., 2006). Regeneration through in vitro culture has become now a viable alternative to the conventional propagation methods. Therefore, the worldwide importance of this flowering plant and to overcome the problem of propagation through a conventional method, an attempt has been made to develop an efficient and reproducible regeneration protocol for the clonal propagation of this plant.

II. Materials and Methods

The present research was carried out in the Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 from the period of February 2016 to June 2017. The planting materials of Chrysanthemum morifolium were collected from Horticulture farm, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh. The nodal segments were used as explants. The nodes were cut as small size (2 to 3 cm) and rinsed with water. The nodal segments were soaked with Tween-20 solution having 10% concentration for 5 min then washed with distilled water several times followed by sterilization with 70% ethanol for 1min. Then the explants were sterilized with 0.2% $HgCl_2$ for 2 min. The explants were rinsed with sterilized distilled water for at least 4 times. The final size of explants was made 0.5-1.0 cm before transferred to the MS media. All these activities were done in a laminar airflow cabinet under aseptic conditions. Explants were cultured on MS medium supplemented with different concentrations of BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and IAA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l). Twenty five treatments were made by the combination of BA and IAA phytohormone. Simple MS medium was used as control treatment. The pH of the medium was adjusted to 5.8. The individual nodal segments were directly inoculated to each of the culture vial containing 25 ml of MS medium. The temperature of the growth room was maintained within 21±1°C by an air conditioner and a 16 hour photoperiod was maintained along with the light intensity of 3000-5000 lux for proper growth and development of culture.

The plantlets were taken out from culture vial after 28 days and dipped in gentle warm water to remove any traces of solidified agar media for acclimatization. Plastic pots (6×6 cm) were kept ready filled with garden soil and compost in the proportion of 1:1 respectively. Immediately after removing solidified agar media from the newly formed root, the plantlets were then transplanted into the pots with special care.

After planting, the plantlets were thoroughly watered and were kept at 23±2 °C with light intensity varied from 3000–5000 lux. The photoperiod was generally 14 hours light and 10 hours dark and 70% RH for 7 days with consecutive irrigation. Then the plants were shifted to shade house with less humidity and indirect sunlight. The top of the pots was covered with a transparent plastic sheet and grew at room temperature and 70% RH for 14 days with periodic irrigation (2 days interval). After 3 weeks, the plants were transferred to the soil following depoting and poting into different pots having bigger size. The plants were watered periodically and upper layer of the soil was mulched occasionally whenever necessary. Data were taken at 14 & 28 days after induction (DAI) of culture.

III. Results and Discussion

The effect of phytohormone on shoot proliferation and elongation from the nodal segment of chrysanthemum was investigated by adding different concentrations of BA and IAA to a basal MS medium. This present experiment showed maximum percentage of shoot (76.00%) within minimum 7.60 days in the treatment BA 2.0 mg/l + IAA 1.0 mg/l which are statistically similar (76.00%) with BA 2.0 mg/l + IAA 0.5 mg/l treatment while the lowest (48.00%) was observed in control (0.0 mg/l)

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treatment (Table 01). This result was partially varied with (Bo et al., 2005) findings where they obtained adventitious shoots of Chrysanthemum of 93.8 % on MS media supplemented with 2.0 mg/1 BA and 2.0 mg/1 NAA.

A combination of BA 2.0 mg/l+ IAA 1.0 mg/l gave the highest number (3.40) of shoot at 28 DAI whereas; the lowest number (1.80) of shoots was found in control treatment (Table 01). Our findings were supported by (Waseem et al., 2009). They used different concentrations of IAA and BAP and found the significant number of shoots of chrysanthemum plantlets from nodal segments.

Table 01: Effect of different concentrations of BA and IAA hormone on shoot regeneration potentiality of chrysanthemum.

Treatments BA+IAA (mg/l)	Percentage of shoot response	Days to shoot	No. of shoot		Length of shoot		No. of leaves	
			14 DAI	28 DAI	14 DAI	28 DAI	14 DAI	28 DAI
Control	48.00^{f}	15.80^{a}	1.00^{d}	1.80^{gh}	0.40^{k}	1.08^{m}	1.20^{j}	2.20^{l-n}
1.0+0.5	68.00^{a-c}	10.80^{ij}	1.60^{bc}	2.60^{c-e}	2.10^{b}	2.56^{c}	4.00^{bc}	7.60^{cd}
1.0+1.0	60.00^{c-e}	10.80^{ij}	1.80^{b}	3.00^{a-c}	1.84^{cd}	2.48^{c}	4.00^{bc}	7.00^{de}
1.0+1.5	52.00^{ef}	11.00^{ij}	1.40^{bcd}	2.80^{b-d}	1.70^{de}	2.32^{de}	3.60^{cd}	6.40^{ef}
1.0+2.0	64.00^{b-d}	11.60^{hi}	1.60^{bc}	3.20^{ab}	1.58^{ef}	2.20^{ef}	3.60^{cd}	6.20^{ef}
1.0+2.5	68.00^{b-d}	11.20^{i}	1.40^{b-d}	3.20^{ab}	1.50^{f}	2.12^{f}	3.20^{de}	6.40^{d-f}
2.0+0.5	76.00^{a}	8.20^{l}	1.80^{b}	3.00^{a-c}	2.36^{a}	3.14^{b}	4.40^{b}	9.80^{b}
2.0+1.0	76.00^{a}	7.60^{l}	2.20^{a}	3.40^{a}	2.48^{a}	3.66^{a}	2.40^{fg}	4.60^{g-i}
2.0+1.5	72.00^{ab}	9.40^{k}	1.60^{bc}	3.00^{a-c}	2.10^{b}	3.02^{b}	4.20^{b}	8.40^{c}
2.0+2.0	68.00^{a-c}	9.80^{jk}	1.40^{b-d}	2.60^{c-e}	2.02^{bc}	2.54^{c}	3.40^{d}	6.40^{d-f}
2.0+2.5	64.00^{b-d}	11.20^{i}	1.40^{b-d}	2.20^{e-g}	1.92^{bc}	2.44^{cd}	2.80^{ef}	5.60^{fg}
3.0+0.5	60.00^{c-e}	12.60^{f-h}	1.40^{b-d}	2.20^{e-g}	1.52^{ef}	2.06^{f}	2.60^{fg}	4.80^{gh}
3.0+1.0	68.00^{a-c}	12.60^{f-h}	1.20^{cd}	2.20^{e-g}	1.4^{ef}	1.90^{g}	6.20^{a}	11.60^{a}
3.0+1.5	72.00^{ab}	12.80^{f-h}	1.40^{b-d}	2.40^{d-f}	1.40^{f}	1.74^{h}	2.60^{fg}	4.00^{h-j}
3.0+2.0	64.00^{b-d}	13.20^{d-g}	1.60^{bc}	2.20^{e-g}	0.98^{g}	1.56^{i}	2.80^{ef}	4.00^{h-j}
3.0+2.5	60.00^{c-e}	13.40^{c-f}	1.40^{b-d}	2.00^{f-h}	0.82^{g-j}	1.46^{i-k}	2.40^{fg}	3.60^{h-k}
4.0+0.5	60.00^{c-e}	13.80^{b-f}	1.20^{cd}	1.80^{gh}	0.68^{ij}	1.32^{kl}	2.20^{gh}	3.20^{j-l}
4.0+1.0	60.00^{c-e}	12.00^{g-i}	1.40^{b-d}	2.20^{e-g}	0.72^{h-j}	1.32^{kl}	2.20^{gh}	3.40^{i-l}
4.0+1.5	52.00^{ef}	13.00^{e-g}	1.20^{cd}	2.20^{e-g}	0.60^{j}	1.22^{lm}	2.20^{gh}	3.40^{i-l}
4.0+2.0	56.00^{d-f}	13.60^{b-f}	1.40^{b-d}	2.20^{e-g}	0.84^{g-i}	1.40^{jk}	1.80^{hi}	4.00^{h-j}
4.0+2.5	56.00^{d-f}	14.80^{ab}	1.40^{b-d}	2.00^{f-h}	0.92^{gh}	1.50^{ij}	1.80^{hi}	3.40^{i-l}
5.0+0.5	48.00^{f}	14.60^{a-c}	1.20^{cd}	1.80^{gh}	0.74^{h-j}	1.34^{kl}	1.80^{hi}	3.00^{j-m}
5.0+1.0	48.00^{f}	13.80^{b-f}	1.40^{b-d}	2.00^{f-h}	0.76^{h-j}	1.16^{m}	1.60^{ij}	2.40^{k-n}
5.0+1.5	48.00^{f}	14.20^{b-e}	1.40^{b-d}	1.60^{h}	0.68^{ij}	1.16^{m}	1.60^{ij}	2.40^{k-n}
5.0+2.0	48.00^{f}	14.60^{a-c}	1.20^{cd}	1.60^{h}	0.66^{ij}	1.22^{lm}	1.40^{ij}	1.80^{mn}
5.0+2.5	48.00^{f}	14.40^{b-d}	1.40^{b-d}	1.80^{gh}	0.60^{j}	1.10^{m}	1.20^{j}	1.40^{n}
CV (%)	19.67	7.14	36.58	25.22	11.16	7.85	21.96	18.27
LSD $_{(0.05)}$	7.932	1.156	0.512	0.487	0.177	0.137	0.455	1.119

In a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT.

The average length of shoot 3.66 cm was noticed from the treatment BA 2.0 mg/l + IAA 1.0 mg/l at 28 DAI while the second highest length of shoot 3.02 cm was observed in 2.0 mg/l BA + 1.0 IAA mg/l (Table 01). The result of our experiment was partially aligned with the findings of (Labade et al., 2016). They found that 1.0 mg/l BAP + 0.1 mg/l IAA treatment gave 5.5 ± 0.51 cm average length of shoot per explants in Chrysanthemum. The result of our experiment was varied due to genotype and culture environment.

The highest number of leaves (6.20 and 11.60) at 14 and 28 days after induction (DAI) respectively were noticed from the BA 3.0 mg/l + IAA 1.0 mg/l treatment which were better performance from rest of other hormone combination (Table 01).

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The present study revealed that the highest percentage (68.00%) of root induction was recorded with 2.0 mg/l+0.5 mg/l, 2.0 mg/l+1.0 mg/l and 2.0 mg/l+2.5 mg/l of BA and IAA respectively (Table 02). The lowest percentage (24.00%) of root induction was found in control treatment (Table 02). The treatment BA 2.0 mg/l + IAA 1.0 mg/l takes minimum 12.20 days for the satisfactory number of root initiation (Table 02). The results of the present study partially agree with the findings of (Nalini, 2012). He cultured shoot tip explants in MS medium containing kinetin 3.0 mg/1 + IAA 2.0 mg/1 and reported it to be the best treatment combination as it produced 67.82% in 17.91 days (minimum).

The highest number of root 4.20 was obtained from BA 2.0 mg/l + IAA 1.0 mg/l concentration followed by 4.00 number of root in 2.0 mg/l BA+1.0 mg/l IAA at 28 DAI. The control treatment gave the lowest response in the case of all parameters of root of chrysanthemum (Table 02).

Table 02: Effect of different concentrations of BA and IAA hormone on root induction potentiality of chrysanthemum.

Treatments BA+IAA (mg/l)	Percentage of root response	Days to root induction	No. of root		Length of root	
			14 DAI	28 DAI	14 DAI	28 DAI
Control	24.00^{g}	29.80^{a}	1.20^{d}	2.00^{h-j}	0.38^{n}	1.04^{j}
1.0+0.5	64.00^{bc}	17.60^{jk}	2.00^{b}	4.00^{ab}	2.14^{cd}	3.34^{bc}
1.0+1.0	64.00^{bc}	18.00^{ij}	2.20^{ab}	3.40^{cd}	1.74^{e}	3.26^{bc}
1.0+1.5	56.00^{cde}	17.60^{jk}	2.40^{a}	3.40^{cd}	1.72^{e}	3.18^{b-d}
1.0+2.0	48.00^{ef}	17.40^{jk}	2.20^{ab}	2.80^{ef}	1.66^{ef}	3.08^{b-e}
1.0+2.5	56.00^{c-e}	18.20^{h-j}	1.60^{c}	2.80^{ef}	1.52^{f}	3.00^{c-e}
2.0+0.5	68.00^{ab}	13.60^{m}	2.00^{b}	3.60^{bc}	2.24^{bc}	4.08^{a}
2.0+1.0	68.00^{ab}	12.20^{n}	2.40^{a}	4.20^{a}	2.50^{a}	4.40^{a}
2.0+1.5	60.00^{b-d}	15.20^{l}	1.60^{c}	3.00^{de}	2.34^{ab}	4.10^{a}
2.0+2.0	60.00^{b-d}	16.40^{kl}	1.40^{cd}	2.60^{e-g}	1.98^{d}	3.74^{ab}
2.0+2.5	68.00^{ab}	17.00^{jk}	1.60^{c}	2.20^{g-i}	1.64^{ef}	3.18^{b-d}
3.0+0.5	64.00^{bc}	19.20^{ghi}	1.40^{cd}	2.40^{f-h}	1.30^{g}	2.72^{c-f}
3.0+1.0	60.00^{bcd}	19.60^{fgh}	1.40^{cd}	2.40^{f-h}	1.22^{g}	2.50^{d-g}
3.0+1.5	63.00^{a}	19.60^{gh}	1.40^{cd}	2.40^{f-h}	1.00^{h}	2.28^{f-h}
3.0+2.0	60.00^{b-d}	19.40^{gh}	1.40^{cd}	2.20^{g-i}	0.88^{h-j}	2.44^{e-g}
3.0+2.5	64.00^{bc}	19.60^{gh}	1.40^{cd}	2.20^{g-i}	1.00^{h}	2.22^{fgh}
4.0+0.5	64.00^{bc}	21.00^{ef}	1.20^{d}	1.60^{jk}	0.84^{h-j}	1.86^{g-i}
4.0+1.0	60.00^{b-d}	20.00^{efg}	1.40^{cd}	2.00^{h-j}	0.90^{hi}	2.06^{f-h}
4.0+1.5	60.00^{b-d}	21.20^{e}	1.20^{cd}	2.00^{h-j}	0.72^{i-l}	2.40^{f-h}
4.0+2.0	52.00^{d-f}	22.80^{d}	1.20^{cd}	1.60^{jk}	0.72^{i-l}	2.14^{f-h}
4.0+2.5	48.00^{ef}	24.00^{cd}	1.40^{cd}	1.80^{i-k}	0.68^{j-l}	2.00^{f-h}
5.0+0.5	48.00^{ef}	23.60^{cd}	1.40^{cd}	1.40^{k}	0.76^{i-k}	1.84^{g-i}
5.0+1.0	48.00^{ef}	24.20^{c}	1.20^{cd}	1.40^{k}	0.60^{k-m}	1.58^{h-j}
5.0+1.5	44.00^{f}	24.80^{bc}	1.00^{d}	1.40^{k}	0.54^{lmn}	1.64^{h-j}
5.0+2.0	44.00^{f}	26.00^{b}	1.20^{cd}	1.40^{k}	0.42^{mn}	1.26^{ij}
5.0+2.5	44.00^{f}	26.00^{b}	1.20^{cd}	1.40^{k}	0.46^{mn}	1.14^{ij}
CV (%)	21.13	12.4	33.75	26.31	11.47	6.30
LSD $_{(0.05)}$	8.231	1.273	0.329	0.465	0.177	0.643

In a column followed by different letter(s) differs significantly whereas figures having common letter(s) do not differ significantly from each other as adjusted by DMRT.

The regenerated plantlets were acclimatized in shade houses and open field conditions (Figure 01). Finally, the survival rate was 75% after acclimatization in open atmospheric conditions (Table 03).

Table 03: Survival rate of *in vitro* regenerated plantlets of Chrysanthemum

Acclimatization	No. of plants transplanted	No. of plants survived	Percentage of survival rate
In shade house with controlled atmosphere	25	20	80
In open atmospheric area	20	15	75

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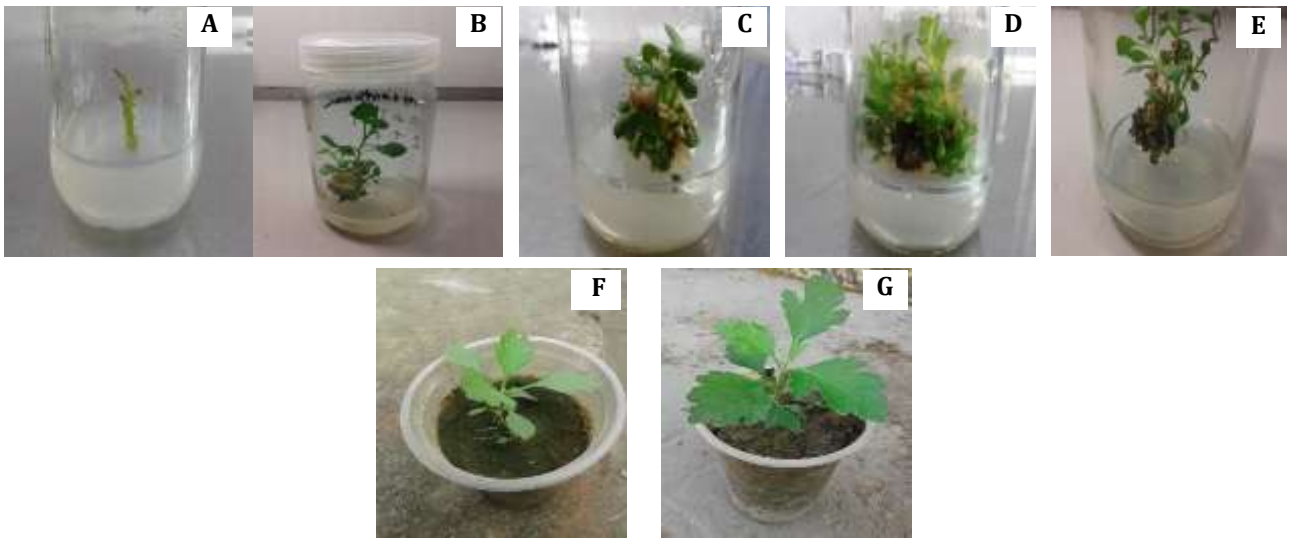

Figure 01. (A) inoculation of explants; (B & C) shoot formation (D) multiple shoot formation (E) root induction; (F & G) plantlet transfer in pot

IV. Conclusion

The results of the present investigation showed an efficient and convenient regeneration protocol of *Chrysanthemum morifolium* through *in vitro* culture. The technique will be readily applicable for large scale clonal propagation and plantation in the sustainable areas for its worldwide importance. Moreover, this protocol indicated that hormonal treatment gave the best results over control treatment in case of shoot and root formation in chrysanthemum.

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