EFFECT OF CULTURE MEDIA AND GROWTH REGULATORS ON *IN VITRO* PROPAGATION OF*CHRYSANTHEMUM INDICUM* L.

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ABSTRACT: A rapid shoot multiplication of Chrysanthemum(*Chrysanthemum indicum* L.).has been optimized in the present study. Apical meristems were used as explants and inoculated on MS media supplemented with various concentrations of growth regulators. The effects of different phytohormones (BAP, NAA, IAA and IBA) were tested for their effect on enhancement of shoot multiplication. MS medium fortified with BAP 1.0 + IAA 0.1 mg/l had a promotory effect on the initiation and multiplication of micro shoots. The optimum shoot formation response was 82% with 5.20 average number of shoots per explants and acquired maximum length of 4.9 cm. Rooting study revealed that MS medium supplemented with IBA 0.1 mg/l were proved to be superior with maximum (85%) rooting, 2.7 roots per explants and 2.5 cm root length. Then plantlets were shifted in Green house for acclimatization.

Key words: *Chrysanthemum indicum* L.; Murashige&Skoog's; Shoot initiation; Root initiation

INTRODUCTION

Chrysanthemums are commonly known as Gul-e-Daudi or Autumn Queen, a genus (*Chrysanthemum*) of about 30 species which behaves both as an annual as well as a perennialflowering plants in the family Asteraceae, native to Asia and northeastern Europe.The genus *Chrysanthemum* comprising of about 100 species is widely distributed in the Northern Hemisphere and all are insect pollinated (Wodehouse, 1930).

Chrysanthemum comes from Greek 'Chrys' meaning golden (the color of the original flowers), and 'anthemon', meaning flower. Chrysanthemum is a highly attractive and charming short day plant, and these are one of the most popular flowers in the world, next to the Rose only. The plant height ranges from 1/3 to 1 m.The plants are erect with lower pannified leaves and further up leaves are oblong, deeply incised and dentate. Leaves on the upper most stems are entire and lanceolate Chrysanthemum flowers bloom in a variety of forms, and can be daisy-like, decorative, pompons or buttons. It has a long shelf life due to low ethylene production during senescence (Bartoli*et al.,* 1997). Chrysanthemum blooms come in a various shapes and sizes and in a wide range of colors. Flowers bloom in early winter with a wide range of color, shape and sizes. They are appreciated for their high keeping quality Also their ability to produce desired grades and types at anytime during the year adds to their popularity.

Chrysanthemum is one of the most important global cut flower and pot plants. Commercial cultivars are usually cultivated by vegetative cuttings or suckers. Traditional breeding and more recently, together with genetic, molecular techniques, has focused on the enhancement of the plant's ornamental value through the improvement of flower color, size and form, vegetative height, growth form and sensitivity to light quality (Silva, 2003).Due to short of the intensive predictable research effort and vast agricultural practices, desired production targets in Chrysanthemum yield and quality enhancement have not been achieved so far. Looking at the potential and promises of plant tissue culture technology effort has been intended for implementing this technology to improve the existing procedures.

MATERIALS AND METHODS

Chrysanthemum plants were collected from the Lawrence garden and Botanical garden of Lahore College for Women University (LCWU), Lahore Pakistan. For surface sterilization all apical meristem were taken from upper portions of the plants. After removing the leaves, apical meristems were separated with the help of fine scissor and washed with tap water for several times to remove the dust particles without damaging the young and delicate tissues. Then explants were washed and cleaned with detergent and later on rinsed with distilled water to remove traces of detergent. At the end, all the explants were dipped in commercial Sodium Hypochlorite solution (15-20%) for 15-20 minute. After this treatment, the explants were rinsed with autoclaved distilled water four-five times, so as to remove and lower the toxic effect of Sodium Hypochlorite.

After surface sterilization, apical meristems were grown on MS(Murashige&Skoog 1962)media supplemented with BAP alone and combination with

NAA and IAA (at concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l) for initiation of multiple shoots. After optimization of the best media these micro shoots were further sub cultured for multiplication. The multiplication media contain selected concentrations of BAP with combinations of IAA (1.0 + 0.1 mg/l). For root induction, newly developed micro shoots were inoculated in varying concentrations of IBA and NAA (at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/l). All media based on MS basic media salts i.e. macronutrients and micronutrients, vitamins, Fe-EDTA, Myoinositol andsucrose 30 g/l. The pH of media was adjusted at 5.57. For solidification of media phytagel with concentration of 1.5 g/l was used before autoclaving at 121^0C and 15 lb/inch^2 for 15-20 minutes.

The cultures of Chrysanthemum were grown in culture room with temperature 20-22 ± 2º C and light intensity of 2000-3000 lux, while the cultured were maintained at photoperiod of 16 hours light and 8 hours dark. Days of initiation for shoots and roots were recorded after 30 days after establishing the culture. Then these plantlets were shifted in green house for acclimatization.

A completely randomized design with 5 replicates was used for the experiment. The data for each parameter were subjected to analysis of variance (ANOVA) using the COSTAT V.63: statistical software (Cohort software, Berkely, California). The mean values were compared by applying Duncan's New multiple range Test at 5% level.

RESULTS AND DISCUSSIONS

For the establishment of aseptic culture ofChrysanthemum different experiments were conducted, different concentrations were used in these experiments.

To induce shoot regeneration apical meristem of Chrysanthemum were inoculated on MS medium supplemented with BAP alone (Table-1) MS media supplemented with five different concentrations of BAP (0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) were tested for their effect on shoot initiation (Fig. a & b). Maximum percentage of shoot initiation (80%) and maximum shoot length (3.67 cm) was observed in BAP1 mg/l. It was noted that higher concentration of BAP failed to manifest its effect on shoot initiation. This finding was also reported by Haq*et al.,* 1998 who confirmed that BAP accelerates the development of apical meristem causing increased number of shoots in Chrysanthemum. Waseem*et al.,* (2009) also reported maximum shoot initiation (93%) and shoot length (5.0 cm) in MS + BAP 1.0 mg/l medium. Karim*et al.,* (2002) also reported similar findings and optimized BAP 1.0 mg/l as the best media of shoot initiation.

The combinations of BAP and NAA were tested for its effect on apical meristem cultures. The maximum response of shoot formation (80%) was observed in BAP 1.0 + NAA 0.1 mg/l (Fig. c & d) with formation of shoots in 6.13 days and had the maximum shoot length 4.8 cm (Table-2). It was noted that lower concentrations of NAA (0.1-0.2 mg/l) with intermediate BAP concentrations (1.0-2.0 mg/l) favoured shoot regeneration and when BAP concentration was increased (i.e. 5.0 mg/l) with lower concentrations of NAA (0.1-0.2 mg/l), the percentage of shoot formation decreased from 75-55%. Ali *et al.,* 2005 observed that when NAA concentrations increased (from 0.4-1.0 mg/l), it suppressed the growth and decreased the multiplication rate while when lower concentration of NAA was used it gave the best result.

The effect of BAP and IAA on shoot initiation and its length was also observed (Fig. e & f). MS medium supplemented with BAP 1.0 and IAA 0.1 mg/l showed the maximum shoot formation (82%) and shoot length 4.9 cm in 5.63 days (Table-3). The percentage of response decreased from 73-40% when concentration of IAA was increased. The result showed that the intermediate levels of BAP along with lower concentrations of IAA (0.1 and 1.0mg/l) had best response on the effect of regeneration from meristems (Table-4). Similar results were reported by Vijaya*et al.,* (1991) and Rout *et al.,* (1997). Karim*et al.,* (2003) suggested that a combination of BAP 1.0 mg/l + IAA 0.1 mg/l produced longest shoots of 4.5 cm by using shoot tip of Chrysanthemum. Parsad*et al.,* (1993) experiments on chrysanthemum micropropagaton reported that different concentrations of BAP + IAA had affected the regeneration of Chrysanthemum plantlets. Bhattacharya *et al.,* (1990) observed 2.1 shoots per explant for 0.1 mg/l IAA in the MS medium for chrysanthemum plantlets.

To initiate roots in newly developed shoots they were inoculated in varying concentrations of NAA (Table-5). The maximum response of root initiation (80%) and maximum root length (2.4 cm) was observed in MS + 1.0 NAA mg/l (Fig. g). These results are supported by the previous work done by of Hoque and Fatima (1995) and Choi *et el.,* (2002). They reported maximum root formation in MS medium supplemented with 0.2 mg/l IBA and 0.2 mg/l NAA. NAA proved useful for root induction.

In order to maximize the survival of *In vitro* derived plants, it is routine practice to acclimatization them under high levels of relative humidity, resulting in high mortality rate after transfer of plants to glass house or field condition.

Table-1. Effect of different concentrations of BAP (mg/l) on shoot formation from shoot apical meristem.

Conc. of BAP mg/l	Time for shoot formation	Percentage of shoot formation	No. of shoots per expalnt	Shoot length (cm)
0.5	9.90^{a}±0.89	60	3.40^{d}±0.14	3.13^{b}±0.05
1	7.36^{b}±0.81	80	4.91^{a}±0.08	3.67^{a}±0.12
2	8.43^{ab}±0.41	70	3.62^{b}±0.12	2.87^{c}±0.05
3	8.97^{a}±0.12	53	3.33^{e}±0.09	2.10^{d}±0.16
4	9.30^{a}±0.08	57	3.52^{c}±0.20	2.00^{d}±0.08

Table-2. Effect of different concentrations of BAP+NAA (mg/l) on shoot formation from shoot apical meristem.

Conc. of BAP+ NAA mg/l	Time for shoot formation	Percentage of shoot formation	No. of shoots per explant	Shoot length (cm)
1.0+0.1	6.13^{c}±0.26	80	4.90^{a}±0.09	4.8^{a}±0.08
1.0+0.2	8.23^{b}±0.12	75	3.72^{c}±0.29	4.6^{ab}±0.14
2.0+0.1	6.3^{c}±0.48	79	4.24^{b}±0.21	4.7^{a}±0.012
2.0+0.2	8.43^{b}±0.41	65	3.61^{d}±0.12	4.4^{b}±0.28
5.0+0.1	9.63^{a}±0.12	60	3.55^{e}±0.15	3.53^{c}±0.20
5.0+0.2	10^{a}±0.14	55	3.52^{f}±0.20	3.26^{c}±0.05

No. of test tubes cultured = 10 ,Each value is mean of three replicate with standard error (mean ± S. E) a, b, c. Mean followed by different letters in the same column differ Significantly at P = 0.05 according to Duncan's new multiple range test.

Table-3. Effect of different concentrations of BAP+IAA (mg/l) on shoot formation from shoot apical meristem.

Conc. of BAP+IAA mg/l	Time for shoot formation	Percentage of shoot formation	No. of shoots per explant	Shoot length (cm)
1.0+0.1	5.63^{c}±0.12	82	5.20^{a}±0.34	4.9^{a}±0.08
1.0+0.2	7.73^{b}±0.17	70	3.62^{d}±0.12	4.5^{bc}±0.21
2.0+0.1	6.50^{c}±0.40	80	4.93^{b}±0.047	4.8^{ab}±0.14
2.0+0.2	8.26^{b}±0.52	75	3.71^{c}±0.29	4.3^{cd}±0.21
5.0+0.1	10.2^{a}±0.55	65	3.53^{cd}±0.20	4.0^{d}±0.21

Table-4. Effect of different concentrations of BAP1+IAA (mg/l) on shoot formation from shoot apical meristem.

Conc. of BAP1+IAA mg/l	Time for shoot formation	Percentage of shoot formation	No. of shoots per explant	Shoot length (cm)
1.0+0.1	5.93^{e}±0.094	80	5.0^{a}±0.30	4.7^{a}±0.12
1.0+0.2	8.16^{c}±0.531	73	3.63^{a}±0.12	4.5^{a}±0.21
1.0+0.4	7.56^{d}±0.047	60	3.44^{d}±0.14	3.9^{b}±0.16
1.0+0.6	8.70^{b}±0.244	60	3.51^{c}±0.15	3.8^{b}±0.16
1.0+0.8	9.00^{ab}±0.004	53	3.33^{e}±0.14	3.4^{c}±0.14
1.0+1.0	9.36^{a}±0.120	40	3.0^{f}±0.05	3.0^{c}±0.26

No. of test tubes cultured = 10 ,Each value is mean of three replicate with standard error (mean ± S. E) a, b, c. Mean followed by different letters in the same column differ Significantly at P = 0.05 according to Duncan's new multiple range

Table-5. Effect of different concentrations of NAA (mg/l) on root formation of *in vitro* regenerated plants.

Conc. of NAA mg/l	Time for Root formation	Percentage of Root formation	Root length (cm)
0.25	34.0^{a}±1.41	40	1.43^{d}±0.12
0.50	33.3^{b}±1.69	70	1.93^{b}±0.05
1.0	29.3^{c}±1.24	80	2.4^{a}±0.08
1.5	34.6^{b}±1.24	60	1.86^{b}±0.04
2.0	35.3^{a}±0.81	50	1.63^{c}±0.12

No. of test tubes cultured = 10 ,Each value is mean of three replicate with standard error (mean ± S. E) a, b, c. Mean followed by different letters in the same column differ Significantly at P = 0.05 according to Duncan's new multiple range test.

A

b

c

E

f

g

H

Fig. (a) and (b) Shoot initiation in MS+ BAP1 medium. (c) and (d) Shoots formation in MS+BAP+NAA medium. (e) and (f) Shoots multiplication in MS+BAP+IAA medium. (g) Roots formation in MS+ NAA medium.

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