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# RAPID *IN-VITRO* PROPAGATION OF *CHRYSANTHEMUM MORIFOLIUM* THROUGH SHOOT BUD EXPLANTS

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**Abstract**

Chrysanthemum (*Chrysanthemum morifolium*) is perennial short day flowering plant. Nodal explants of Chrysanthemum were cultured on simple Murashige and Skoog (MS) media, MS media supplemented with different concentrations of 6-Benzyl adenine (6-BA) alone and in combination with different concentrations of Naphthalene acetic acid (NAA) or Thidiazuron (TDZ) for shoot initiation and the regenerated shoots were sub-cultured on MS, ½ MS and ¼ MS media for rooting. No shoot induction was recorded in explants cultured on simple MS, but the phyto-hormones addition to MS media resulted in 100% shoot induction. In case of MS media supplemented with 6-BA, maximum shoot buds (12) and shoot length (6.06 cm) explant$^{-1}$ were recorded when 44.39 µM 6-BA was added to MS media. In case the MS media was supplemented with 6-BA+NAA, maximum (11)shoot buds explant$^{-1}$ were recorded in explants cultured in MS+88.79 µM 6-BA+10.74 µM NAA media. Among the different concentrations of 6-BA+TDZ added to MS media, maximum (6.67) shoot buds explant$^{-1}$ were recorded for explants planted in MS+88.79 µM 6-BA+9.08 µM TDZ. The regenerated shoots were then sub cultured on MS, ½ MS and ¼ MS media for rooting. In that case, there was non-significant effect on root length. However, maximum (15.78) roots were recorded for those cultured in ½ MS media. Thus it is concluded that supplementing MS media with 44.39µM 6-BA is the best concentration for rapid shoot regeneration and½ MS is optimal for the *In vitro* rooting of *Chrysanthemum morifolium.*

**Key words:** Chrysanthemum, Micropropagation, Phytohormones, 6-BA, NAA, TDZ.

## Introduction

Chrysanthemum (*Chrysanthemum morifolium*) commonly known as Queen of the East, belongs to the family *Asteraceae*. It is a highly attractive short day flowering plant, which though perennial, is treated like a seasonal blooming in late autumn. Chrysanthemums are grown throughout the world for cut flowers as well as pot plants. Chrysanthemum cut flowers are popular for flower arrangements because of their long vase life. Chrysanthemum is a complex hybrid, which is grown from seeds and segregates into many diverse flower forms (Ihsan *et al*., 2007).

Chrysanthemum is native to China and was first cultivated as a flowering herb back in the 15th century BC. It was believed that this plant had the power of life. Its boiled roots were used as a headache remedy, young sprouts and petals were eaten as salad and leaves were brewed for a festive drink (Bose *et al.,* 2003). Chrysanthemum is not only a source of beautification but also a flower that has extremely useful medicinal characteristics. The flowers are used as blood purifiers, and in Chinese medicine, they are consumed for making a cooling summer beverage. Chrysanthemum is excellent for curing inflammation. It is also utilized to improve eyesight and cure a wide range of eye disorders such as eyesore, redness, night blindness and other related problems. Researchers have also demonstrated chrysanthemum potential and efficacy in treating angina by dilating coronary arteries. It acts as an antibiotic against a variety of pathogens (Sassi *et al.,* 2008). Chrysanthemum has the ability to inhibit abnormal growth, and it has been proved that 15 compounds isolated from the flowers, showed strong inhibitory effects against abnormal cells (Hu *et al.,* 1994). The primary chemical extracts of Chrysanthemum include flavonoids, betaine, choline and vitamin B1. Chrysanthemum is known as anti-pyretic and antihypertensive. Its flowers have been used to relieve hypertension, pacify liver, expel wind and clear eyesight. The flowers are also said to be effective for wind-heat syndrome with symptoms of fever and sore throat. The extract of a fresh plant can be applied to skin infections (Marongiu *et al.,* 2009). In China, people use chrysanthemum petals as a salad with a perception of its effect in life longevity. People prepare tea (tisane) from the dried petals of the Chrysanthemum. The tisane has many medicinal uses, including an aid in recovery from influenza, acne and as a cooling herb. The tisane can help in the cure of sore throat and reduce fever. The juice of the petals and leaves is extracted and used to make wine (Campbell, 1995; Collins *et al.,* 1997).

In 1952 Morel and Martin initiated tissue culture studies on chrysanthemum (Ihsan *et al*., 2007). Most of the work was done on optimization of micropropagation protocols through callogenesis processes using different organs such as leaf, shoot tip and floret explants and obtained adventitious shoots (Bush *et al*., 1976; Dejong & Custers, 1986; Malaure *et al*., 1991, Sutter & Langhans, 1981). Recently some work has been done on the effect of culturing media and plant hormones on propagation of chrysanthemum from shoot apex (Zafarullah *et al.,* 2013).

Being cultivated for more than 2000 years, chrysanthemum importance and demand has never been diminished. Currently, it is the world's second economically important floricultural crop, following rose (Teixeira, 2003). *In vitro* propagation techniques have been increasingly used for threatened plants conservation (Chee *et al.,* 2015), as it's the best choice to produce plants in large scale (Kazmi *et al.,* 2015). Due to chrysanthemum high popularity and demand, it has become one of the top commercial targets for micropropagation and thus tissue culture can be utilized for its large scale production (Nalini, 2012).

There is a need to establish quick multiplication techniques for its commercial production to ensure an easy supply of chrysanthemum plants to meet the large scale demand. The surrounding nutritional, hormonal, osmotic, and gas environments control the development of somatic embryos. Therefore, it is very important to optimize the culture growth and development environment for successful propagation (Zhen *et al.*, 2015). Keeping in view this importance, the present study was designed to develop a rapid mass propagation method for chrysanthemum.

## Materials and Methods

The research work was conducted at Post-harvest Physiology and Technology of Ornamental Plants Beijing key laboratory, Department of Ornamental Horticulture, China Agricultural University, Peking, People Republic of China. The tissue cultured plants used in this experiment were propagated in the same lab.

**Culture media:** Nodal explants were collected from tissue cultured plants produced in laboratory through micro-propagation. Murashige & Skoog (1962) medium was used as a basal medium. This contained all the nutritional components essential for the growth and development of chrysanthemum cultures. First different concentrations of phyto-hormones were added to the basal MS media (Table 1). Then 30 g sucrose was added and volume was increased with double distilled to the final volume (Hussain *et al*. 2018). The Medium pH was adjusted at 5.8 by adding 0.01N HCl/NaOH and 0.8% (8g $l^{-1}$) was added and mixed before autoclaving for media solidification.

The nodal explants were transferred to simple MS media, MS media supplemented with different concentrations of 6-Benzylaminopurine (6-BA) alone or in combination with different concentrations of Naphthalene acetic acid (NAA) or Thidiazuron (TDZ) for shoot initiation, as shown in Table 1. The regenerated shoots were then sub cultured on MS, ½ MS or ¼ MS media for rooting.

**Table 1. Phytohormones concentrations used for *In vitro* propagation of *Chrysanthemum morifolium*.**

| Shoot induction media | BAP | NAA | TDZ |
|---|---|---|---|
| | Concentration (µM) | | |
| MS | 0 | 0 | 0 |
| MS+6-BA | 22.20 | -- | -- |
| | 44.39 | -- | -- |
| | 88.79 | -- | -- |
| | 177.58 | -- | -- |
| | 355.16 | -- | -- |
| | 710.32 | -- | -- |
| MS+6-BA+NAA | 44.35 | 5.37 | -- |
| | 88.79 | 10.74 | -- |
| | 177.58 | 21.48 | -- |
| | 266.37 | 32.22 | -- |
| | 355.16 | 42.96 | -- |
| | 710.32 | 85.92 | -- |
| MS+6-BA+TDZ | 44.35 | -- | 4.54 |
| | 88.79 | -- | 9.08 |
| | 177.58 | -- | 18.16 |
| | 266.37 | -- | 27.24 |
| | 355.16 | -- | 36.32 |
| | 710.32 | -- | 72.64 |

**Growing conditions and statistical analysis:** The cultures were kept under sixteen hour photoperiod and eight hour dark period cycle in growth room at 23 ± 2°C temperature. The study was conducted in controlled environment using completely randomized design (CRD). The experiment was replicated three times with 15 cultures treatment$^{-1}$ each time. Analysis of variance (ANOVA) technique was used in order to know any significant differences among the treatments. In case of significant differences, the means were further compared for individual differences through least significant difference (LSD) test.

**Data collection and analysis:** The data for shoot initiation were recorded after 45 days of explants inoculation into simple MS and MS supplemented with different concentrations of 6-BA alone or in combination with different concentrations of NAA and TDZ. The shoots produced were sub cultured on MS, ½ MS or ¼ MS for rooting and the data were recorded after four weeks. Microsoft Office 2007 and statistix-9 software's were used to analyze the data.

## Results

### 1. Shoot response

**Shoot induction:** Nodal explants were cultured on MS media alone and MS media supplemented with 18 different concentrations of 6-BA alone and in combination with different concentrations of NAA or TDZ. No shoot induction occurred in nodal explants cultured on to simple MS media (Table 2, Fig. 1A), but addition of all concentrations of phytohormones resulted in 100% shoot induction (Table 2, Figs. 1B-1S). The explants responded differently to various concentrations of phytohormones added to MS media. Addition of 6-BA to MS media induced shoots, and maximum (12) shoot buds explant$^{-1}$were recorded (Table 2, Fig. 1C) when MS media was supplemented with 44.39 µM (µ.moll$^{-1}$) 6-BA, while further increase in6-BA concentration significantly declined shoot buds explant$^{-1}$as shown in Table 2 and Figs. 1D-1G. As auxins and cytokinins have synergistic effect on each other (Ross and Salisbury, 1984), the explants were also cultured on MS media supplemented with different concentrations of 6-BA in combination with NAA or TDZ. Addition of 6-BA+NAA, to MS media induced shoots, and maximum shoot buds (11) explant$^{-1}$ were observed at MS + 88.79 µM 6-BA+10.74 µM NAA (Table 2, Fig. 1I). With further increase in the concentration of6-BA+NAA, the number of shoot buds explant$^{-1}$ gradually decreased (Table 2 and Figs. 1J-1M). In case of 6-BA+TDZ combinations, more shoot buds (6.67) explant$^{-1}$ were recorded in MS media supplied with 88.79 µM 6-BA + 9.08 µM TDZ , while with the increase or decrease in 6-BA+TDZ concentrations, the number of shoot buds explant$^{-1}$ declined (Table 2 and Figs. 1N-1S).

**Shoot length (cm):** The shoot length was also significantly affected by different concentrations of

phytohormones. In case of 6-BA concentrations, the length of shoots increased from 3.88 cm to 6.06 cm when 6-BA dose increased from 22.20 µM to 44.39 µM respectively (Table 2 and Figs. 1B, 1C), while further increase in 6-BA concentration resulted in gradual decline in shoot length, as shown in Table 2 and Figures 1D-1G. When different concentrations of 6-BA+NAA in combinations were added to MS media, maximum shoot length (4.36 cm) was recorded at MS+88.79 µM 6-BA+10.74 µM NAA (Table 2 and Fig. 1I), while further increase or decrease in the dosage of 6-BA+NAA caused a decline in shoot length (Table 2 and Figs. 1H-1M). In case of MS media supplemented with different concentrations of6-BA+TDZ, maximum shoot length (2.38 cm) was recorded at 88.79 µM 6-BA+ 9.08 µM TDZ combination (Table 2, Fig. 1O), while minimum shoot length(1.02 cm)was recorded at 710.32 µM 6-BA + 72.64 µM TDZ dose (Table 2 and Fig. 1S).

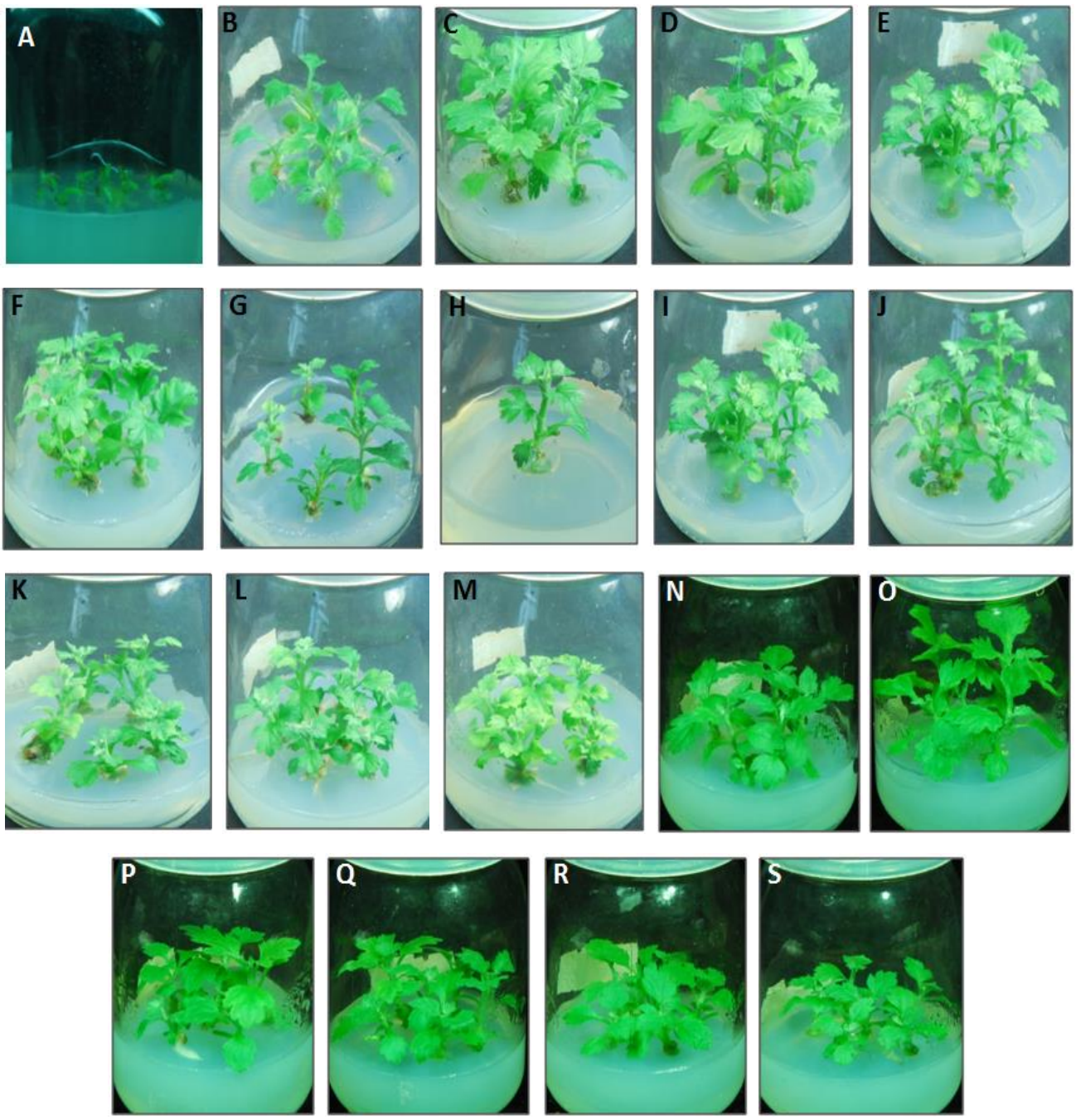


Fig. 1. Effect of different concentrations of 6-BA alone and in combination with different concentrations of NAA or TDZ.
Note: Shoot induction in response to A) MS media, B) MS media + 22.20 µ.M 6-BA, C) MS + 44.39 µ.M6-BA, D) MS media + 88.79 µ.M 6-BA, E) MS media + 177.58 µ.M 6-BA, F) MS media + 355.16 µ.M 6-BA, G) MS media + 710.32 µ.M 6-BA, H) MS media + 44.35 µ.M 6-BA + 5.37 µ.M NAA, I) MS media + 88.79 µ.M 6-BA+10.74µ. M NAA, J) MS media + 177.58 µ.M 6-BA + 21.48 µ.M NAA, K) MS media + 266.37 µ.M 6-BA + 32.22 µ.M NAA, L) MS media + 355.16µ.M 6-BA + 42.96 µ.M NAA, M) MS media + 710.32 µ.M 6-BA + 85.92 µ.M NAA, N) MS media + 44.35 µ.M 6-BA + 4.54 µ.M TDZ, O) MS media + 88.79 µ.M 6-BA + 9.08 µ.M TDZ, P) MS media + 177.58µ.M 6-BA + 18.16 µ.M TDZ, Q) MS media + 266.37 µ.M 6-BA + 27.24 µ.M TDZ, R) MS media + 355.16 µ.M 6-BA + 36.32 µ.M TDZ, and S) MS media + 710.32 µ.M 6-BA + 72.64 µ.M TDZ recorded after 45 days of culturing.

**Table 2. Effect of various concentrations of 6-BA alone and in combination with different concentrations of NAA or TDZ on shoot induction of *Chrysanthemum morifolium*.**

| Media | Shoot buds | Shoot length (cm) | Shoots explant$^{-1}$ |
|---|---|---|---|
| MS | 0 l | 0 l | 0 f |
| MS + 22.20 µM6-BA | 5.34 jk | 2.99 f | 1 e |
| MS + 44.39 µM6-BA | 12.00 a | 6.06 a | 1 e |
| MS + 88.79 µM6-BA | 10.08 c | 4.93 b | 1 e |
| MS + 177.58 µM6-BA | 8.08 ef | 4.04 cd | 1 e |
| MS + 355.16 µM6-BA | 8.00 ef | 3.02 f | 1 e |
| MS + 710.32 µM6-BA | 5.75 jk | 2.04 h | 1 e |
| MS + 44.35 µM 6-BA + 5.37 µM NAA | 9.00 d | 3.90 d | 1 e |
| MS + 88.79 µM 6-BA + 10.74 µM NAA | 11.00 b | 4.36 c | 1.08 e |
| MS + 177.58 µM 6-BA + 21.48 µM NAA | 9.25 cd | 3.45 e | 1.50 d |
| MS + 266.37 µM 6-BA + 32.22 µM NAA | 8.75 de | 2.68 fg | 1.75 c |
| MS + 355.16 µM 6-BA + 42.96 µM NAA | 7.08 gh | 2.39 g | 2.69 b |
| MS + 710.32 µM 6-BA + 85.92 µM NAA | 7.09 gh | 1.46 ij | 3.33 a |
| MS + 44.35 µM 6-BA + 4.54 µM TDZ | 4.92 k | 2.01 h | 1 e |
| MS + 88.79 µM 6-BA + 9.08 µM TDZ | 6.67 hi | 2.38 g | 1 e |
| MS + 177.58 µM 6-BA + 18.16 µM TDZ | 6.00 ij | 1.88 h | 1 e |
| MS + 266.37 µM 6-BA + 27.24 µM TDZ | 5.58 jk | 1.53 i | 1 e |
| MS + 355.16 µM 6-BA + 36.32 µM TDZ | 5.50 jk | 1.15 jk | 1 e |
| MS + 710.32 µM 6-BA + 72.64 µM TDZ | 5.25 jk | 1.02 k | 1 e |

Note: Data recorded after 45 days of culturing. Means followed by different letters in their respective columns are significantly different at $p \leq 0.05$ according to Least Significance Difference (LSD) test

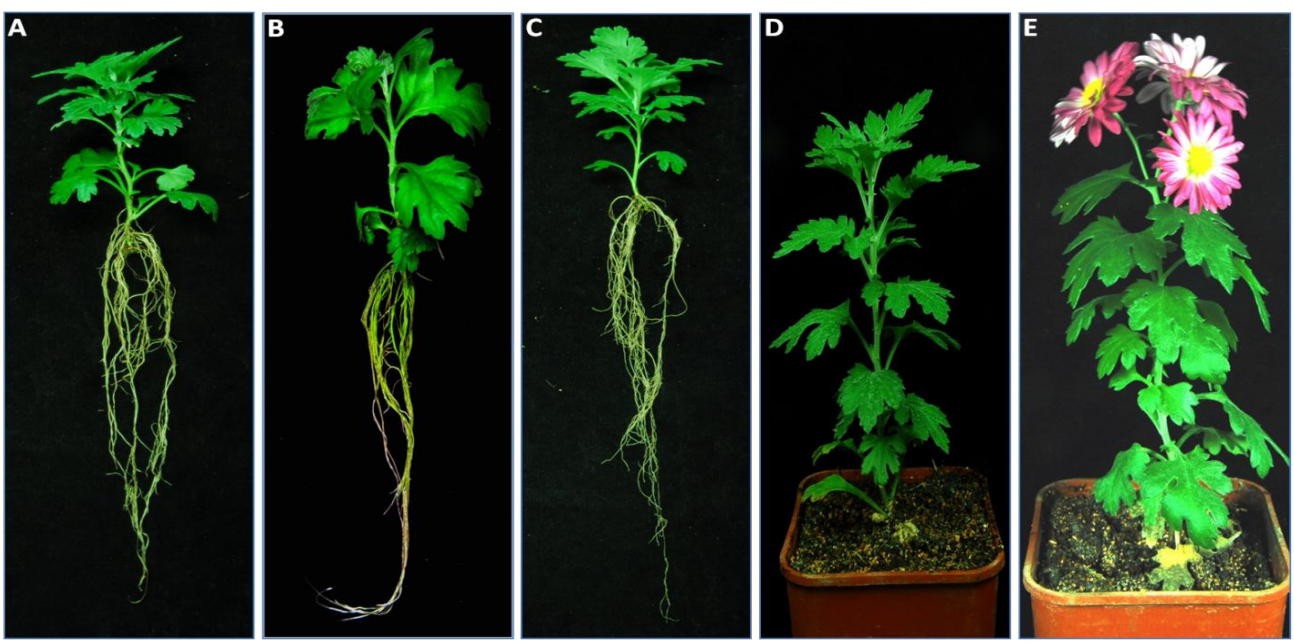


Fig. 2. Different MS media effect on *Chrysanthemum morifolium* rooting. Note: Rooting in response to A) MS media, B) ½ MS media, and C) ¼ MS media, D) at fully grown vegetative stage after transplanting to peat + vermiculite (1:1) media, and E) at flowering stage.

**Table 3. Effect of different MS media on rooting of *Chrysanthemum morifolium*.**

| Media | Root number | Root length (cm) |
|---|---|---|
| MS | 12.56 ab | 12.88 |
| ½ MS | 15.78 a | 13.11 |
| ¼ MS | 11.45 b | 13.53 |

Note: Data recorded after 27 days of sub culturing. Means followed by different letters in the root number column are significantly different at p≤0.05 according to Least Significance Difference (LSD) test

**Shoot multiplication:** Shoot multiplication was also observed for various concentrations of 6-BA alone or in combination with NAA, TDZ added to MS media. No shoot multiplication was recorded for different concentrations of 6-BA (Table 2 and Figs. 1B-1G) as well as different combined concentrations of 6-BA + TDZ added to MS (Table 2 and Figs. 1N-1S). However, shoot multiplication did occur when MS media were supplemented with different concentrations of 6-BA + NAA. Maximum shoots (3.33) explant$^{-1}$ were recorded at the highest dose of MS + 710.32 µM 6-BA + 85.92 µM NAA (Table 2, Fig. 1M). The shoot multiplication decreased with the decrease in combined 6-BA + NAA concentrations.

**2. Rooting response**

The regenerated shoots produced were further cultured for rooting on MS, ½ MS and ¼ MS media without addition of any plant growth regulators for rooting. Maximum roots shoot$^{-1}$ (15.78) were produced by regenerated shoots in ½ MS media, closely followed by MS media (12.56 roots shoot$^{-1}$), while ¼ MS produced minimum (11.45) roots shoot$^{-1}$ (Table 3, Fig. 2B). However, no significant differences were found among the root lengths (Table 3, Figs. 2A, 2B and 2C). Overall, the plants grown on ¼ MS media looked weaker than those observed in MS and ½ MS media. Thus, being maximum root producer and economical at the same time, ½ MS media could be the best option for rooting in chrysanthemum rapid in-vitro propagation.

**3. Hardening and transfer to soil media**

The plantlets produced were washed with tap water to completely remove MS media from their roots. Then the plantlets were placed in a tray containing double distilled water (DD water). The plantlets were sprayed with water, then covered with polythene sheet and placed in long day conditions (16 hours light and 8 hours dark) at 23±2$^{o}$C for three days. The plantlets were then transplanted to soil media, containing peat and vermiculite (1:1 by volume) and watered subsequently. In the current study 100% plants survival was recorded with above mentioned transplantation procedure (Figs. 2D and 2E).

**Discussion**

The difference in shoot buds, shoot length and shoots explant$^{-1}$ may be due to endogenous cytokinins in the explants and culture conditions. Cytokinins promote chloroplast development, synthesis, increase cell expansion in leaves in dicots, increase nutrients sink activity, promote cell division and organ development (Ross and Salisbury, 1984). Auxins and cytokinins both are growth regulators and have positive effect over each other. For embryogenesis, auxins and cytokinins are added to the medium for growing plants *In vitro*. If high cytokinins-to-auxins ratio is maintained, certain cultured cells divide and multiply producing more cells and developing into buds, stems and leaves (Imtiaz *et al*., 2014). In the present study, for rapid shoot regeneration, MS media supplemented with 44.39 µM6-BAwas an optimum concentration as compared to higher concentrations of 6-BA or in combination with NAA/TDZ concentrations. The decrease in number of shoot buds explant$^{-1}$ at higher concentrations or combined concentrations may be due to the residual effects of hormones, which accumulated in the cultured explants and the use of PGRs. These may have increased endogenous cytokinins concentration that caused toxic effects and reversed the growth processes (Imtiaz et al., 2014). These results are in line with those of Zafarullah *et al*. (2013), who found that higher concentrations of BAP supplemented to MS media decreased regeneration. Similar results were also observed by Waseem *et al*. (2009) and Karim *et al*. (2002). Moreover, Ali *et al*. (2005) and Zafarullah *et al*. (2013) also observed, that lower NAA concentrations in combination with 6-BA concentrations resulted in more shoot buds explant$^{-1}$than higher combined concentrations added to MS media. Whereas for 6-BA + TDZ combined concentrations the response was almost similar but lower than 6-BA + NAA combined concentrations added to MS media.

It is well known that auxins are produced in the meristem, move downwards and promote adventitious roots development on stems (Ross & Salisbury, 1984). To initiate rooting, the regenerated shoots were sub cultured to simple MS, ½ MS and ¼ MS media instead of auxin addition as practiced by Fatima *et al*. (1995), Choi *et al*. (2002) and Zafarullah *et al*. (2013). In the present study, ½ MS media was found optimal for chrysanthemum rooting. Here a root induction of more than 95% was achieved and 15.78 roots plantlet$^{-1}$ with 13.11 cm root lengths were produced. These results are in contrast to the findings of Zafarullah *et al*. (2013), who got 80% root regeneration with 2.4 cm length at MS + 1.0 mg l$^{-1}$ NAA, and Fatima *et al*. (1995) and Choi *et al*. (2002), who reported 0.2 mg l$^{-1}$ IBA and 0.2 mg l$^{-1}$ NAA as optimum concentrations added to MS media for optimum root regeneration.

**Conclusion**

In the present study, MS media supplemented with 44.39 µM6-BA produced maximum number of shoot buds (12) with maximum shoot length (6.06 cm nodal-explant$^{-1}$) of *Chrysanthemum morifolium.* In case of rooting, maximum roots (15.78) with 13.11 cm root lengths were observed in plantlets cultured on½ MS media. In light of the above results, it is concluded that MS media supplemented with 44.39 µM 6-BA is the best dosage for shoot induction and ½ MS is an optimal and economical media for rooting in order to achieve rapid *In vitro* propagation of *Chrysanthemum morifolium*.

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**References**

Ali, A., A. Munawar and F.A. Siddiqui. 2005. *In vitro* propagation of turmeric (*Curcuma longa* L.). *Int. J. Biol. Biotechnol*., 1(4): 511-518.

Bose, T.K., L.P. Yadav, P. Pal, V.A. Parthasarathy and P. Das. 2003. Commercial Flowers vol. II, Naya Udyog, Kolkata, India.

Bush, S.R., E.D. Earle and R.W. Langhans. 1976. Plantlets form petal segments, petal epidermis and shoot tips of periclinal chimera *Chrysanthemum morifolium* "Indianapolis". *Amer. J. Bot*., 63: 729-737.

Campbell, D.L. 1995. The tea book. Gretna: Pelican Publishing, pp. 116.

Chee, F.M., X. Rathinam, M. Danial, C.K. Lam, M.H. Qui and S. Subramaniam. 2015. Effect of methyl-jasmonate on 9-methoxycanthin-6-one content in *Eurycoma longifolia* (Tongkat Ali) Root culture. *Pak. J. Bot*., 47(3): 897-904.

Choi, D.C., S.Y. Seo, J.M. Kim, J.S. Choi and Y.G. Choi. 2002. Plant regeneration and test of kanauycin concentration through the leaf explants culture in chrysanthemum. A symposium: Technologies for manipulation quality and productivity traits in horticultural crops. *26th Int. Horticult. Cong*., pp. 90.

Collins, R. A., Ng, T. B., W.P. Fong, C.C Wan, and H.W. Yeung. 1997. A comparison of human immunodeficiency virus type 1 inhibition by partially purified aqueous extracts of Chinese medicinal herbs. *Life Sci*., 60: 345-351.

DeJong, J. and J.B.M. Custers. 1986. Induced changes in growth and flowering *of Chrysanthemum* after irradiation and *In vitro* culture of pedicles and petal epidermis. *Euphytica*, 35: 137-184.

Fatima, M. and M.I. Hoque. 1995. *In vitro* multiple shoot regeneration in *Chrysanthemum morifolium* Ramat. *Plant Tissue Cult*., 5(2): 153-162.

Hu, C.Q., K. Chen, Q. Shi, R.E. Kilkuskie and Y.C.K.H. Cheng. 1994. Anti-AIDS agents, 10. Acacetin-7-O-beta-Dgalactopyranoside, an anti-HIV principle from *Chrysanthemum morifolium* and a structure-activity correlation with some related flavonoids. *J. Nat. Prod*., 57: 42-51.

Hussain, M., N.I. Raja, H. Rashid, Z. Mashwani, A. Mehmood and M. Iqbal. 2018. Establishment of an efficient protocol for plantlets regeneration via direct and indirect organogenesis in Citrus reticulate blanco (kinnow mandarin). *Pak. J. Bot.*, 50(3): 1203-1210.

Ilahi, I., M. Jabeen and N. Sadaf. 2007. Rapid clonal propagation of chrysanthemum through embryogenic callus formation. *Pak. J. Bot*., 39(6): 1945-1952.

Imtiaz, M., A.M. Khattak, N. Ara, A. Iqbal and H.U. Rahman. 2014. Micropropagation of *Jatrophacurcas* L. through shoot tip explants using different concentrations of phytohormones. *The J. Anim. & Plant Sci*., 24(1):229-233.

Karim, M.Z., M.N. Amin, M.A.K. Azad, F. Begum, M. M. Rehman, M. M. Islam and R. Alam. 2002. Effect of different plant growth regulator on *In vitro* shoot multiplication of *Chrysanthemum morifolium*. *J. Biol. Sci*., 3(6): 553-560.

Kazmi, S.K., S. Khan, N. Kabir, A.A. Mirbahar, M. Raziq and N. Kausar. 2015. Eembryogenic callus induction, somatic embryogenesis, regeneration and histological studies of kinnow mandarin (*Citrus reticulata blanco* L.) from nucellar embryo and epicotyl region. *Pak. J. Bot*., 47(1): 305-310.

Malaure, R.S., G. Barclay, J.B. Power and M.R. Davey. 1991. The production of novel plants from florets *of Chrysanthemum morifolium* using tissue culture; Shoot regeneration from ray florets and somaclonal variation exhibited by the regenerated plants. *J. Plant Physiol*., 130-139.

Marongiu, B., A. Piras, S. Porcedda, S. Porcedda, E. Tuveri, S. Laconi, D. Deidda and A. Maxia. 2009. Chemical and biological comparisons on supercritical extracts of *Tanacetum cinerariifolium* (Trevir) Sch. Bip., with three related species of *Chrysanthemums* of Sardinia (Italy). *Nat. Prod. Res*., 23: 190-199.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Plant Physiol*., 15: 473-497.

Nalini, R. 2012. Micropropagation of chrysanthemum (*Chrysanthemum morifolium*) using shoot tip as explant. *Inter. J. Food, Agri. and Vet. Sci*., 2(2): 62-66.

Ross, C.W. and F. W. Salisbury. 1984. *Plant physiology*. Colorado USA Edi. 3, pp. 312-333.

Sassi, A.B., F. Harzallah-Skhiri, N. Bourgougnon and M. Aouni. 2008. Antimicrobial activities of four Tunisian *Chrysanthemum* species. *Indian J. Med. Res*., 127: 183-192.

Sutter, E. and R.W. Langhans. 1981. Abnormalities in *Chrysanthemum* regenerated from long term cultures. *Ann. Bot*., 48: 559.

Teixeira da Silva. 2003.Chrysanthemum organogenesis through thin cell layer technology and plant growth regulator control. *Asian J. Plant Sci*., 2(6): 505-514.

Waseem, K., M.S. Jillani and M.S. Khan. 2009. Rapid plant regeneration of Chrysanthemum (*Chrysanthemum morifolium* L.) through shoot tip culture. *Afr. J. Biotech*., 8(9): 1871-1877.

Zafarullah, S. Ilyas, S. Naz, F. Aslam and F. Manzoor. 2013. Effect of culture media and growth regulators on *In vitro* propagation of *Chrysanthemum indicum* L. *Pak. J. Sci*., 65(4): 462.

Zhen, Y., J. Chen, Q. Chen and J. Shi. 2015. Elemental analyses of calli and developing somatic embryo of hybrid *Liriodendron*. *Pak. J. Bot*., 47(1): 189-196.

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