www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 47-50
© 2022 TPI
www.thepharmajournal.com
Received: 26-05-2022
Accepted: 30-06-2022

Ganisetti Anitha
M. S. Swaminathan School of Agriculture, Centurion University, Paralakhemundi, Odisha, India

Mukund Shiragur
M. S. Swaminathan School of Agriculture, Centurion University, Paralakhemundi, Odisha, India

Sandhyarani Nishani
M. S. Swaminathan School of Agriculture, Centurion University, Paralakhemundi, Odisha, India

Pushpa Latha G
M. S. Swaminathan School of Agriculture, Centurion University, Paralakhemundi, Odisha, India

Corresponding Author
Ganisetti Anitha
M. S. Swaminathan School of Agriculture, Centurion University, Paralakhemundi, Odisha, India

In vitro propagation of chrysanthemum through petals

Ganisetti Anitha, Mukund Shiragur, Sandhyarani Nishani and Pushpa Latha G

Abstract
Chrysanthemum (*Chrysanthemum grandiflora* Tzvelev) is one of the commercial crops in India. Studies were conducted for standardization of micro propagation protocol from ray florets of chrysanthemum. The experiments revealed that the highest percentage of aseptic culture and lowest percentage of contamination was observed in treatment with mercury chloride (0.1%) and ethanol (70%), the maximum callus percentage was obtained in treatment MS medium with BAP 4 mg l^{-1} and NAA 1 mg l^{-1}, highest percentage of shoot proliferation and more number of micro shoots per clump were seen in MS medium with 5 mg l^{-1} BAP and 1 mg l^{-1} NAA. Number of days taken for rooting was less in half MS medium with IBA 1.5 mg l^{-1}. Number of roots per shoot were more in half MS medium with IBA 1.5 mg l^{-1}. Half MS medium with IBA 1 mg l^{-1} resulted more root length in Poornima Red.

Keywords: Chrysanthemum cultivar Poornima red, petals, callus, MS medium

Introduction
Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one of the important commercial cut flower and pot plant, commonly called as 'Autumn Queen'. Chrysanthemum ranks second in the global cut flower market after rose (Datta and Gupta) [4]. In floriculture industry, chrysanthemum has large demand and popularity because of its range of flower forms, colours and their growth habits. *In vitro* propagation will be useful for rapid plant multiplication, germplasm conservation, elimination of pathogens, genetic manipulations, and for secondary metabolite production. Plant tissue culture has emerged as an approaching tool and forms the backbone of plant biotechnology and the better quality planting material is a basic need of growers for accelerating the productivity. (Chebet *et al.*,) [2] The success of the *in vitro* propagation depends on numerous factors like genotype, type of media, plant growth regulators and type of explants, which should be used during the process (Kim *et al.*,) [7]. The present article emphasizes the protocol standardization for rapid multiplication of chrysanthemum cultivars through *in vitro*.

Material and Methods
Standardization of protocol for organogenesis was carried out in the Tissue Culture Unit, Department of Biotechnology and Crop Improvement during 2018-19. For the study, chrysanthemum cultivar Poornima Red was selected and the ray florets were used as explants. Flowers were collected from healthy mother plants which were grown in well maintained poly house. Flowers were washed with running tap water thoroughly for 15 minutes followed by washing with sterile water for 2 to 3 times before culture under laminar air flow chamber served as control. Similarly flowers were treated with different chemicals sodium hypochlorite, mercuric chloride and ethanol in different concentrations with specific time period. Finally 3-4 times rinses with sterile water. After sterilization, ray florets were peeled off from the flower and cut horizontally. Basal portion was placed adaxially on Murashige and Skoog medium (Murashige and Skoog, 9) with 30g/l^{-1}sucrose, 8g/l^{-1} agar and Benzyl amino purine (BAP, at 2.0, 3.0, 4.0 mg l^{-1}), Kinetin (KN, at 2.0, 3.0, 4.0 mg l^{-1}) in combination with Naphthalene acetic acid (NAA, at 1.0 mg l^{-1}) for callus initiation. The pH of the medium was adjusted to 5.7 before autoclaving at 121 °C for 20 minutes. After callus formation, callus was transferred to the MS medium with BAP (4.0, 5.0 and 6.0 mg l^{-1}) and NAA (1.0 mg l^{-1}). After formation of shoots, shoots were transferred to the half MS medium either with Indole butyric acid (0.5, 1.0, 1.5 mg l^{-1}) or NAA (0.5, 1.0 and1.5mg l^{-1}) for rooting. Media was supplemented with 30g l^{-1} sucrose as a carbon source and 7g l^{-1} agar-agar as a gelling agent. pH was maintained in between 5.7-6. Media was autoclaved in 121 °C temperature and 15 lb pressure

for sterilization. In rooting media activated charcoal has been added at 2g l^{-1}. All the media were kept and observed for contamination for minimum three days before using for culture. Baby jar bottles (250 ml) with autoclavable polypropylene caps were used as culture containers. Culture room maintained at a temperature of 25 ± 2 °C with uniform light intensity (ca 1000 lux) was provided by fluorescent tubes (7200ºK) over a light and dark cycle of 16 and 8 hours respectively. Each treatment had 6 replications arranged in a completely randomized design.

Results and Discussion

The data on percentage of aseptic culture was recorded one week after culture initiation in all three selected chrysanthemum cultivars. The highest aseptic culture was noticed in mercury chloride and ethanol treatment with 86.60 percent aseptic culture, respectively. No aseptic culture was recorded in untreated control (Table 1). Significantly, the lowest contamination (5.00%) was recorded when treated with mercuric chloride and ethanol (Table 1).

The highest percentage of callus (93.75%) induction was observed in MS medium with BAP 4 mg l^{-1} and NAA 1 mg l^{-1} in all varieties of chrysanthemum. Whereas, control (MS media without growth regulators) treatment showed zero percentage of callus induction (Table 2). These results shows that BAP combined with NAA may be important for callus induction. Obukosia *et al*. [11] also reported the effect of auxin in callus production. NAA acts as an auxin to induce cell development and enlargement at low concentration. Cell enlargement is associated with an increase in activity of enzymes which will affect the cell wall plasticity and new cell wall materials (Cleland, 3). Nahid *et al.* [10] in chrysanthemum reported that, the induction of callus formation in chrysanthemum was maximum in MS medium with 2 mg l^{-1} BA and 0.1 mg l^{-1} NAA. Verma *et al.* [14] described that the 81.67% callus formation was seen in MS medium with 4 mg/l BAP and 1 mg/l NAA in chrysanthemum.

The highest percentage of micro shoot proliferation 79.00% in Poornima Red observed in treatment MS medium with BAP 5 mg l^{-1} and NAA 1 mg l^{-1}. The highest number of micro shoots per clump (22.30 in Poornima Red) was observed in treatment MS medium with BAP 5 mg $l^{-1}$and NAA 1 mg l^{-1} followed by treatment BAP 6 mg l^{-1} and NAA 1 mg l^{-1} (6.16). Whereas, there was no emergence of micro shoots in remaining treatments which were used in this experiment (Table 3). Successful shoot regeneration is important in organogenesis of any crop. Cytokinins are generally used for inducing shoot proliferation, combination of auxin and cytokinin was shown effective for shoot regeneration (Kumar *et al.*,) [8]. Highest percentage of shoot proliferation and a greater number of micro shoots per clump were seen in MS medium with 5 mg l^{-1} BAP and 1 mg l^{-1}. These results were quite similar to the results obtained by Verma *et al.*, [14] and Chakrabarty *et al.*, [1] in chrysanthemum. Shoot regeneration might be due to the concentration of BAP (cytokinin) which will induce the axillary branching and production of multiple shoots. Poornima Red has shown good response for shoot proliferation in MS medium with 5 mg l^{-1} BAP and 1 mg l^{-1} NAA but, the percentage of response will be varied with variety, it might be due to the capacity of genotype for uptake of growth regulator concentration.

Significant differences were found among treatments with respect to number of days taken for rooting, number of roots per shoot and root length. Number of days taken for rooting was less in half MS medium with IBA 1.5 mg l^{-1}. Number of roots per shoot was more in half MS medium with IBA 1.5 mg l^{-1}. These results were quite close to the results obtained by Swarna *et al.*, [13] in chrysanthemum. Gautheret [5] suggested first the importance of auxin in root induction, Hoque [6] was used IBA at different concentrations in MS medium for root induction on *in vitro* regenerated shoots in chrysanthemum.

Root length was varied with cultivar to cultivar and concentration of growth regulators (IBA and NAA). Half MS medium with IBA 1 mg l^{-1} resulted more root length and half MS medium with IBA 1.5 mg l^{-1} resulted more root length. Similarly Swarna *et al.*, [13] reported that, high mean root length was recorded in media containing 1.0 mg l^{-1} IBA and 1.5 mg l^{-1} IBA in chrysanthemum and Shatnawi *et al.* [12] reported that maximum root length was obtained by using IBA or NAA in chrysanthemum.

Significant differences were found among treatments with respect to number of days taken for rooting, number of roots per shoot and root length. It might be due to the capacity of genotype for uptake of growth regulator concentration and difference in endogenous growth regulators.

Table 1: Effect of surface sterilants on aseptic culture and contamination percentage in *in vitro* culture of chrysanthemum

S. No	Treatments	Aseptic culture (%)	Contamination (%)
1	Control	0.00 (0.28)	100.00 (89.70)
2	Sodium hypochlorite and ethanol	25.00 (29.70)	55.00 (47.90)
3	Mercury chloride and ethanol	86.60 (72.40)	5.00 (9.36)
4	Ethanol (75%)	22.50 (28.1)	65.80 (54.30)
S. Em±		3.25	2.53
C. D. at 1%		13.09	10.2

*Figures in parenthesis indicates arcsine transformed values

Table 2: Effect of different growth regulators on callus induction in *in vitro* culture of chrysanthemum cv. Poornima Red

S. No	Treatments	Callus induction (%)
1	Control	0.00 (0.28)
2	BAP 2 mg/l+ NAA 1 mg/l	71.25 (57.58)
3	BAP 3mg/l+ NAA 1 mg/l	76.25 (60.83)
4	BAP 4 mg/l+ NAA 1 mg/l	91.25 (72.79)
5	Kinetin 2 mg/l+ NAA 1 mg/l	51.25 (45.72)
6	Kinetin 3 mg/l+ NAA 1 mg/l	57.50 (49.31)
7	Kinetin 4 mg/l+ NAA 1 mg/l	82.50 (65.27)
S. Em±		1.64
C. D. at 1%		6.55

*Figures in parenthesis indicates arcsine transformed values

Table 3: Effect of different growth regulators on microshoot proliferation and number of microshoots from callus in *in vitro* culture of chrysanthemum cultivars

S. No	Treatments	Microshoot proliferation (%)	Number of microshoots
1	Control	0.00 (0.28)	0.00 (0.28)
2	BAP 4mg/l+ NAA 1 mg/l	43.00 (40.98)	6.03 (14.18)
3	BAP 5mg/l+ NAA 1 mg/l	79.00 (62.73)	22.30 (28.15)
4	BAP 6 mg/l+ NAA 1 mg/l	66.00 (54.33)	6.16 (14.28)
S. Em±		2.23	0.61
C. D. at 1%		8.58	2.45

*Figures in parenthesis indicates arcsine transformed values

Table 4: Effect of different growth regulators on days taken for rooting, number of roots and root length in *in vitro* culture of chrysanthemum cv. Poornima Red

S. No	Treatments	Number of days taken for rooting	Number of roots	Root length (cm)
1	Control	19.97	5.10	6.83
2	½ MS + NAA 0.5 mg/l	14.73	8.91	6.60
3	½ MS + NAA 1 mg/l	15.53	8.00	6.90
4	½ MS + NAA 1.5 mg/l	16.96	8.20	7.90
5	½ MS + IBA 0.5 mg/l	11.40	8.03	8.66
6	½MS + IBA 1 mg/l	12.23	12.10	8.90
7	½ MS + IBA 1.5 mg/l	10.96	12.60	7.33
S. Em±		0.97	0.22	0.28
C. D. at 1%		4.07	0.93	1.16

*Figures in parenthesis indicates arcsine transformed values

Conclusion

Highest percentage of aseptic culture and lowest percentage of contamination was observed in treatment with mercury chloride (0.1%) and ethanol (70%), the maximum callus percentage was obtained in treatment MS medium with BAP 4 mg l^{-1} and NAA 1 mg l^{-1}, highest percentage of shoot proliferation and more number of micro shoots per clump were seen in MS medium with 5 mg l^{-1} BAP and 1 mg l^{-1} NAA in all three chrysanthemum. Rooting was good in half MS medium with IBA 1.5 mg l^{-1}. In this study, we standardized an efficient protocol for plantlet regeneration from callus using petals as explants which will be helpful for commercial propagation of contaminant free plants, in other biotechnological techniques which will be helpful for production of new varieties.

Authors' contribution

Conceptualization of research (Mukund S.); Designing of the experiments (Ramanagouda SH, Seetharamu GK); Contribution of experimental materials (Patil, BC); Execution of field/lab experiments and data collection (Anitha G.); Analysis of data and interpretation (Sandhayarani N); Preparation of the manuscript (Anitha G, Mukund S).

Declaration: We do not have any conflict of interest.

Acknowledgments: Approved for publication by the Head of the Department, Department of Floriculture and Landscape Architecture. Funding for this project was provided by the University of Horticultural sciences, Bagalkot, Karntaka. We thank Dean of Kittur Rani Channamma College of Horticulture, Arabhavi for provided facilities for conducting this experiment.

References

1. Chakrabarty D, Mandal AKA, Datta SK. Management of chimera through direct shoot regeneration from florets of chrysanthemum (*Chrysanthemum morifolium* Ramat.). J Hort. Sci. Biotech. 1999;74:293-296.
2. Chebet DK, Okeno JA, Mathenge P. Biotechnological approaches to improve horticultural crop production. Acta Horticulturae. 2003;625(1):473-477.
3. Cleland R. Cell wall extension. Plant Physiol. 1971;22:197-222.
4. Datta SK, Gupta VN. Year round cultivation of garden chrysanthemum (*Chrysanthemum morifolium* Ramat.) through photoperiodic response. Sci. Cult. 2012;78:71-77.
5. Gautheret RJ. Sur Ia possibility de realiserIa culture indefinie des tissue de tubercules de carrotle. Acad. Sci. 1939;208:118-121.
6. Hoque MI. *In vitro* multiple shoot regeneration in *Chrysanthemum morifolium* Ramat. Plant Tissue Culture. 1995;5(2):153-162.
7. Kim CK, Chung JD, Jee SO, Oh JY. Somatic embryogenesis from in vitro grown leaf explants of *Rosa hybrida* L. African Journal of Plant Biotechnology. 2003;5(3):169-172.
8. Kumar S, Kanwar JK, Sharma DR. *In vitro* regeneration of *Gerbera jamesonii* bolus from leaf and petiole explants. J P. Biochem. Biot. 2004;13:73-75.
9. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962;15:473-497.
10. Nahid JS, Shyamali S, Kazumi H. High frequency shoot regeneration from petal explants of *Chrysanthemum morifolium* Ramat. *in vitro*. Pak. J Biol. Sci. 2007;10(9):3356-3361.
11. Obukosia SD, Kimani E, Wathaika K, Mutitu E, Kimani PM. Effect of growth regulators and genotypes on pyrethrum *in vitro*. *In vitro* cell division. Biol. Plant. 2005;41:162-166.
12. Shatnawi M, Al-Fauri A, Megdadi H, Kair M, Al-Shatnawi SRS, Abu-Romman AAL. *In vitro* multiplication of *Chrysanthemum morifolium* Ramat. and it is responses to NaCl induced alinity. Jordan J biol. sci. 2010;3(3):101-110.
13. Swarna RJ, Yeasmin D, Rahman Md. M, Alam Md. F. Callus induction and indirect organogenesis in *Chrysanthemum morifolium* Ramat. Int. J Biosci.

2016;9(3):139-149.
14. Verma AK, Prasad KV, Singh SK, Kumar S. *In vitro* isolation of red coloured mutant from chimeric ray florets of chrysanthemum induced by gamma-ray. Indian J Hort. 2012;69(4):562-567.