DOI : 10.5958/0974-0112.2021.00007.4

Indian J. Hortic. 78(1), March 2021: 46-52

Standardization of *in vitro* regeneration protocol in annual chrysanthemum

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ABSTRACT

An efficient in vitro regeneration protocol of annual chrysanthemum (*Chrysanthemum coronarium* L.) was standardized using leaf explants. Leaf segments (0.25 cm2) were taken from three weeks old in vitro raised plantlets and cultured on MS basal medium containing BAP, NAA and Kinetin at different concentrations. Maximum survival of micro shoots (86.67%) and shoot regeneration (82.79%) was achieved in half-strength MS medium supplemented with BAP (0.5 mg/l) and NAA (1.0 mg/l) (T6). Half-strength MS medium supplemented with GA3 (0.5 mg/l) (T3) showed thick shoots (5.78 cm) with dark green leaves, and the highest rooting (90.00%) was recorded in the treatment, including half-strength MS basal medium supplemented with NAA (0.5 mg/l) (T3). The newly regenerated plantlets recorded maximum plant survival (76.60%) in a medium containing cocopeat, perlite and vermiculite (1:1:1) (T2) supplemented with half-strength MS inorganic broth. The study concluded that the established protocol might produce true-to-type plants that are otherwise difficult in the species. Furthermore, this protocol may help for large-scale multiplication of desired types to fulfil the demand for quality planting material for *C. coronarium*.

Keywords: *Chrysanthemum coronarium,* Growth regulators, Rooting, Acclimatization, Protocol

INTRODUCTION

Chrysanthemum coronarium L. is an annual herb belong to family *Asteraceae* and is known popularly as garland or annual chrysanthemum. It is widely distributed in the Mediterranean region, Japan, China, and Philippines (Sanchez-Monge, 14). Annual chrysanthemum has two-pinnatisect oblong leaves with big capitula, usually having white and yellow colored florets. Botanical extract of this species had showed strong and selective allelopathic activity against weeds (Hosni *et al*., 5), and nematodes (Bar-Eyal *et al*., 1). It is being cultivated in Indian subcontinent throughout the year for white and yellow coloured loose flowers. It is highly cross-pollinated species and propagated by seeds. Among its different flower forms *viz*., single, semi-double and double type, mostly semi double and double types are preferred in the market. However, maintaining such true to the type is difficult by conventional methods due to self incompatibility and heterozygosity due to out crossing. Therefore, development of homozygous lines is not possible by conventional methods.

Regeneration through *in vitro* culture has now become viable alternative to conventional propagation methods. *In vitro* propagation offers large scale multiplication of true to type disease free plants from limited explants within a short period of time. To meet the high demand for semi double/double chrysanthemums, tissue culture can be utilized for its large-scale production. Shoot regeneration from leaf and stem explants has been studied in *Chrysanthemum morifolium* (Jacobsen *et al.,* 6).A rapid shoot multiplication protocol has been developed through apical meristem inoculated on MS medium supplemented with various concentrations of growth regulators in *Chrysanthemum indicum* L.(Zafarullah *et al*., 15). However, not much information is available on efficient protocol for *in vitro* regeneration of annual chrysanthemum plants for use *per se* or for use in other biotechnologically led crop improvement related aspects. Therefore, the present study was conducted with an objective to develop standard protocol for mass scale *in vitro* regeneration of annual chrysanthemum.

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MATERIALS AND METHODS

The present study was carried out during 2017-18 at Central Tissue Culture Laboratory, Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi. Surface sterilization treatment and culture medium for *in vitro* seed germination was standardized during our previous experiments (Pooja *et al*., 12). Further, the seeds were germinated *in vitro* and leaf explants were taken from *in vitro* raised seedlings for regeneration to get infection free seedlings**.** To optimize culture conditions for leaf regeneration from cultured tissues, leaf disc explants (0.25 cm^2) were excised from young and mature leaves of 3 weeks old plants grown *in vitro* culture containing half strength MS medium (Murashige and Skoog,10), 30 g/l sucrose, 7.0 g/l agar powder as basal medium

supplemented with different combinations of cytokinins viz, BAP (6-Benzylaminopurine),Kinetin and Auxin i.e. NAA (1-Naphthalene acetic acid).A total of nineteen treatment combinations were tested to find out optimum treatment combination for shoot regeneration. The treatment T_0 was devoid of all growth regulators. The other treatments including T_1(BAP 0.25 mg/l and NAA 0.25 mg/l);T_2(BAP 0.25 mg/l and NAA 0.50 mg/l);T_3(BAP 0.25 mg/l and NAA 1.0 mg/l);T_4(BAP 0.50 mg/l and NAA 0.25 mg/l);T_5(BAP 0.50 mg/l and NAA 0.50 mg/l);T_6(BAP 0.50 mg/land NAA 1.0 mg/l);T_7(BAP 1.0 mg/l and NAA 0.25 mg/l);T_8(BAP 1.0 mg/land NAA 0.50 mg/l);T_9(BAP1.0 mg/l and NAA 1.0 mg/l);T_{10}(NAA 0.25 mg/l and Kinetin 0.25 mg/l);T_{11}(NAA 0.25 mg/l and Kinetin 0.5 mg/l);T_{12}(NAA 0.25 mg/l and Kinetin 1.0 mg/l);T_{13}(NAA 0.50 mg/l and Kinetin 0.25 mg/l); T_{14}(NAA 0.50 mg/land Kinetin 0.5 mg/l);T_{15}(NAA 0.50 mg/l and Kinetin 1.0 mg/l);T_{16}(NAA 1.0 mg/l and Kinetin 0.25 mg/l);T_{17}(NAA 1.0 mg/l and Kinetin 0.5 mg/l) and T_{18}(NAA 1.0 mg/l and Kinetin 1.0 mg/l).

To achieve shoot elongation, all the treatments T_0 to T_5 involved half strength of MS salts. The treatment T_0 was devoid of GA_3 (Gibberellic Acid) whereas the treatments T_1 to T_5 were supplemented with GA_3at 0.1mg/l, 0.25mg/l, 0.5mg/l, 1.0mg/l and 2.0mg/l respectively for shoot elongation. Fully developed micro-shoots with expanded dark green foliage were selected for *in vitro* root induction. For this purpose, all the treatments T_0 to T_6 consisted of half strength of MS salts, sucrose (60g/l) and agar (7g/l).Along with these components, growth regulators such as IBA (Indole-3-butyric acid), NAA, IAA (Indole-3-acetic acid) were supplemented wherein the treatment T_0 was devoid of any growth regulator and other treatments had varying concentrations of these hormones viz,T_1(IBA 0.5 mg/l);T_2(IBA 1.0 mg/l);T_3(NAA 0.5 mg/l);T_4(NAA 1.0 mg/l);T_5(IAA 0.5 mg/l);T_6(IAA 1.0 mg/l).The plantlets were acclimatized in varying treatments using a mixture of different pot media namely viz., T_0 (Cocopeat: Soilrite: Perlite);T_1(Peatmoss: Soilrite: Perlite);T_2(Cocopeat: Perlite: Vermiculite);T_3(Peatmoss: Perlite: Vermiculite) in 1:1:1 ratio. For the supplementation of essential nutrients to harden the plants, liquid half-strength MS inorganic salts devoid of calcium, organic components, sucrose and plant growth regulators were used. The experiments were laid down in completely randomized design (CRD). In most of the experiments, three replications were taken to record the data. However, replications were increased according to the treatments. The complete data was analyzed using online OPSTAT software developed by Haryana Agriculture University, Hisar, Haryana. All the percentage data was subjected to angular transformation before statistical analysis.

RESULTS AND DISCUSSION

Growth regulators such as BAP, NAA and Kinetin were used in different combinations and concentrations to study their influence on leaf segment regeneration (Table 1). The leaf segments inoculated on basal medium supplemented with 0.5 mg/l BAP and 1.0 mg/l NAA (T_6) exhibited maximum survival of leaf segments (86.67%) followed by the treatment T_{16} involving basal medium supplemented with NAA 1.0 mg/l and Kinetin 0.25 mg/l. None of leaf segments survived in control (T_0).In case of callus formation, highest callus formation (82.56%) was observed in the treatment T_{15} consisting of half strength MS supplemented with NAA(0.50 mg/l) and Kinetin (1.0 mg/l) and was found at par with T_{16}, T_6, T_5, T_{11} and T_{12}. However, lowest callusing (73.58%) was found in the leaf segments inoculated on the culture medium having half strength MS basal salts supplemented with BAP (0.25 mg/l) and NAA (0.50 mg/l) in the treatment T_2.However, there was no callus formation in control T_0.Callusing was recorded earliest (6.45 days) in the treatment T_3 containing half strength MS basal salts supplemented with BAP 0.25 mg/l and NAA 1.0 mg/l however, it was found to be at par with treatment T_4, T_7, T_8, T_{10}, T_{14}, T_{16} and T_{17}. Shoot regeneration was found highest (82.49%) in the treatment T_6 (basal medium supplemented with 0.5 mg/l BAP and 1.0 mg/l NAA) followed by T_5 (basal medium supplemented with 0.50 mg/l BAP and 0.50 mg/l NAA) and T_{16}(basal medium supplemented with NAA 1.0 mg/l and Kinetin 0.25 mg/l). Early shoot regeneration was found in the treatment T_2 and T_{12} (27.22 days) however, these two treatments were found at par with all other treatments except T_1, T_5, T_6, T_{11} and T_{16}. Number of shoots per explant was significantly higher in the treatment containing basal medium supplemented with 0.5 mg/l BAP and 1.0 mg/l NAA (T_6) i.e. 7.89, 19.11 and 46.00 at 15 days, 30 days and 45 days after inoculation stages respectively (Fig 1A).Cytokinins promote cell division/expansion and thereby regulating the organogenesis process. In the absence of cytokinins, auxins alone might induce non regenerating callus and roots. Cytokinins (BAP or Kinetin) at lower concentration might be enough for callus induction but may not be enough for prolific plant regeneration. An optimum combination of both auxins and cytokinins synergistically affect the process of shoot and root regeneration from leaf segments. Lee *et al.* (8) reported regeneration of adventitious shoots at high frequency (73%) when leaf discs were inoculated on MS medium containing BA (2.5 µM) and NAA (2.5 µM) in garland chrysanthemum. Kaul *et al.* (7) used MS basal medium supplemented with 5 µM each of BAP and NAA for regeneration of adventitious shoots from leaf and stem explants of

Table 1. Effect of different growth regulators on shoot regeneration in annual chrysanthemum

Treatments(s)	Survival (%)	Callusing (%)	Days to callusing (days)	Shoot Regeneration (%)	Days to shoot regeneration (days)	No. of shoots/ explant (15 days)	No. of shoots/ explant (30 days)	No. of shoots/ explant (45 days)
T_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_1	72.22(58.23)*	76.06(60.75)	6.56	78.42(62.32)	33.89	3.22	6.44	13.67
T_2	71.11(57.53)	73.58(59.06)	7.56	76.98(61.34)	27.22	2.67	7.78	16.33
T_3	70.00(56.79)	74.19(59.47)	6.45	78.45(62.32)	30.55	7.22	14.33	33.45
T_4	75.55(60.39)	73.65(59.13)	7.00	75.88(60.61)	28.89	4.22	8.00	18.55
T_5	78.89(62.85)	80.08(63.75)	8.78	80.77(63.99)	37.22	3.22	8.22	17.44
T_6	86.67(68.66)	80.45(63.81)	8.89	82.79(65.58)	36.11	7.89	19.11	46.00
T_7	77.78(61.87)	76.63(61.11)	7.11	78.93(62.69)	28.89	3.22	7.22	17.56
T_8	71.11(57.77)	75.67(60.47)	7.33	78.84(62.64)	32.22	4.33	5.89	13.33
T_9	76.66(61.21)	76.04(60.68)	7.44	76.72(61.14)	28.33	2.44	4.11	13.11
T_{10}	73.34(58.97)	77.50(61.69)	7.34	79.20(62.86)	28.89	1.44	3.22	12.33
T_{11}	72.22(58.23)	79.03(62.73)	8.45	77.67(61.79)	33.34	5.22	7.56	18.44
T_{12}	76.67(61.33)	78.29(62.25)	7.89	78.59(62.43)	27.22	2.22	4.89	15.67
T_{13}	74.44(59.69)	77.02(61.34)	7.66	77.85(61.96)	29.44	4.00	8.89	18.22
T_{14}	75.56(60.36)	76.77(61.17)	7.22	77.62(61.78)	32.78	3.67	7.44	15.00
T_{15}	74.44(59.62)	82.56(65.36)	7.55	74.37(59.57)	31.11	2.89	4.78	8.22
T_{16}	84.44(66.77)	80.98(64.18)	7.00	81.98(64.94)	36.11	3.78	7.89	13.89
T_{17}	71.11(57.65)	75.66(60.55)	7.34	79.26(62.93)	30.00	5.11	11.45	26.56
T_{18}	74.45(59.65)	74.55(59.79)	7.56	74.13(59.43)	31.67	5.00	9.00	19.33
CD#	6.39	4.50	0.94	3.37	5.46	0.65	0.73	1.82

*Figures mentioned in the parenthesis are transformed values; # Mean difference at 5% level of significance

chrysanthemum. A study carried out by Bhattacharya *et al.*(2) on *in vitro* regeneration in *Chrysanthemum morifolium* Ramat cv. Birbal Sahni showed that leaf callus regenerated 2-3 shoots after three weeks when cultured on MS medium supplemented with 0.1 mg/l IAA and 0.2 mg/l BAP. Naing *et al*. (11) reported that leaf explants of *morifolium* cv. Vivid Scarlet cultured on MS medium supplemented with a combination of 1 mg/l BA and 2 mg /l NAA under light conditions without any initial dark period produced the highest number of shoots (12.3) per explant.

Shoot elongation was induced on half strength MS medium supplemented with different concentrations of GA_3. Highly desirable medium (5.78 cm) and thick shoots with well developed dark green leaves were recorded after 45 days in the treatment T_3 containing 1/2 strength MS medium supplemented with 0.5 mg/l GA_3 (Table 2, Fig1B) At higher concentrations of GA_3 the shoots were lanky with light green leaf was found in the treatment T_4 (half strength MS salts + 1.0 mg/l GA_3) and T_5 (half strength MS salts + 2.0 mg/l GA_3) compared to control T_0 (4.0 cm). The *in vitro* proliferated micro shoots having optimum length, thickness with well developed leaves are suitable for development of strong roots. The optimum concentration of gibberellic acid in the culture medium is highly essential for the growth and development of shoots. At a lower level it produces stunted, weak and underdeveloped shoots whereas, higher level produces lean, lanky and very long shoots. According to Salunkhe *et al.* (13), gibberellic acid stimulates elongation and development of micro shoots and these strong shoots are suitable for rooting.

In the present study, highest rooting (71.54%) in minimum duration (6.33 days) with more number of primary roots (5.78) and better root length(3.52 cm) after 15 days was recorded on half strength MS medium supplemented with sucrose (60 g/l), agar (7 g/l) and NAA (0.5 mg/l) in treatment T_3 (Table 3, Fig 1C) followed by treatment T_2 (84.33%) in minimum duration (7.78 days) cultured on half strength MS medium supplemented with sucrose (60g/l),agar (7g/l) and IBA (1.0mg/l).Strength of basal medium, sucrose level and supplementation with growth regulators are

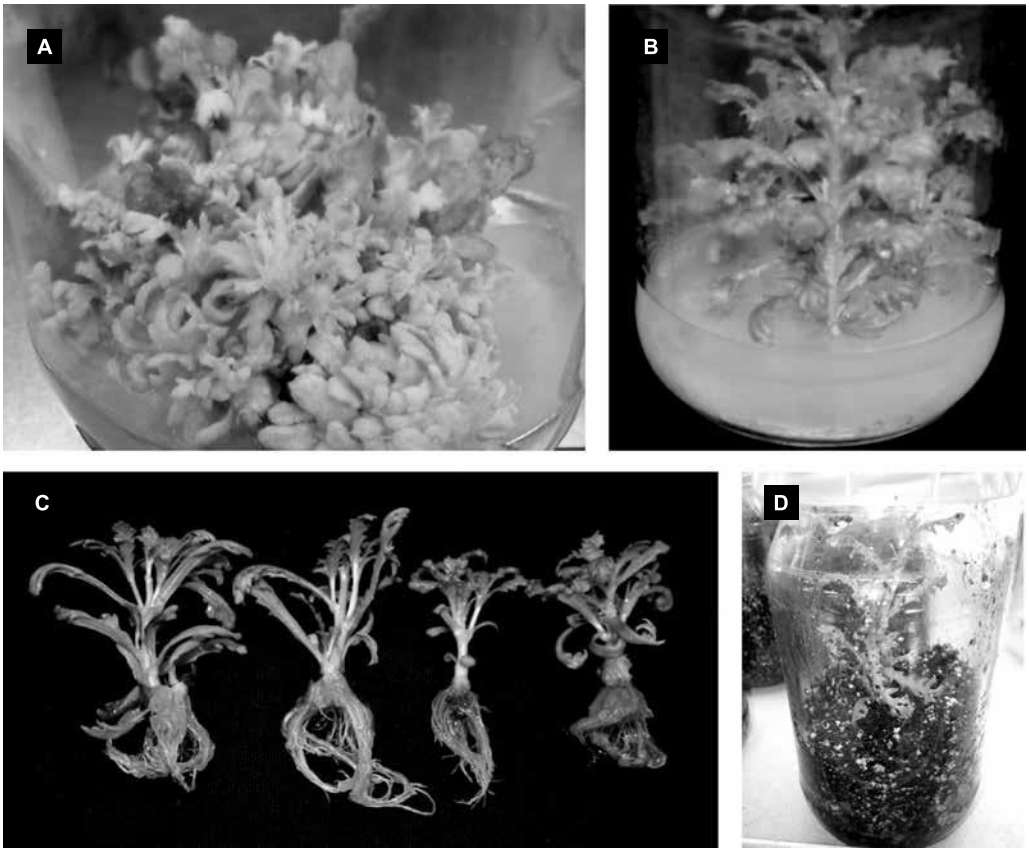

Fig. 1. *In vitro* shoot regeneration on MS medium +BAP (0.50 mg/l) + NAA (1.0 mg/l) (T_6)(A); *In vitro* shoot elongation after 45 days of culturing on MS mediumGA_3 0.5mg/l(T_3)(B); *In vitro* root induction on MS (1/2 strength) + sucrose (60 g/L) + 7 g/L agar + NAA (0.5 mg/l) T_3after 15 days of culture (C); *In vitro* plantlets showing maximum survival in cocopeat, perlite and vermiculite (1:1:1) (T_2)in annual chrysanthemum (D)

Table 2. Effect of GA_3 on shoot elongation in annual chrysanthemum

Treat.	Shoots length (cm) after 45 days	Visual Observations
T_0	4.00	Light green shoots
T_1	4.56	-
T_2	4.67	-
T_3	5.78	Medium height thick shoots with dark green well-developed leaves
T_4	8.55	Lanky shoots with Light green leaves
T_5	9.22	Lanky shoots with Light green leaves
CD#	0.66	-

*Figures mentioned in the parenthesis are transformed values; # Mean difference at 5% level of significance

important for successful rhizogenesis in micro shoots raised *in vitro*. The rooting of micro shoots could be improved only after addition of auxins into the rooting medium (Davies, 3). MS medium devoid of auxins produced thin, long and comparatively inferior roots to those induced by auxins. The micro propagated plants may be unable to absorb water and nutrients when they are transferred from high osmotic strength of full MS medium to a lower osmotic strength environment in soil and leads to the death of the tissues. Root initiation and growth are high energy requiring processes that can occur only at the expense of available metabolic substrates, mainly sugars. Higher concentration of sucrose induces a greater number of roots and higher carbohydrate stocks, but shorter plants with reduction of photosynthetic pigment

Table 3. Effect of different concentrations of auxins on *in vitro* root induction in annual chrysanthemum

Treatments(s)	Rooting (%)	Duration for root initiation (days)	No. of primary roots after 15 days	Root length after 15 days cm)	Remarks
T_0	81.00 (64.16)	7.89	4.33	2.42	Very thin and long roots
T_1	83.44 (65.97)	6.78	4.78	2.18	Thick grey roots with callus
T_2	84.33 (66.67)	7.78	5.00	2.49	Thick grey roots with more callus
T_3	90.00 (71.54)	6.33	5.78	3.52	White, thick and medium long roots
T_4	82.33 (65.12)	8.22	4.11	2.53	-
T_5	80.78 (63.97)	7.89	4.22	2.35	-
T_6	82.33 (65.12)	7.56	4.89	2.63	-
CD#	1.87	0.92	0.62	0.57	

*Figures mentioned in the parenthesis are transformed values; # Mean difference at 5% level of significance

content compared to plants grown on less sucrose medium (Martins *et al*., 9).

The field environment has considerably lower relative humidity, higher light intensity and infectious atmosphere which are harmful to delicate micro propagated plants in comparison to the *in vitro* conditions. In the present experiment, highest plant survival (76.60%) was obtained in medium consisting of cocopeat, perlite and vermiculite in 1:1:1 ratio in the treatment T_2 (Table 4, Fig 1D) supplemented with 1/2 strength of inorganic MS basal salts. whereas it was 68.91% in those transferred to control T_0 containing cocopeat, soilrite, vermiculite (1:1:1 ratio + double distilled water). The *in vitro* raised plants possess leaves which are incapable of sufficient photosynthesis, stomata unable to close and have poorly developed cuticular waxy layer on the surface of aerial parts .It is imperative that for a species grown *in vitro* is passed through an acclimatization process in order to ensure that sufficient number of plants survive and grow vigorously when transferred to soil (Hazarika, 4). Stepwise protocol developed for *in vitro* regeneration cycle of annual chrysanthemum is represented in Fig 2.

It can be concluded from the current study that the maximum number of micro shoots and shoot regeneration was obtained from half strength MS supplemented with 0.5 mg/l BAP and 1.0 mg/l NAA. Greater shoot elongation was found on half strength MS medium supplemented with 0.5 mg/l GA_3 and maximum per cent of rooting was obtained on half strength MS medium supplemented with 60 g/l sucrose, 7 g/l agar and 0.5 mg/l NAA. After root formation plantlets were grown on substrate medium consisting of cocopeat, perlite and vermiculite in 1:1:1 ratio supplemented with liquid half-strength MS inorganic salts resulted highest plant survival percentage which is important for hardening and successful growth of plantlets under field conditions. Therefore, the standardized protocol may be utilized for large scale multiplication of true to type plants, so as to fulfill the demand of quality planting material of *C. coronarium.*

AUTHORS' CONTRIBUTION

Conceptualization of research (GK); Designing of the experiments (GK, SP); Contribution of experimental materials (AKT); Execution of field/lab experiments and data collection (PA); Analysis of data and interpretation (PA, GK); Preparation of the manuscript (PA, GK,SP)

Table 4. Effect of different potting media on acclimatization of *in vitro* raised plants in annual chrysanthemum

Treat.	Survival (%) after 15 days	Shoot length (cm) after 15 days	Shoot length (cm) after 30 days	Root length (cm) after 15 days	Root length (cm) after 30 days	Days req. for hardening
T_0	68.91 (56.09)	7.71	12.90	12.33	17.85	26.02
T_1	71.19 (57.52)	7.82	12.24	11.37	17.60	25.38
T_2	76.60 (61.05)	8.54	13.51	13.31	18.39	27.98
T_3	71.47 (57.69)	7.91	12.65	12.35	17.74	26.22
CD#	1.53	N/A	N/A	N/A	N/A	N/A

*Figures mentioned in the parenthesis are transformed values; # Mean difference at 5% level of significance

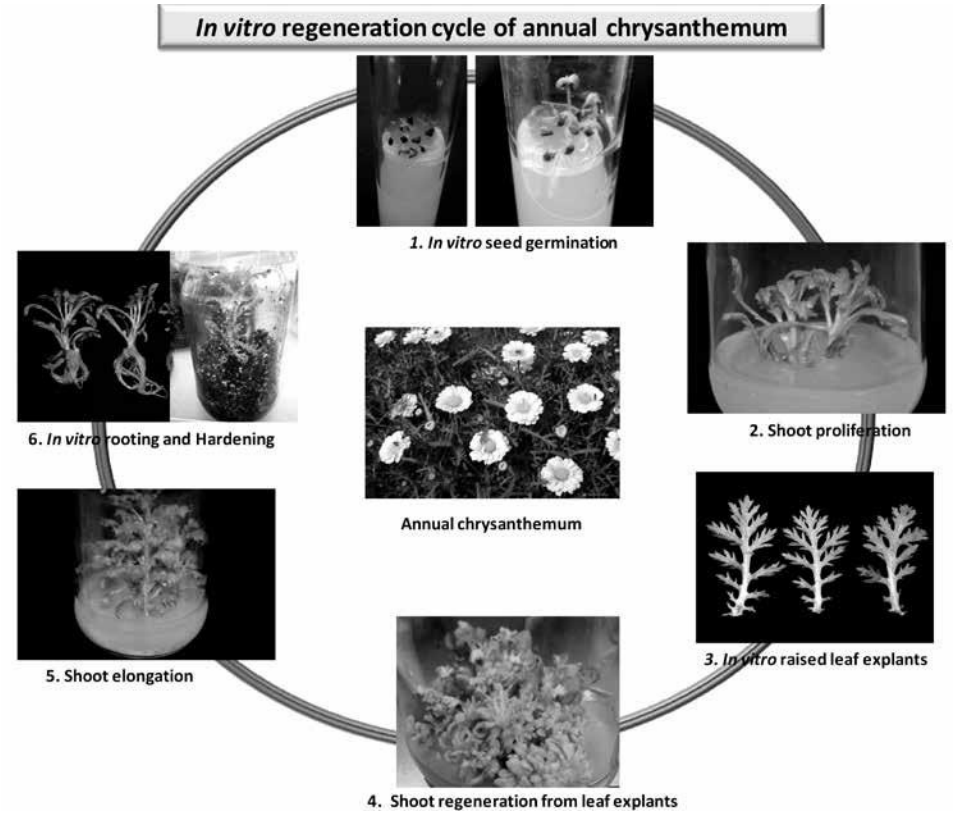

Fig. 2. *In vitro* regeneration cycle of annual chrysanthemum using leaf segments

DECLARATION

The authors declare that there is no conflict of interest

ACKNOWLEDGEMENT

Author would like to express sincere gratitude to the ICAR, New Delhi for providing JRF fellowship during M.Sc. research programme for the present study.

REFERENCES

1. Bar-Eyal, M., Sharon, E. and Spiegel, Y. 2006. Nematicidal activity of *Chrysanthemum coronarium*. *Eur. J. Plant Pathol.* **114**: 427-33.

2. Bhattacharya, P., Dey, S., Das, N. and Bhattacharyya, B. C. 1990. Rapid mass propagation of *Chrysanthemum morifolium* by callus derived from stem and leaf explants. *Plant Cell Rep.* **9**: 439-42.

3. Davies, D. R. 1980. Rapid propagation of roses *in vitro*. *Sci. Hortic.* **13**: 385-89.

4. Hazarika, B. N. 2003. Acclimatization of tissue-cultured plants. *Curr. Sci.* pp. 1704-12.

5. Hosni, K., Hassen, I., Sebei, H. and Casabianca, H. 2013. Secondary metabolites from *Chrysanthemum coronarium* (Garland) flower heads: Chemical composition and biological activities. *Ind. Crops. Prod.* **44:** 263-71.

6. Jacobsen, H. J. and Himstedt, J. P. 2000. Shoot regeneration from stem and leaf explants of chrysanthemum (*Dendranthema* × *grandiflorum*). **In:** *In vitro Culture and Horticultural Breeding. Proc. Intl. Symp.* 2-7 July, 2000, Tampere, Finland, **560:** 421-24.

7. Kaul, V., Miller, R. M., Hutchinson, J. F. and Richards, D. 1990. Shoot regeneration from stem and leaf explants of *Dendranthema* × *grandiflorum* Tzvelev. (Syn. *Chrysanthemum morifolium* Ramat.). *Plant Cell Tissue Organ Cult.* **21**: 21-30.

8. Lee, T., Michele Huan, E. E, and Eng Chong, P. 1997. High frequency shoot regeneration from leaf disc explants of garland chrysanthemum (*Chrysanthemum coronarium*). *In vitro Pl. Sci.* **126**: 219-26.

9. Martins, J. P. R., Pasqual, M., Martins, A. D. and Ribeira, S. F. 2015. Effects of salts and sucrose concentrations on'*In vitro*' propagation of' *Billbergia zebrina*' (Herbert) Lindley. (Bromeliaceae). *Australian J. Crop Sci.* **9**(1): 85.

10. Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* **15**(3): 473-97.

11. Naing, A. H., Jeon, S. M., Han, J. S., Lim, S. H., Lim, K. B. and Kim, C. K. 2014. Factors influencing *in vitro* shoot regeneration from leaf segments of chrysanthemum. *Com. Ren. Biol.* **337**(6): 383-90.

12. Pooja, A., Panwar, S., Tiwari, A. K. and Kumar, G. 2019. Sterilization, germination and shoot proliferation from *Chrysanthemum coronarium* L. seeds. *J. Crop Weed*. **15**(2): 79-84.

13. Salunkhe, D. K., Bhat, N. R. and Desai, B.B. 1990. Rose. **In**: *Postharvest biotechnology of flowers and ornamental plants*. *Springer,* Berlin, Heidelberg, pp. 47-66.

14. Sanchez-Monge, E. 1991. Flora agrı´cola. Tomo I. Ministerio de Agricultura, *Pesca y Alimentacio´n Secretarı´a General Te´cnica*, Madrid, p. 1294.

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

Received : August, 2020; Revised : January, 2021;
Accepted : February, 2021