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INDIRECT *IN VITRO* PLANT REGENERATION FROM RAY FLORET EXPLANTS OF A LOCAL CHRYSANTHEMUM GENOTYPE (*Chrysanthemum* × *morifolium* RAMAT.)

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ABSTRACT

An efficient *in vitro* regeneration protocol is essential for rapid multiplication and large scale production of aseptically regenerated chrysanthemum planting material. The present study was undertaken to develop an effective protocol for plant regeneration and acclimatization of a local chrysanthemum cultivar (*Chrysanthemum morifolium* cv. CO 1) using petal explants. Healthy flower petals were excised from freshly opened flowers and cultured under aseptic conditions on Murashige and Skoog (MS) medium supplemented with suitable plant growth regulators. The cultured explants exhibited regeneration responses under controlled environmental conditions, leading to callus induction and shoot regeneration. Regenerated shoots were subsequently transferred to rooting medium, followed by primary and secondary hardening under protected conditions. The hardened plantlets established successfully under nursery conditions, with 80% survival at 4 weeks after transfer and exhibited normal vegetative growth. The protocol demonstrates the potential of petal explants as a source for *in vitro* propagation of this local chrysanthemum genotype.

Key words : Chrysanthemum, Micropropagation, Petal explants, *in vitro* culture, Acclimatization.

Introduction

Chrysanthemum (*Chrysanthemum* × *morifolium* Ramat.) is one of the most important ornamental flowering crops cultivated worldwide for its remarkable diversity in flower colour, shape, size and extended blooming period. It is widely grown as a cut flower, loose flower, potted plant and landscape ornamental, contributing significantly to the global floriculture industry (De and Bhattacharjee, 2011). Globally, chrysanthemum ranks among the top cut-flower crops. In India, chrysanthemum holds immense commercial importance because of continuous demand for garlands, floral decorations, religious offerings and landscaping (Datta, 2003).

Conventionally, chrysanthemum is propagated through stem cuttings, suckers and division of rooted stools; however, these methods are often constrained by low multiplication rates, seasonal dependence, and the transmission of systemic pathogens, resulting in poor planting material and reduced productivity (Rout *et al.*,

2006). Plant tissue culture has emerged as an effective alternative for the rapid multiplication of chrysanthemum under controlled conditions (Hoque and Fatema, 1995); when combined with meristem-tip culture and pathogen indexing, it can also support the production of virus-tested planting material (Rout *et al.*, 2006). In addition to commercial propagation, *in vitro* regeneration serves as an important tool for germplasm conservation, genetic transformation, mutation breeding (Mandal *et al.*, 2000), and crop improvement programs (Murashige, 1974; George *et al.*, 2008).

The success of *in vitro* regeneration depends on several factors, including genotype, explant type, nutrient medium, plant growth regulators and culture conditions. Among the various explants used for chrysanthemum regeneration, petal (ray floret) explants have shown considerable potential because of their regenerative ability and responsiveness to organogenesis under suitable culture conditions (Teixeira da Silva, 2003; Mandal and Datta, 2005; Barakat *et al.*, 2010; Naing *et al.*, 2014). Murashige

and Skoog (1962) medium remains the most widely used basal medium for chrysanthemum tissue culture owing to its balanced nutrient composition and its ability to support efficient shoot regeneration and plant development. Regeneration efficiency varies considerably among cultivars, and genotype-specific regeneration responses have been reported even within petal/ray floret systems (Naing *et al.*, 2014), necessitating the development of protocols specific to local germplasm (Rout *et al.*, 2006).

Recent work has continued to refine ray-floret regeneration systems in named chrysanthemum cultivars, reporting complete protocols from callus induction through acclimatization (Jadhav *et al.*, 2024) and a comprehensive review has consolidated progress across chrysanthemum *in vitro* regeneration approaches, genotype effects and direct versus indirect organogenesis (Eisa *et al.*, 2022). The present study is distinguished from Jadhav *et al.* (2024) in genotype, explant handling and hormonal strategy: it addresses a separate, locally maintained cultivar (cv. CO 1) (Thamodharan *et al.*, 2024) *in vitro*, uses intact rather than wounded petal explants and evaluates a BAP–NAA-based regeneration system rather than the kinetin-based system optimized for TQP-06, allowing a genotype- and cytokinin-specific comparison of regeneration efficiency within the ray-floret/petal explant system. Therefore, the present study was undertaken to develop an efficient *in vitro* regeneration and acclimatization protocol for a local cultivar of *Chrysanthemum* × *morifolium* (cv. CO 1) using petal explants, to establish a reliable system for rapid multiplication and future biotechnological applications.

Materials and Methods

Plant material and Explant preparation

The present investigation was carried out at the Department of Plant Breeding and Genetics, Vanavarayar Institute of Agriculture, Pollachi, Tamil Nadu, India. The planting material used in the study was *Chrysanthemum morifolium* cv. CO 1, procured from VPR Nursery, Pollachi. The cultivar was identified based on its floral morphology: flower colour yellow; capitulum radiate and flat-topped; and ray florets strap-shaped, flat and straight, with a bright yellow margin. Healthy, freshly opened flowers were collected from vigorously growing mother plants maintained under protected cultivation. Ray petals were excised and used as explants for *in vitro* regeneration.

Surface sterilization

Flowers were initially washed under running tap

water for 15 minutes to remove dust and surface contaminants. Explants were then treated with a solution of 10% sodium hypochlorite (150 µL) and two to three drops of Tween-20 in a small volume of sterile water, and agitated on an orbital shaker for 20 minutes. This was followed by surface sterilization with 1% mercuric chloride for 1 minute, performed aseptically in a laminar air-flow cabinet. Following sterilization, explants were rinsed three to four times with sterile distilled water to remove traces of sterilant. The basal portion of the ray petals was trimmed and inoculated onto the culture medium with the adaxial surface in contact with the medium. The contamination and viable explant percentages were 15% and 85%, respectively.

Culture medium and Culture conditions

Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with 30 g L⁻¹ sucrose and 8 g L⁻¹ agar was used for culture initiation. Different concentrations of 6-benzylaminopurine (BAP; 2.0, 3.0 and 4.0 mg L⁻¹) or kinetin (KN; 2.0, 3.0 and 4.0 mg L⁻¹), each in combination with 1.0 mg L⁻¹ α-naphthalene acetic acid (NAA), were used for callus induction and shoot regeneration. The medium pH was adjusted to 5.8 before autoclaving at 121°C and 15 psi for 20 minutes. Before use, all media were incubated for three days to confirm freedom from microbial contamination. Cultures were maintained in 250 mL culture bottles fitted with autoclavable polypropylene caps and incubated at 25 ± 2°C under a 16 h light/8 h dark photoperiod, at a light intensity of approximately 1000 lux provided by cool white fluorescent lamps. Well-developed shoots were excised and transferred to half-strength MS medium containing either indole-3-butyric acid (IBA; 0.5, 1.0 and 1.5 mg L⁻¹) or α-naphthalene acetic acid (NAA; 0.5, 1.0 and 1.5 mg L⁻¹) for root induction. Activated charcoal (2 g L⁻¹) was added to the rooting medium to improve root development.

Experimental Design and Statistical analysis

The experiment was laid out in a Completely Randomized Design (CRD). Each culture bottle contained one explant and was treated as the experimental unit; three explants (bottles) constituted one replication, with six replications per treatment (18 explants evaluated per treatment). Cultures were randomized within the growth room and the entire experiment was repeated independently once.

Acclimatization of Regenerated Plantlets

A total of 25 well rooted plantlets were carefully removed from the culture vessels for acclimatization. Adhering agar medium was gently washed off using tap

water and residual traces were removed with a fine needle. For primary hardening, plantlets were transferred to polybags containing a sterile potting mixture of red soil, sand and farmyard manure (2:1:1, v/v/v). Plantlets were maintained under a protected environment at 80% relative humidity, 25°C and 1500 lux light intensity, with irrigation provided once daily using a rose can. Primary hardening was carried out for 4 weeks, followed by 3 weeks of secondary hardening, before transfer to the nursery. Survival after transfer to the nursery was 80% (*i.e.*, 20/25 plantlets) at 4 weeks after transfer.

Results and Discussion

Effect of Plant growth Regulators on Callus induction and Shoot Regeneration

Petal explants cultured on MS medium supplemented with different concentrations of BAP or kinetin in combination with NAA responded differently for callus induction and shoot regeneration (Table 1). Callus initiation was observed from the basal cut surface of the petal explants within 10–15 days of inoculation. The callus was initially creamy white and compact, gradually turning green on subsequent subculture. Among the treatments tested, MS medium supplemented with 4.0 mg L⁻¹ BAP + 1.0 mg L⁻¹ NAA (T₃) numerically produced the callus induction (94.4%) and shoot regeneration (88.9%), with an average of 8.4 shoots per explant. Lower concentrations of BAP and kinetin were associated with comparatively reduced regeneration responses.

The response observed with BAP may be attributable to its role in stimulating cell division and shoot bud differentiation. Comparable cytokinin–auxin responses

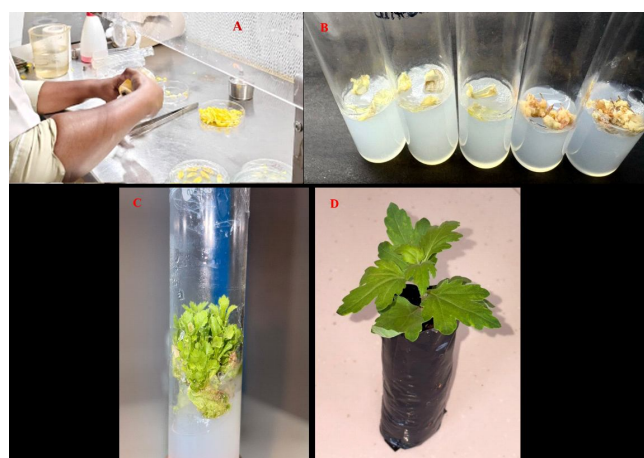


Fig. 1 : *In vitro* regeneration of chrysanthemum. (A) Inoculation of petal explants on MS medium; (B) Callus induction; (C) Regenerated shoots/shoot clusters on MS medium; (D) Hardened plant.

have been reported in chrysanthemum shoot organogenesis using nodal and leaf explants (Waseem *et al.*, 2011; Naing *et al.*, 2014) and for petal/ray-floret explants specifically, in named cultivars (Barakat *et al.*, 2010; Thangmanee and Kanchanapoom, 2011; Jadhav *et al.*, 2024).

Root Induction

Well-developed shoots (3–4 cm long) were transferred to half-strength MS medium supplemented with different concentrations of IBA or NAA. Root initiation was observed within 10–14 days. Regenerated shoots developed healthy white roots, which subsequently became profusely branched. Half-strength MS medium supplemented with 1.0 mg L⁻¹ IBA (R2) numerically gave the rooting response, with 94.4% rooting, 6.7 roots per

Table 1 : Effect of plant growth regulators on callus induction and shoot regeneration (mean ± SE, n = 18 explants/treatment).

Treatment	BAP (mg L ⁻¹)	KN (mg L ⁻¹)	NAA (mg L ⁻¹)	Callus induction 30 DAI, % (n/N) mean ± SE	Shoot regeneration 60 DAI, % (n/N) mean ± SE	Shoots/explant mean ± SE	Group†
T ₁	2.0	-	1.0	72.2 ± 10.6 (13/18)	66.7 ± 11.1 (12/18)	4.50 ± 0.48	b
T ₂	3.0	-	1.0	83.3 ± 8.8 (15/18)	77.8 ± 9.8 (14/18)	6.79 ± 0.81	a
T ₃	4.0	-	1.0	94.4 ± 5.4 (17/18)	88.9 ± 7.4 (16/18)	8.38 ± 0.67	a
T ₄	-	2.0	1.0	66.7 ± 11.1 (12/18)	61.1 ± 11.5 (11/18)	3.91 ± 0.46	b
T ₅	-	3.0	1.0	77.8 ± 9.8 (14/18)	72.2 ± 10.6 (13/18)	5.23 ± 0.51	b
T ₆	-	4.0	1.0	83.3 ± 8.8 (15/18)	72.2 ± 10.6 (13/18)	6.08 ± 0.42	a

†Means followed by different letters within a column differ significantly (p<0.05, DMRT).

Table 2 : Effect of different concentrations of auxins on root induction (mean $\pm$ SE, n = 18 explants/treatment).

Treatment	IBA (mg L ⁻¹)	NAA (mg L ⁻¹)	Rooting 90 DAI, % (n/N) mean $\pm$ SE	Roots/shoot mean $\pm$ SE	Group† (roots/shoot)	Root length (cm) mean $\pm$ SE	Group† (length)
R1	0.5	-	83.3 $\pm$ 8.8 (15/18)	4.80 $\pm$ 0.47	a	4.21 $\pm$ 0.11	b
R2	1.0	-	94.4 $\pm$ 5.4 (17/18)	6.71 $\pm$ 0.51	a	5.80 $\pm$ 0.21	a
R3	1.5	-	88.9 $\pm$ 7.4 (16/18)	6.00 $\pm$ 0.53	a	5.29 $\pm$ 0.16	a
R4	-	0.5	72.2 $\pm$ 10.6 (13/18)	3.92 $\pm$ 0.47	b	3.79 $\pm$ 0.18	b
R5	-	1.0	83.3 $\pm$ 8.8 (15/18)	4.60 $\pm$ 0.62	b	4.09 $\pm$ 0.17	b
R6	-	1.5	77.8 $\pm$ 9.8 (14/18)	4.21 $\pm$ 0.38	b	3.89 $\pm$ 0.14	b

†Means followed by different letters within a column differ significantly (p<0.05, DMRT).

shoot, and a mean root length of 5.8 cm (Table 2). Auxin-mediated rooting is widely reported in chrysanthemum, with IBA frequently found to be more effective than NAA for inducing healthy root systems.

Acclimatization and survival

The hardening procedure described in Section 2.5 resulted in 80% plantlet survival at 4 weeks after transfer to nursery conditions, with established plants exhibiting normal vegetative growth (Fig. 1). This indicates that the combination of gradual humidity reduction during primary and secondary hardening and a red soil sand farmyard manure potting mixture was suitable for this genotype.

Conclusion

This study outlines a petal (ray floret) based *in vitro* regeneration and acclimatization protocol for chrysanthemum cv. CO 1, with MS medium supplemented with 4.0 mg L⁻¹ BAP + 1.0 mg L⁻¹ NAA giving the highest numerical callus induction and shoot regeneration, and half-strength MS with 1.0 mg L⁻¹ IBA giving the highest numerical rooting response; regenerated plantlets established at 80% survival at 4 weeks after transfer to nursery conditions.

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Author contributions

All authors contributed to the conceptualization and design of the study. Data collection, laboratory work and

analysis were carried out by the authors and the manuscript was drafted and reviewed collectively. All authors read and approved the final manuscript.

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