



Acclimatization and field evaluation of micropropagated plants of chrysanthemum cv. 'Arka Swarna'

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ABSTRACT

Chrysanthemum cv. 'Arka Swarna' was micropropagated using shoot-tip and nodal microcuttings on MS medium containing 3% sucrose and 0.25% Phytigel® in the absence of externally supplied plant growth regulators, yielding 90 - 100% rooted plantlets, or in medium containing 1 µM benzyladenine or kinetin yielding 20 - 32% plantlets within 2 - 4 weeks of subculture. The stocks were acclimatized employing sachet technique wherein the rooted plantlets (2.5 - 4 cm) were planted in polythene bags of 5"×9" filled to one-third height with planting mixture. The closed bags with 1 - 5 plants were incubated under conditions similar to the *in vitro* stocks. The plantlets recorded 90 - 100% establishment within 4 weeks. The *ex vitro* established plants were evaluated in the field a month later by direct planting, or after one month in a field nursery-bed, along with conventional suckers. While field establishment (80 - 95%) was not significantly influenced by the treatments, micropropagated plants put through the nursery appeared to be the best among the three treatments in vegetative growth, floral characteristics and flower yield, demonstrating advantage of micropropagation over conventional propagation for shy-suckering chrysanthemums.

Key words : Acclimatization, chrysanthemum, *Dendranthema grandiflora*, hardening, micropropagation

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora*) is the second largest cut flower crop grown all over the globe and one of the most popular commercial flower crops in India (Kher, 1988). Conventionally the crop is propagated through suckers, which is quite slow for rapid multiplication of new varieties and exotic introductions, particularly, if the variety does not actively sucker. Micropropagation is an alternate approach for rapid cloning of chrysanthemums (Ben Jaacov and Langhans, 1972; Rout and Das, 1997; Teixeira da Silva, 2004). An elite Pompon type chrysanthemum cv. Arka Swarna with attractive golden flowers was developed at the Indian Institute of Horticultural Research, Bangalore, valued for both cut and loose flowers (Janakiram and Rao, 2001), but is a shy suckering type. Panicker *et al* (2007) evolved a micropropagation protocol for cv. 'Arka Swarna' which involved culture of shoot-tip and nodal segments on MS basal medium devoid of any plant growth regulators. The microcuttings gave rise to a single shoot coupled with rooting, yielding 90 - 100% rooted plantlets within 2 - 4 weeks, all of which were suitable for acclimatization. The cultures showed reduction in rooting with cytokinins in the medium bringing

down the yield of hardenable plantlets to 20-32% at 1 µM benzyl adenine (BA) or kinetin. No rooting was observed above 5 µM. All the cultures showed covertly residing endophytic bacteria with no apparent adverse effect (Panicker *et al*, 2007).

Tissue culture derived plants are very delicate and need to go through slow transition from the protected *in vitro* condition to the *ex vitro* environment, termed as hardening or acclimatization (Preece and Sutter 1991; Ravindra and Thomas, 1995; Thomas, 1998). Further, a successful micropropagation protocol warrants that performance of tissue culture derived plants is matched to or is better than conventionally propagated plants. The present study was taken up to assess establishment of micropropagated chrysanthemum plants *ex vitro* and to test field performance of tissue-culture derived plants in comparison to conventionally propagated plants.

MATERIAL AND METHODS

Plant material

Studies were carried out using micropropagated plantlets of chrysanthemum cv. 'Arka Swarna' which

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initiated *in vitro* from field had been derived nodal explants and were further propagated *in vitro* for a period of one year, as described in an earlier study (Panicker *et al.*, 2007). Rooted plantlets were derived from shoot-tip and nodal segments after cultured on MS basal medium supplied with 3% sucrose and gelled with 2.5 gl^{-1} Phytigel® (Sigma Chemical Co, St. Louis, USA) with no added plant growth regulators, or, on medium supplied with 1 μM BA/ kinetin. Such plantlets, with a shoot height of 2.5 - 4 cm, 3 - 5 nodes and 1 - 10 or more roots, were used for acclimatization.

Acclimatization

Sachet method, as described by Ravindra and Thomas (1995), was employed for acclimatization. Rooted plantlets were briefly washed under tap water and planted in potting mixture comprising 2:1:1 parts of autoclaved sand, soil and SoilriteTC⁰ (Karnataka Explosives, Ltd., Perlite Division, Bangalore) filled to one-third capacity in polythene bags (9 inch height \times 5 inch width). These in turn, were provided with drainage holes and watered to field capacity. Only a single plantlet was used per bag unless mentioned otherwise. The upper open end of the polybag was closed with a staple pin and the sachets were incubated under ambient conditions (25-30°C) and provided with 16 h of light (30-50 $\mu\text{E m}^{-2} \text{s}^{-1}$) using cool, white fluorescent lamps (Thomas, 1998). The top of the sachet was opened two weeks after planting and *ex-vitro* establishment % was recorded one month from planting. The plants were shifted to a glasshouse and placed there for another a month, or, were transplanted to a nursery bed, under shade-nets (approx. 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ light). Planting of upto five rooted plantlets per polythene bag and pruning of *in-vitro* formed roots at planting (Thomas and Ravindra, 1997) was also tried.

Field evaluation of micropropagated and conventionally propagated plants

Acclimatized plants were evaluated in field either directly (one month from opening sachets) or after planting in a nursery for a month, along with conventionally propagated suckers.

T1: Tissue culture derived, one-month old plants post acclimatization, planted directly in field (Code: TC-D)

T2: Tissue culture derived, acclimatized plants planted in nursery bed under shade and after a month transplanted to experimental field (Code: TC-N)

T3: Conventionally propagated suckers from mother plants raised in nursery for a month and subsequently transplanted to experimental field (Code: CP-S).

The experiment was laid out in completely randomized design (CRD) in a single bed of red loamy soil supplemented with sand and farm yard manure. Six replications with four plants constituted one replication. Percent establishment was recorded taking into account the whole population of plants in a treatment. Other plant growth variables (plant height, plant spread, number of primary branches and buds) were recorded from all four plants in a replication and floral characteristics (flower diameter, number of ray florets and flower weight) were recorded on five flowers from five representative plants in each replication. Flower yield / plant and duration of flowering were also assessed.

Statistical analysis

Statistical analysis was performed as per Gomez and Gomez (1984). CRD design was followed for the field experiment on account of accommodating all the treatments and their replications in a uniform bed. Percent values were subjected to arc-sine transformation before statistical analysis to stabilize the variance.

RESULTS AND DISCUSSION

The shoot-tip and nodal segments that were cultured on MS basal medium gave rise to a single shoot 2 - 3.5 cm long coupled with rooting in 90 - 100% microcuttings, and 20 - 32% rooting in medium supplied with 1 μM BA or kinetin with shoot height of 2 - 2.5 cm. No rooting was observed at higher BA/ kinetin (5 - 20 μM) cones.

Micropropagated plants showed 90 - 100% establishment in sachet method of acclimatization in different batches (Fig. 1A). The top of the sachet was opened, exposing the plants to ambient environment, 10-14 days after planting. The plants kept in the same bags for one month or more displayed good growth (Fig. 1B).

Tissue culture derived plants showed relatively better field establishment over conventional suckers, although, the effect was not significant (Table 1). Overall, tissue culture derived plants were comparable to or superior to conventionally propagated plants, depending on whether these were planted directly (TC-D) or after going through a nursery (TC-N). TC-N plants outperformed TC-D plants in plant height, plant spread, number of flower buds, flower diameter, number of florets, flower weight and yield per plant. Compared to conventionally propagated plants, TC-N plants were better in respect of flower diameter, flower weight, number of florets and yield per plant. TC plants,



Fig 1. Acclimatization of micropropagated chrysanthemum through sachet technique (A) and the established plants showing growth during their maintenance in the same sachets (B). (Bar = approx. 10 cm)



Fig 2. Performance of chrysanthemum plants in the field following direct planting of acclimatized plants (A), acclimatized plants after a month in field nursery (B), and, conventional sucker-derived plants at one month in field nursery (C)

Table 1. Performance of tissue culture derived and conventionally propagated plants of chrysanthemum cv. Arka Swarna

Treatment	Field establishment (%)	Plant height (cm)	No. of branches	Plants pread (cm)		Flower buds (no.)	Flower diameter (cm)	Florets (no.)	Flowering duration (days)	Flower wt.(g)	Floweryield/ plant(g)	Sucker (no.)
				N-S	E-W							
TC Plant-Direct (TC-D)	95(84.0)	58.5	13.9	27.5	30.3	76.3	4.66	215.1	41.80	3.53	269.71	8.55
TC Plant-Nursery (TC-N)	95(84.0)	64.5	15.2	33.1	37.4	100.9	5.22	222.1	44.60	5.00	500.78	9.00
Conventional Sucker (CP-S)	80(69.0)	67.5	16.0	30.0	34.5	87.5	4.21	191.5	39.20	2.75	241.92	5.95
Significance	NS	**	NS	**	**	**	**	**	**	**	**	**
SED ±	7.14	1.22	3.21	0.90	0.54	2.84	0.07	1.29	0.42	0.25	18.77	0.26
CD ($P = 0.05$)	30.86	5.77**	5.25	4.29**	2.56**	13.50**	0.31**	6.13**	2.01**	0.20**	81.02**	0.78**

whether planted directly or after pre-nursery, displayed significantly higher sucker production than conventional sucker derived plants. Overall, tissue culture-derived plants transplanted after a span in the nursery bed appeared to be better than those in the other two treatments (Fig. 2).

In a subsequent trial, it was found that pruning *in-vitro* formed roots made planting into the sachets easier with no apparent negative effects on establishment (90 - 100%) or growth. Upto five plantlets could be planted per sachet, giving similar establishment rate as with single

plants. Post establishment (2-3 weeks), these could be transplanted to the nursery bed under shade, thus serving as a source of plants for subsequent field planting.

The present study demonstrates suitability of micropropagation for rapid clonal propagation of shy-suckering 'Arka Swarna' based on results from field evaluation. A phase of one month in nursery bed subsequent to primary acclimatization was desirable in micropropagated chrysanthemum. Performance of acclimatized plants was not satisfactory in direct field-planting, possibly due to their delicate nature and delay in adjusting to field conditions. Tissue cultured plants of pepper, cardamom and vanilla also have shown similar results of better performance compared to conventionally propagated material (Rathy *et al*, 2005; Kuruvilla *et al*, 2005; Madhusoodanan *et al*, 2005). Planting as many as five rooted plantlets in a polythene bag and their transplantation to nursery appeared feasible, serving as an alternative to conventional sucker propagation in this shy-suckering cultivar.

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