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Research Article

MICROPROPAGATION OF CHRYSANTHEMUM (*CHRYSANTHEMUM MORIFOLIUM*) USING SHOOT TIP AS EXPLANT

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Chrysanthemum has been cultivated for more than 2000 years and today, it is the world's second most economically important floricultural crop following the rose (Teixeira da silva, 2003). Due to high popularity and demand for chrysanthemum it has become one of the first commercial targets for Micro propagation and thus tissue culture can be utilized for its large scale production. Regeneration of chrysanthemum plantlets was obtained by shoot tips as explant with different concentrations and combinations of auxins (IAA, IBA) and cytokinin (Kinetin), for the formation of micro-shoots, which were sub-cultured for development of roots. Fully developed plantlets were successfully transferred to suitable growing media for acclimatization. Among the various treatments for shoot regeneration, MS medium with Kinetin 3.0 mg l^{-1} + IAA 2.0 mg l^{-1} was found to be the best treatment combination as it produced 67.82 per cent success in 17.91 days. For root production, MS medium supplemented with IBA 1.0mg l^{-1} was found to be the best treatment as it produced 82.12 per cent success in 19.19 days.

Key Words*: Chrysanthemum morifolium, MS medium, Kinetin, IAA, IBA*

INTRODUCTION

Chrysanthemum is commonly known as Autumn Queen. It belongs to the family Compositeae (Asteraceae). It is highly attractive and charming short day plant, which behaves both as an annual as well as perennial flowering herb. The plant height ranges from 1-3 feet. The leaves are alternate and toothed, roots are adventitious and the stem is woody solid. The flowers bloom in early winter with a wide range of color, shape and sizes. The flower color ranges from white and cream through the shades of yellow, pink, bronze, red, deep purple and green (Arora, 1990). The common commercially available chrysanthemum is *Chrysanthemum morifolium* Ramat. It is native to the northern hemisphere, chiefly Europe and Asia. In the southern parts of the country, it is mostly grown in farmer's fields for supplying loose flowers to the market for garlands, hair decoration by the ladies and for offering to God. While yellow coloured flowers are preferred in the south, in the north, various hues of red, purple, yellow and white are found to be grown in abundance. Chrysanthemum accounts for 35 per cent of the total cut flower production. According to a report of the Flowers and Plants Association (2001), chrysanthemum is the second most important cut flower by sales value. Tissue culture, an important area of biotechnology can be used to improve the productivity of planting material through enhanced availability of identified planting stock with desired traits. Micro propagation is one of the important contributions of plant tissue culture to commercial plant propagation and has vast significance. Micro propagation is the true to type propagation of selected genotypes using *in vitro* culture techniques. This technique provides a rapid reliable system for the production of large number of genetically uniform disease free plantlets.

Chrysanthemum is propagated vegetatively either through root suckers or terminal cuttings. This conventional process of shoot cutting is very slow. Clonal propagation through *in vitro* culture can enhance multiplication many fold.

MATERIALS AND METHODS

Experiments on "Micro propagation of Chrysanthemum (*Chrysanthemum morifolium)*" were carried out at the Tissue Culture unit of the Department of Biotechnology in Adhiparasakthi Agricultural College,

Kalavai. Healthy and vigorously growing plants of *Chrysanthemum morifolium* maintained in the Floriculture unit of the Department of Horticulture were used for the study. Shoot tips were collected from 25 to 30 days old plants and thoroughly washed with 0.1 per cent teepol followed by washing with sterilized distilled water thrice. The explants were then surface sterilized with 70 per cent ethanol for three minutes, followed by washing with sterilized distilled water, thrice. The outer leaves in each explants were removed and inner most shoot tip, measuring 0.5 to 1.0 cm in length, having 1 or 2 leaf primordia were excised. Then explants were inoculated (Fig.1,) on Murashige and Skoog(1962) medium supplemented with various concentration of IAA(Indole acetic acid) and Kinetin to obtain maximum shoot production (Kushal Sing and Arora,1994). The inoculated tubes were kept in the culture room, maintaining a temperature of 25 ± 2^{0}C and a humidity of 75 per cent. The light dark cycle of 16 hrs and 8 hrs was maintained with 2000-3000 lux intensity. Multiple shoots obtained directly and were elongated then they were separated from each other and rooted successfully on full strength MS medium supplemented with various concentrations of IAA and IBA (Indole buteric acid). Fully developed plantlets were removed from the culture tubes and freed from agar by washing gently with glass double distilled water and then transferred to small perforated plastic containers having 1:1 ratio vermiculite and sand media.

RESULTS AND DISCUSSION

Growth regulators have become an integral part of all *in vitro* studies. The background of this addition lies in excellent recognition of the mechanism of hormonal control of tissue differentiation and plant growth during early stages of development. Fruitful results of *in vitro* culture techniques lies in the addition of several growth regulators in sophisticated sequence, to induce the formation of shoots and roots in undifferentiated tissues (Krikorian, 1982). Two principal classes of growth regulators are used in tissue culture studies, namely auxins and cytokinins. Auxin is required by most plant cells for division and root initiation, whereas cytokinin promotes cell division, shoot proliferation and shoot morphogenesis.

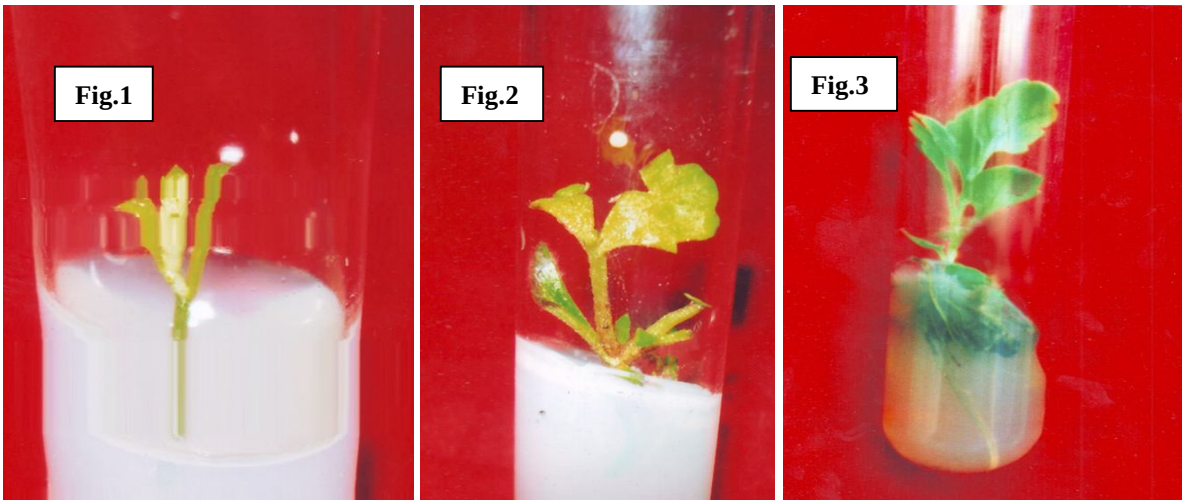

Figure 1: Shoot tip of chrysanthemum in MS medium; Figure 2: Shoot regeneration from shoot tip in chrysanthemum; Figure 3: Root regeneration from shoot tip in *Chrysanthemum*

Table1: Effect of Kinetin+IAA on shoot regeneration (per cent) and duration (days) for shoot regeneration from shoot tips in *Chrysanthemum morifolium*

Treatments (MS +Kinetin+IAA) (mg l^{-1})	Culture response (per cent)	Duration (days)
T_1 (0.0+0.0)	00.00	00.00
T_2 (1.0+0.0)	27.08	31.08
T_3 (2.0+0.0)	28.23	30.31
T_4 (3.0+0.0)	32.45	29.92
T_5 (0.0+1.0)	36.23	28.10
T_6 (1.0+1.0)	39.41	27.23
T_7 (2.0+1.0)	54.67	22.63
T_8 (3.0+1.0)	57.65	21.82
T_9 (0.0+2.0)	43.71	26.91
T_{10} (1.0+2.0)	48.92	24.73
T_{11} (2.0+2.0)	65.11	18.33
T_{12} (3.0+2.0)	67.82	17.91
T_{13} (0.0+3.0)	46.81	25.81
T_{14} (1.0+3.0)	60.34	20.65
T_{15} (2.0+3.0)	62.23	19.45
T_{16} (3.0+3.0)	51.03	23.34
SED	0.50	0.07
CD (p = 0.05)	1.01	0.14

It can be observed from the data in the Table 1 that the effects of different concentrations and combinations of Kinetin+ IAA on the culture response of shoot tips were significant and ranged from 27.08 to 67.82 per cent among the treatments. It is interesting to note that there was no response when the explants were cultured in the MS medium without any supplementation, suggesting that it is essential to add growth regulators exogenously to obtain desirable results. Among the plant growth regulators tried, a combination Kinetin+ IAA, T_{12} (Kinetin 3.0 mg l^{-1} + IAA 2.0 mg l^{-1}) recorded the highest culture

Table 2: Effect of auxins on root regeneration (per cent) and duration (days) for root regeneration from shoot tips in *Chrysanthemum morifolium*

Treatments (MS+auxins) (mg l^{-1})	Culture response (per cent)	Duration (days)
T_1 (MS alone)	0.00	0.00
T_2 IAA 1.0	73.43	21.30
T_3 IAA 2.0	69.21	22.51
T_4 IAA 3.0	57.81	24.42
T_5 IBA 1.0	82.12	19.19
T_6 IBA 2.0	73.29	21.52
T_7 IBA 3.0	61.92	23.35
SED	0.58	0.08
CD (p = 0.05)	1.16	0.16

response of 67.82 per cent in the duration of (17.91) days and promoting maximum shoot formation in the explants (Fig. 2.) when compared to the individual treatments. T_2 (MS medium supplemented with Kinetin alone at 1.0 mg l^{-1}) recorded the least culture response of 27.08 per cent in the duration of (31.08) days. The results of the present study are in conformity with the earlier findings of Nagoor Meeran (1995) and Karim and Amin (2003) in chrysanthemum. The micro shoots obtained from *in vitro* cultures were further differentiated on MS medium supplemented with various concentrations of auxins. Among the treatments, IBA 1.0 mg l^{-1} (Table 2) proved to be the best for promoting root regeneration (Fig.3.) when compared to the other auxins tried. Similar results have been obtained by Minas (2008) in chrysanthemum.

The results of the experiment indicates that chrysanthemum can be multiplied in large scale through micro propagation .Farmers face many difficulties while raising chrysanthemum in nursery in open field condition, due to the climatic condition prevailing in that particular area ,pest and disease incidence .This study clearly indicates that the above problems can be overcome by micro propagation of chrysanthemum.

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