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Rapid Multiplication of *Chrysanthemum morifolium* Through *in vitro* Culture

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Abstract: An efficient protocol was developed for direct regeneration, multiplication and rooting under in *vitro* conditions of *Chrysanthemum.* The frequency of multiple shoot regeneration response was 95 and 91%, for nodal segments and shoot tips, respectively when cultured on the medium containing MS + 1.0 mg l^{-1} BAP. Efficient rooting was achieved on half strength of MS+ 0.2 mg l^{-1} IBA. *In vitro* raised plantlets were transferred to potted soil and finally transferred to the field.

Key words: *Chrysanthemum morifolium*, micro propagation, rapid multiplication, propagation, *in vitro* culture

Introduction

Chrysanthemum morifolium is one of the common ornamental plants of Bangladesh belonging to the family Composite. It is a perennial herb, weak and lateral branching, herbaceous angular and present wooly. It is important not only for its outstanding aesthetic beauty and a long lasting capability but also because of its good potentials for marketing as cut flowers and potted plants to many countries.

Chrysanthemum are propagated vegetatively either through root suckers or terminal cuttings. This conventional process of shoot cutting is very slow. For commercial purpose, we need large scale production. Due to the high popularity and demand for chrysanthemum it becomes one of the first commercial targets for micro propagation (Levin *et al.*, 1988). Ben-Jaacov and Langhans (1972), described *in vitro Chrysanthemum* micro propagation from shoot tips and shoot initiated callus. Bhattacharya *et al.* (1990) reported rapid mass propagation of *Chrysanthemum morifolium* through callus derived from leaf and stem explants. Through the use of tissue culture, it is possible to obtain a large number of plants from one explant (Bajaj *et al.*, 1992). It represents the optimum efficiency in terms of vegetative plant propagation and allows a large number of propagules to be produced in a relatively short period under controlled conditions, throughout the year in a relatively small space.

The present study was undertaken to establish a protocol for large-scale clonal propagation of *Chrysanthemum* through *in vitro* culture.

Materials and Methods

The experiment was conducted at Plant Tissue Culture Laboratory, Department of Botany, University of Rajshahi, Bangladesh during the period of 1997 to 1998. The shoot tips and nodes were used as experimental plant materials. The explants were collected from 4 months old grown from the stem cuttings at the Botanical garden of Rajshahi University and they were washed thoroughly under running tap and distilled water.

The material was then taken into laminar flow cabinet and surface sterilized with 0.1% $HgCl_2$ for different durations. After sterilization, the explants were planted on the surface of the semisolid MS, MMS_1 or MMS_2 medium gelled with 6 gm l^{-1} agar, 30 gm l^{-1} sucrose. The pH of the medium was adjusted to 5.7 and autoclaved at 121°C for 20 minutes. All the cultures were incubated at 25± 2°C and culture was kept under a 14 hours photoperiod fluorescent tube light. The materials were subcultured at 3-4 weeks intervals. The shoots were separated and individual shoots were placed in the rooting medium. After hardening plantlets were transferred to soil for establishment.

Results and Discussion

Different concentrations of BAP and Kn (viz. 0.1, 0.2, 0.5, 1.0, 2.0, 5.0,10.0 mg l^{-1}) were used in MS media for shoot regeneration from nodal and shoot tip explants of *Chrysanthemum.*

Proliferation of axillary shoot from the nodal and shoot tip segments of the mature plants and *in vitro* raised shoot origin was remarkably influenced by types and concentrations of the cytokinin used. Among different concentrations used, best response towards shoot proliferation from nodal and shoot tip explants was obtained on MS + 1.0 mg l^{-1} BAP (Table 1). Roest and Bokelmann (1975) obtained similar results when they used 1.0 mg l^{-1} BAP in the medium for shoot regeneration of *Chrysanthemum.* Among the two explants used, nodal explant showed comparatively better response than shoot tip explants towards shoot regeneration. Similar results have been published by Hoque *et al.* (1995) while working on multiple shoot regeneration for carnation. For nodal explant, the highest degree of axillary shoot proliferation was found on medium containing 1.0 mg l^{-1} of the cytokinin (BAP) and 95% of the explants proliferation with 5.3± 0.2 shoots. On the other hand, for shoot tip explants the highest degree of axillary shoot proliferation was found on medium containing 1.0 mg l^{-1} of the cytokinin (BAP) and 91% of the explants proliferation with 4.2± 0.3 shoots (Fig. 1A, B). The effectiveness of cytokinin BAP was proved to be superior to that of Kn in regeneration of shoots from both the explants. It is in agreement with those of Hutchinson (1981) who reported that MS media supplemented with BA have been satisfactory for many species and cultivars of crop plants for their *in vitro* propagation. Superiority of BA over other cytokinins in producing *in vitro* shoots has also been confirmed in other plants like *Rosmarinus officinalis* (Misra and Chatruvedi, 1984), *Arachis hypogaea* (Mhatre *et al.*, 1985) and *Atropa beladona* (Benjamin *et al.*, 1987). The proliferation efficiency of nodal explants was significantly higher than that of shoot tip explant, when evaluated after five-six weeks of proliferation. Micro cuttings prepared from *in vitro* proliferated shoots with 3-5 cm length were cultured on MMS_1 medium with 0.1,0.2,0.5 and 1.0 mg l^{-1} of IBA, NAA and IAA supplemented for root formation. Percentage of root induction and number of roots per shoots were highly influenced by concentration and type of the auxins. Among different concentrations of auxin supplemented in media lowest rooting was obtained with IAA and highest with 0.2 mg l^{-1} IBA (Table 2). Similar observations were found on C*hrysanthemum morifolium* (Hoque *et al.*, 1995; Khan *et al.*, 1994). NAA showed effective results in the induction of root. Using NAA at different concentrations on MS medium, several workers (Earle and Langhans, 1974a) reported root induction in *Chrysanthemum.* On medium with 0.2 mg l^{-1} concentration of the auxin the cultured shoot cuttings produced the highest number of roots per micro shoots and that were 9.1± 0.2 for IBA, 8.9± 0.1 for NAA 7.2± 0.8 for IAA (Fig. 1C). The maximum length of the longest root was 5.3± 0.1 on 0.2 mg l^{-1} IBA supplemented medium. Healthy and well established rooted plantlets were transferred to soil for acclimatization. Among the regenerates transplanted 75% of them

Table 1: Effects of cytokinin for *in vitro* shoot proliferation from the nodal and shoot tip explant of *C. morifolium* on MS medium. There were 10-15 explants in each treatment. Data were recorded after 6-8 weeks of culture

	Node				Shoot tip			
Growth regulators (mg l^{-1})	Percent of explant responded	No. of usable shoots per culture	No. of nodes per shoot	Average length of shoot (cm)	Per cent of explant responded	No. of usable shoots per culture	No. of nodes per shoot	Average length of shoot (cm)
BAP								
0.1	67	4.4± 1.0	4.2± 1.2	4.0± 1.0	64	4.1± 1.0	4.1± 1.3	3.9± 1.0
0.2	74	4.1± 0.5	4.9± 1.0	4.3± 0.5	74	4.2± 0.3	4.2± 1.2	4.3± 0.5
0.5	85	4.5± 0.5	5.6± 0.5	6.1± 1.4	86	4.1± 0.8	5.0± 0.8	5.6± 0.4
1.0	95	5.3± 0.2	7.5± 1.5	7.0± 0.5	91	4.2± 0.3	5.3± .07	5.8± 0.2
2.0	81	3.9± 0.4	4.6± 0.5	4.4± 0.6	79	4.0± 0.3	4.5± .04	5.0± 0.4
5.0	71	3.4± 0.5	3.1± 1.0	3.0± 0.2	68	3.3± 0.5	4.0± 0.4	3.1± 1.5
10.0	64	3.1± 1.5	2.7± 0.5	2.5± 1.0	63	3.3± 0.7	3.1± 0.5	3.0± 1.4
Kn								
0.1	40	2.2± 0.0	2.5± 1.0	2.4± 0.5	38	2.1± 0.8	2.6± 1.8	2.5± 0.5
0.2	51	2.3± 0.5	3.1± 0.5	2.6± 0.3	50	2.4± 0.6	3.0± 1.5	2.5± 0.3
0.5	60	2.4± 1.0	3.7± 0.1	3.3± 0.5	60	3.5± 0.1	4.1± 0.6	4.2± 0.5
1.0	66	2.8± 0.5	4.0± 1.5	3.5± 0.4	57	3.1± 1.5	4.0± 0.6	3.8± 0.2
2.0	50	2.3± 0.4	3.0± 1.0	2.6± 0.2	49	2.8± 0.2	3.0± 0.1	3.1± 0.9
5.0	46	2.1± 0.2	2.6± 0.5	2.3± 0.2	45	2.7± 0.3	2.3± 0.7	2.3± 0.6
10.0	30	1.9± 1.0	2.0± 0.5	1.6± 0.5	29	2.0± 1.5	2.1± 0.9	2.1± 0.9

Fig. 1: *In vitro* shoot regeneration in *Chrysanthemum morifolium*
A. Multiple shoot regeneration from nodal explants on MS medium containing 1.0 mg l^{-1} BAP
B. Initiation of multiple shoots from shoot tip explants on MS medium supplemented with 1.0 mg l^{-1} BAP
C. Root formation in regenerated shoots in ½ strength MS+ 0.2 mg l^{-1} IBA
D. Regenerated plantlets established in the soil

Table 2: Effects of different auxins on formatting of root from the *in vitro* grown micro shoots cultured on MMS1 medium fortified with 6 gm l^{-1} agar. There were 10-15 micro shoots cultured for each treatment and data were collected after 4-6 weeks

Growth regulators (mg l^{-1})	% of cutting rooted	No. of roots per rooted cutting	Average length of roots (cm)	Days to emergence of roots
IBA				
0.1	90	7.0± 0.1	5.1± 0.1	5-12
0.2	100	9.1± 0.2	5.3± 0.1	5-10
0.5	100	7.0± 0.5	5.1± 0.2	7-9
0.0	91	6.0± 0.3	4.2± 0.1	7-15
NAA				
0.1	90	6.6± 0.4	4.9± 0.2	8-12
0.2	95	8.9± 0.1	5.0± 0.1	10-12
0.5	91	6.1± 0.1	4.4± 0.1	5-12
1.0	88	5.0± 0.2	3.9± 0.1	7-10
IAA				
0.1	68	5.9± 0.1	4.0± 0.2	10-15
0.2	70	7.2± 0.8	3.9± 0.1	7-12
0.5	65	5.0± 0.1	3.5± 0.4	8-10
1.0	60	3.5± 0.5	3.0± 0.8	6-14

survived and acclimatized successfully on the soil (Fig. 1D). The results of the present study indicate that using shoot tip as well nodal explants is possible to multiply *Chrysanthemum* on a large scale on MS medium supplemented with BAP and Kn. This protocol can be used for commercial rapid propagation of *Chrysanthemum* to earn foreign exchange and also to meet the local demand.

References

Bajaj, Y.P.S., 1992. A suggested method for *in vitro* long term storage at 4°C of *Chrysanthemum* and petunia germplasm. Plant Tissue Cult., 3: 57-58.

Ben-Jaacov, J. and R.W. Langhans, 1972. Rapid multiplication of *Chrysanthemum* plant by stem tip proliferation. Hort. Sci., 7: 289-290.

Bhattacharya, P., S. Dey, N. Das and B.C. Bhattacharya, 1990. Rapid mass propagation of Chrysanthemum morifolium by callus derived from stem and leaf explants. Plant Cell Rep., 9: 439-442.

Benjamin, B.D., P.C. Roja, M.R. Heble and M.S. Chandha, 1987. Multiple shoot cultures of *Atropa belladonna*: Effect of physico-chemical factors growth and alkaloid formation. J. Pl. Physiol., 129-135.

Hutchinson, J.F., 1981. Tissue culture propagation of fruit trees. In: Proc. Symp. on Tissue Culture of Economically Important Plants, A.N. Rao (Ed.) Singapore, pp: 113-120.

Hoque, M.I., M. Fatema, R. Hashem and R.H. Sarker, 1995. *In vitro* plant regeneration in C*hrysanthemum morifolium* Ramat. Annual Tissue Cult. Conf. (Dhaka, Mach 19). Plant Tissue Cult., pp: 92.

Khan, M.A., D. Khanam, K.A. Ara and A.K.M.A. Hossain, 1994. *In vitro* plant regeneration in *Chrysanthemum morifolium* (Ramat). Plant Tissue Cult., 4: 53-57.

Levin, R., V. Gaha, B. Tal, S. Hirsch, D. Denola and I. Vasil, 1988. Automated plant tissue culture for mass propagation. Biotechnol., 6: 1035-1040.

Mhatre, M., V.A. Bapat and P.S. Rao, 1985. Micropropagation of protoplastculture of peanut (*Arachis hypogaea* L.) Cur. Sci., 54: 1052-1056.

Misra, P. and H.C. Chaturavedi, 1984. Micropropagation of *Rosmarinus officinalis* L. Plant Cell, Tissue and Organ Cult., 3: 163-168.

Roest, S. and G.S. Bokelman, 1975. Vegetative propagation of *Chrysanthemum morifolium* Ramat *in vitro.* Sci. Hort., 3: 317-330.