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Effects of Different Plant Growth Regulator on *in vitro* Shoot Multiplication of *Chrysanthemum morifolium*

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Abstract: Shoot multiplication of ***Chrysanthemum morifolium*** was achieved from the nodal and shoot tip explants of mature plant using MS medium with different concentrations and combinations of growth regulators. Maximum frequency of explants produced axillary shoot and the highest number of shoots per explant were obtained when MS medium fortified with 1.0 mg l^{-1} BAP.The combination BAP+GA_3 was found effective result. But Kinetin (Kn) showed low performance for producing multiple shoots. The degree of shoot formation was affected by explant types and the exogenous hormonal regime in the medium.

Key words: Multiplication, ***in vitro***, growth regulator, ***Chrysanthemum morifolium***

Introduction

Chrysanthemum morifolium Ramat, commonly called as guldaudi or autumn queen, belongs to the family Composaetae (Arora, 1990). It 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 folds (Sauvaire and Galzy, 1978). It has become now a viable alternative to the conventional propagation methods. There are many reports on tissue culture of chrysanthemum from different countries. Battacharya ***et al.*** (1990) reported rapid mass propagation of ***Chrysanthemum morifolium*** through callus derived from leaf and stem explants. Khan ***et al.*** (1994) have shown that ***in vitro*** mass propagation of some local cultivars is possible from only shoot tip explants. Ben -Jaacov and Langhans (1972), Earle and Langhans (1973) described ***in vitro*** chrysanthemum micropropagation from shoot tips and shoot initiated callus. Levin ***et al.*** (1988) utilized tissue culture technique for large scale production of chrysanthemum. Amin ***et al.*** (1997) demonstrated that axillary and adventitious bud multiplication of Chrysanthemum was possible from the nodal, shoot tip and petiole explants.

Micropropagation using axillary shoot proliferation from nodal and shoot tip culture is the most desirable and safe as micropropagules to minimise genetic variation.The formation of healthy shoots and its higher rates of multiplication is one of the prerequiste of an economically viable micropropagation protocol. Therefore the attempts of this present study was to determine the effect of different growth regulators on shoot formation and multiplication over the cultural period. The objective of present study has been to determine the optimum cultural conditions for production of genetically stable multiple shoots from both the explants.

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.The culture media described by Murashige and Skoog (1962) was supplemented with cytokinin, auxins and gibberellic acid in different concentrations and combinations were used for the growth and multiplication of axillary shoot the culture of explants. Normally cultures were grown on media having 3% carbon source (Sucrose) and 0.6% gelling agent (agar). The pH of the medium was adjusted to 5.7 and autoclaved at 121^{0}C for 20 min. All the cultures were incubated at 25± 2^{0}C and culture was kept under a 14 h photo period fluorescent tube light. The materials were subcultured at 3-4 weeks intervals.

Results and Discussion

Effect of cytokinin

Surface sterilized shoot tip and nodal segments were cultured on MS media supplemented with different concentrations of cytokinin. After, 6 weeks of culture, media supplemented with different doses followed to induce proliferation of axillary shoot from the explant. .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 BAP (Table 1) For nodal explant, the highest degree of axillary shoot proliferation was found on medium containing 1.0mg/l of the cytokinin (BAP) and 95% of the explants proliferation with 5.3±0.2 shoots (Fig. A and B). 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. When concentrations of BAP increased from 1.0 to 5.0 mg l^{-1} and 10.0 mg l^{-1} then the percentage of explant responded decreased to 71 and 64%. Number of shoots per culture, number of nodes per shoot and shoot length were also found to decrease considerably in 5.0 and10.0 mg l^{-1} BAP containing media.The cultured explants did not produce considerable number of shoots per culture and growth of shoots was not satisfactory on the Kn augmented medium. For nodal explant, the highest degree of axillary shoot proliferation was found on medium containing 1.0 mg l^{-1} of the cytokinin (Kn) and 66% of the explants proliferation with 2.8±0.5 shoots (Table 1). 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 (Kn) and 57% of the explants proliferation with 2.1±0.5 shoots. The effectiveness of cytokinin BAP was proved to be superior to that of Kn in regeneration of shoots from both the explants. Roest and Bokelmann (1975) obtained similar results when they used 1.0 mg l^{-1} BAP in the medium for shoot regeneration of chrysanthemum It is in agreement with those of Hutchinson (1981) who reported

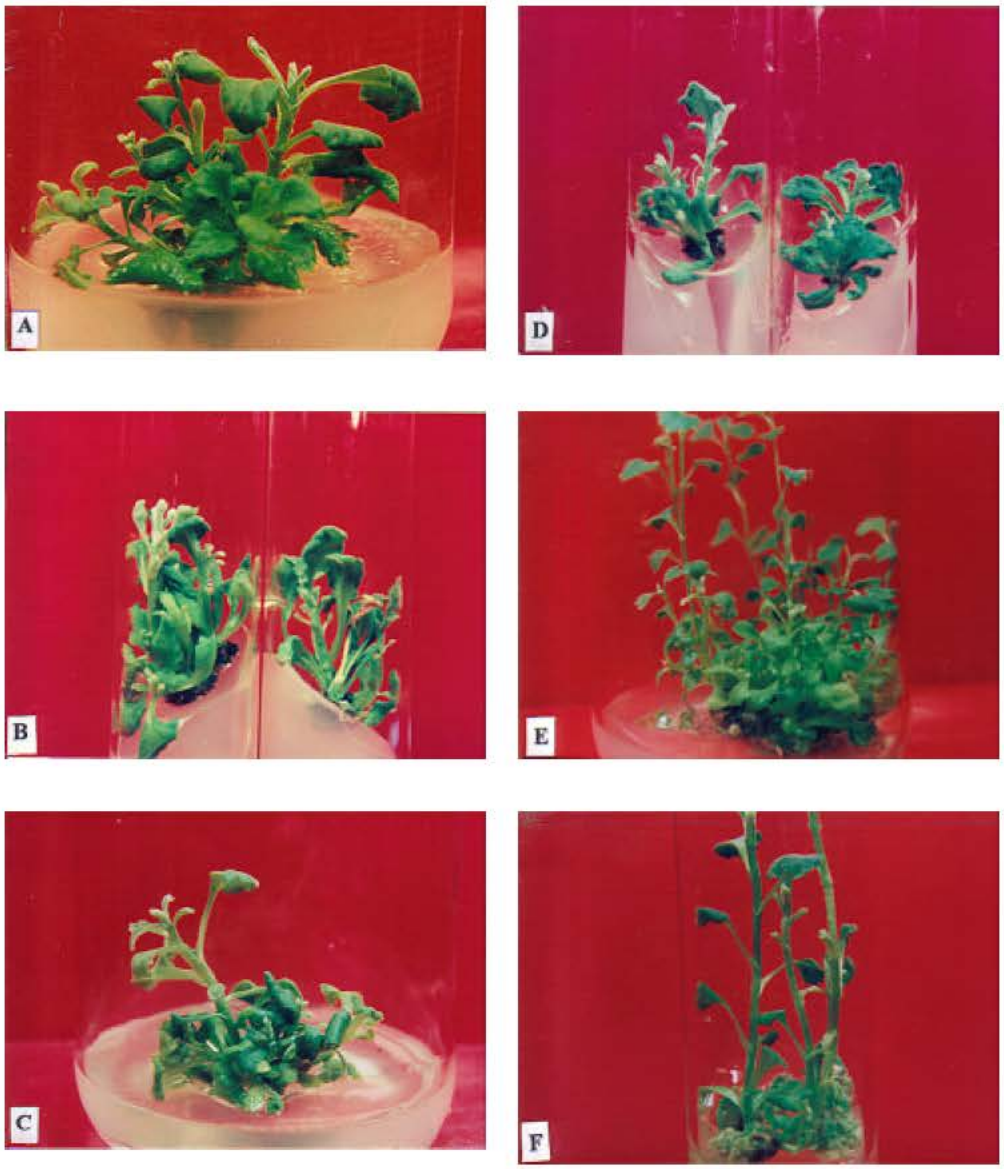

Fig. 1: A-E. In vitro shoot Multiplication from nodal and shoot tip explant of *C. morifolium*.
A-B: Development of multiple shoots from nodal and shoot tip explant on MS+BAP1 mg/l.
C-D: Shoot formation from nodal and shoot tip explant on MS medium containing BAP 10. mg/l + 0.1mg/l IAA.
E-F: Shoot multiplication from nodal and shoot tip explant on MS + BAP 1.0 mg/l + 0.1 mg/l GA3.

that MS media supplemented with BAP have been satisfactory for many species and cultivars of crop plants for their ***in vitro*** propagation. Superiority of BAP over other cytokinins in producing ***in vitro*** shoots has also been confirmed in other plants like ***Rosmarinus officinalis*** (Misra and Chatruvedi, 1984), ***Arachis hypogaea*** (Matre ***et al.***, 1985), ***Atropo beladona*** (Benjamin ***et al.***, 1987).

Effect of cytokinin with auxin

Cytokinin with auxin also play important role for shoot regeneration. In the preliminary experiment different concentrations of BAP in combinations with different auxin were tested for shoot proliferation. Among these different growth regulator combinations, BAP+IAA showed the better proliferation results than other combinations viz. BAP+IBA and BAP+NAA. In the later combination,BAP+IBA and BAP+NAA, the cultured explant produce fast growing callus that hindered the shoot proliferation rate. For this reason, only BAP+IAA combination was used for this experiment to find out proper balance between cytokinin and auxin for proliferating shoots from nodal and shoot tip explant of Chrysanthemum. In this experiment, both the explants were cultured on MS medium supplemented with different concentrations of BAP.(0.5,1.0 and 5.0 mg l^{-1}) in combination with IAA (0.1, 0.2, 0.5 and 1.0 mg l^{-1}). Among sixteen growth regulator combinations used in this experiment the cultured explant produced axillary shoots with roots in eleven combinations. The other five BAP+IAA combinations failed to proliferate axillary shoot but produced callus. On the shoot proliferation medium containing 1.0 mg l^{-1} BAP along with a lower concentrations of IAA showed better results for all growth parameters and both the explants. For both the explant, the highest degree of axillary shoot proliferation was found 74 and 69% medium containing 1.0 mg l^{-1} BAP+0.1 m/l IAA (Table 2). The maximum number of shoot per culture, highest length of shoots were 4.0±0.3, 3.9±0.1 and 5.7 ±0.4, 4.5±1.6 cm, respectively (Fig. C and D). Bhattacharya ***et al.*** (1990) obtained desirable morphogenetic responses from nodal segments, shoot tips and from leaf and stem calli of *C.* ***morifolium*** on MS medium containing 0.1 mg l^{-1} IAA+ 0.2 mg l^{-1} BAP. Multiple shoot regeneration in ***Chrysanthemum morifolium*** was observed by Hoque ***et al.*** (1995) from shoot tip and nodal explants when they were cultured on MS medium containing either BAP or a combination of IAA, NAA and Kn. Several reports are also available using different concentrations of IAA and BAP on MS medium for obtaining multiple shoot regeneration from different explants of chrysanthemum (Gertsson and Anderson,1985; Lazar and Cachita-Cosma,1983).

Effect of BAP with GA_3

Nodal and shoot tip explants from ***in vitro*** grown shoots of a particular experiment were taken and cultured on MS medium supplemented with various concentrations and combinations of BAP (viz., 0.5, 1.0, 2.0, 5.0 mg l^{-1}) and GA_3 (viz., 0.1, 0.2, 0.5, 1.0 mg l^{-1}). Results of sixteen different combinations of these growth regulators are summarized and presented in the Table 3. Among different combinations used, best result was observed on the medium supplemented with 1.0 mg l^{-1} BAP+0.1 mg/ l GA_3 after 7-8 weeks of culture. On this growth regulators combination 90 and 85% explants produced 5.2±0.5, 5.0±0.6 shoots per culture for both the explants, respectively (Fig. E and F). The average length of the shoots were 7.9±1.3 cm and

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 shoot per culture	No. of node per shoot	Average length of shoot (cm)	Percent of explant responded	No. of usable shoot per culture	No. of node per shoot	Average length of shoot (cm)
BAP								
0.1	67	4.4±1.0	4.1±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	4.2±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	4.3±0.6	79	4.0±0.3	4.4±.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

Table 2: Effects of cytokinin and auxin 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 shoot per culture	No. of node per shoot	Average length of shoot (cm)	Percent of explant responded	No. of usable shoot per culture	No. of node per shoot	Average length of shoot (cm)
BAP+IAA								
0.5+0.1	41	2.7± 0.5	4.9±0.7	4.1±0.3	40	2.6±1.5	4.5±0.5	4.0±0.7
+0.2	32	2.3±0.4	4.6± 0.3	3.9± 0.2	30	2.2±0.8	4.3± 0.1	3.8±1.5
+0.5	-	-	-	-	-	-	-	-
+1.0	-	-	-	-	-	-	-	-
1.0+0.1	74	4.0±0.3	5.7± 0.4	5.7± 0.5	73	3.9±0.1	5.0±1.5	4.5±1.6
+0.2	64	3.6±0.5	4.1± 0.1	3.7±0.4	62	3.5±1.5	4.6±0.3	3.6±1.5
+0.5	52	3.0±0.2	3.7± 0.2	3.0±0.2	51	3.0±0.1	3.5±0.1	3.0±1.0
+1.0	-	-	-	-	-	-	-	-
2.0+0.1	71	3.0±0.1	4.3±0.5	3.6±0.2	70	3.7±0.3	4.1±0.4	3.5±0.5
+0.2	67	2.9±0.2	4.0±0.2	3.2±0.1	66	3.6±0.4	4.0±1.0	3.1±0.8
+0.5	59	2.7±0.1	3.8± 0.5	3.0±0.3	57	3.4±0.6	3.7±0.3	3.0±0.1
+1.0								
5.0+0.1	65	2.8±0.2	4.0± 0.2	3.6±0.4	65	3.6±1.5	4.0±0.2	3.5±0.5
+0.2	59	2.5±0.2	3.7± 0.5	2.9±0.4	58	3.5±0.5	3.8±0.2	3.0±1.5
+0.5	45	2.8±0.4	3.0± 0.5	2.0±0.2	44	3.6±1.5	3.1±0.2	2.9±0.1
+1.0	-	-	-	-	-	-	-	-

Table 3: Effects of cytokinin (BAP) and giberellic acid (GA_3) 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 shoot per culture	No. of node per shoot	Average length of shoot (cm)	Percent of explant responded	No. of usable shoot per culture	No. of node per shoot	Average length of shoot (cm)
BAP+GA3								
0.5+0.1	83	4.3±0.5	6.4±0.4	6.2±0.5	82	3.1±0.8	6.2±1.5	6.1± 0.9
+0.2	81	4.8±0.4	6.3±0.5	6.0± 0.3	80	4.2±1.5	6.1±1.4	6.0± 0.1
+0.5	76	4.7±0.5	6.0±0.2	6.1± 0.3	74	4.0±1.5	6.0±0.1	6.0±1.5
+1.0	69	4.0±0.5	5.8±0.3	5.7±0.6	67	3.8±0.1	5.9±0.1	5.8± 0.5
1.0+0.1	90	5.2±0.5	7.9±1.5	7.4±1.3	88	5.0±0.6	6.1±1.5	7.1± 0.8
+0.2	89	5.0±0.3	7.6±.3	7.0±1.2	82	4.8±0.1	6.0±0.5	6.2± 0.1
+0.5	84	4.9±0.7	6.6±0.2	6.9±1.3	83	3.9±0.1	6.6±0.1	6.8± 0.1
+1.0	74	4.3± 0.3	5.9±0.5	6.0±1.2	73	3.2±0.8	5.9±0.1	6.0± 1.0
2.0+0.1	83	4.3±0.7	6.2±0.2	6.1±0.3	81	3.1±0.9	6.1±0.9	6.1± 0.5
+0.2	74	4.2±0.5	5.8±0.3	6.1±0.4	72	3.2±0.8	5.7±0.2	6.0± 0.3
+0.5	72	3.9±0.1	5.0±0.2	5.8±0.4	71	3.0±0.2	5.6±0.1	5.9± 1.0
+1.0	70	4.0±0.8	4.8±0.2	5.1±0.9	69	3.9±0.3	4.7±0.1	5.0± 0.2
5.0+0.1	70	3.9±0.1	4.0±0.3	5.0±0.4	69	3.5±0.5	4.8±0.5	5.1±0.6
+0.2	69	3.8±0.2	3.9±0.4	4.6±0.4	67	3.3±03	4.7±0.3	4.0±0.1
+0.5	65	3.4±0.4	3.6±0.3	3.1±0.7	64	3.1±0.5	4.6±0.2	5.0±0.5
+1.0	55	3.0±0.3	3.1±0.5	3.2±0.8	50	3.0±0.5	3.0±0.5	3.1±0.2

7.1±0.8 cm. When the medium containing higher concentrations of BAP and GA_3 then the percentage of explant responded, number of usable shoot per culture, number of node per shoot, average shoot length decreased gradually with the increase of the growth regulator concentrations. Rey and Mroginski (1985) reported best shoot proliferation on NAA+Kn+GA_3 combination for ***in vitro*** plantlet regeneration but our study BAP+GA_3 combination was found to produce better proliferation than other combinations. Addition of GA_3 to the BAP containing media increased shoot length but it decreased shoot multiplication. This could be attributed that GA_3 enchanced length increment at the cost of multiplication rate of shoot. It is contrary to the report by Wilna de Winnar (1988) that GA3 with BA-NAA formulation stimulated both proliferation and elonggation of shoot. In the present study GA3 stimulated shoot elongation but not shoot bud proliferation. This result is in consistent with the findings of Conover and Litz (1978).

It was found that the responses of nodal segments and shoot tip explant for shoot multiplication was not equal.Among the explants studied the nodal segment were found to be the best for shoot multiplication in comparison to the shoot tip explants. This differential response with regards to morphogenic development from chrysanthemum explants may be due to the genotypic differences of the plant materials used in the present investigation with that Bhttachrya ***et al.*** *(*1990). This effect can be attributed to the prsence of axillary buds at more advanced stage and absence of apical dominance in the nodal explant.

Among different growth regulators tested for shoot multiplication BAP gave the maximum number of shoots. GA_3 affected shoot height greatly. Shoot height was reduced with an increase in the concentration levels of different cytokinins. It can be concluded from the present results that among the different treatments with cytokinins and auxins either singly or in combinations MS+BAP medium is more effective for shoot multiplication than other combinations.

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