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In vitro Shoot Multiplication of *Chrysanthemum morifolium* as Affected by Sucrose, Agar and pH

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Abstract: Effects of sucrose, agar and pH on ***in vitro*** shoot multiplication of Chrysanthemum were studied. Nodal explant from the ***ex vitro*** grown plant was used as the test material. For optimum shoot induction and multiplication in MS medium containing BAP+ sucrose 30 gm l^{-1}, agar 6 gm l^{-1} and pH 5.5-6.0 proved more effective. The media having 30 gm l^{-1} sucrose showed the highest percentage of explant responded to shoot proliferation and that was 100%. This sucrose concentration also showed the optimum result for number of usable shoots per culture, number of node $shoot^{-1}$ and average length of shoots and the values were 5.4±0.6, 5.1±0.8 and 5.6±0.4 cm. The highest proliferation response of the explant was observed on MS medium having 6 gm l^{-1} of agar and the frequency was 100%. Among different level of pH, the highest percentage of explant showing proliferation was observed on the media adjusted to pH 5.5 and 6.0. The results presented here proved to be suitable for the ***in vitro*** shoot multiplication of ***Chrysanthemum morifolium***.

Key words: ***In vitro***, multiplication, optimum, effect, multiple shoot

Introduction

Chrysanthemums are showy flowers and recently its popularity has increased not only for their outstanding aesthetic beauty but also for their good potential as cut flowers to many countries of the world (Erler and Siegmund, 1986). Due to the high popularity and demand for chrysanthemum it becomes one of the first commercial targets for micro propagation (Levin ***et al.***, 1988). For commercial micropropagation of *C. **morifolium*** requires to develop protocol which will be able to produce multiple shoots in a shorter period of time and also technically feasible. ***In vitro*** growth and shoot multiplication may be affected by sucrose, agar and pH of the shoot induction media.

Sucrose is used as source of C and energy for optimum proliferation and growth of the ***in vitro*** grown cultures. Agar is used in the tissue culture media as a gelling agent. But it has also some effects on growth and development of the culture depending on its concentration and brand. The lower and higher pH level hindered multiple shoot proliferation.

Therefore the attempts of this present study was to determine the effects of sucrose and agar and pH on ***in vitro*** shoot formation and multiplication of Chrysanthemum over the cultural period. The objective of this present study has been to determine the optimum cultural conditions for production of genetically stable multiple shoots from 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 ***in vitro*** cultures were established from nodal explant. 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 2-10 gm l^{-1} agar, 10-50 gm l^{-1} sucrose. The pH of the medium was adjusted to 4.5-6.5 and autoclaved at 121^{0}C for 20 min. All the cultures were incubated at 26±1^{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

Sucrose is an important factor for ***in vitro*** shoot proliferation. In this experiment different concentration of sucrose in MS medium were used for multiple shoot regeneration and development. Nodal segment were taken from ***in vitro*** cultures that grew on a particular medium composition for the present investigation. Nodal segments were cultured on MS medium having 0.2 mg l^{-1} BAP at five different concentrations of sucrose viz.10, 20, 30, 40 and 50 gm l^{-1} and with a control treatment of without sucrose. After 6 weeks of culture percentage of explant showing proliferation, number of total shoots culture^{-1}, number of usable shoots culture^{-1}, number of nodes shoot^{-1}, average length of shoots were as shown Table 1.

Among the different sucrose concentrations in MS medium, the media having 30 gm l^{-1} sucrose showed the highest percentage of explant responded to shoot proliferation and that was 100%. The medium having 30 gm l^{-1} sucrose also produced the optimum result for number of usable shoots culture^{-1}, number of nodes culture^{-1} and average length of shoots and the values were 5.4±0.6, 5.1±0.8 and 5.6±0.4 cm (Fig 1.A). The medium containing 50 gm l^{-1} sucrose showed the lowest percentage of explant number showing proliferation, number of usable shoots culture^{-1}, number of nodes shoot^{-1} and average shoot length, and they were 59%, 4.0±1.0, 3.6±0.1 and 4.5±0.5cm. From the present investigation it was observed that different concentrations of sucrose affected ***in vitro*** growth of ***C.morifolium*** shoots variously. Complete inhibition of sprouting and development of shoots of the culture on sucrose-free medium confirm the essentiality of a easily accessible energy source in the proliferation medium. The ***in vitro*** grown shoots despite being green, do not rely on photosynthesis and grow as heterotrophs (Bhojwani and Razdan, 1983). Inhibition of chlorophyl synthesis and shoot growth on sucrose deficient medium have also been reported by Amin and Jaiswal (1989). At 40 gm l^{-1} and 50 gm l^{-1} sucrose concentrations, the shoot size was bigger but its number decreased and root growth was inhibited. The present findings also indicates that the sucrose not only acts as a carbon cum energy source in the medium but also acts as an osmoticum (Brown ***et al.***, 1979 and Skirvin, 1981) and different concentrations of it act as one of the controlling factors for the induction and growth of shoots.

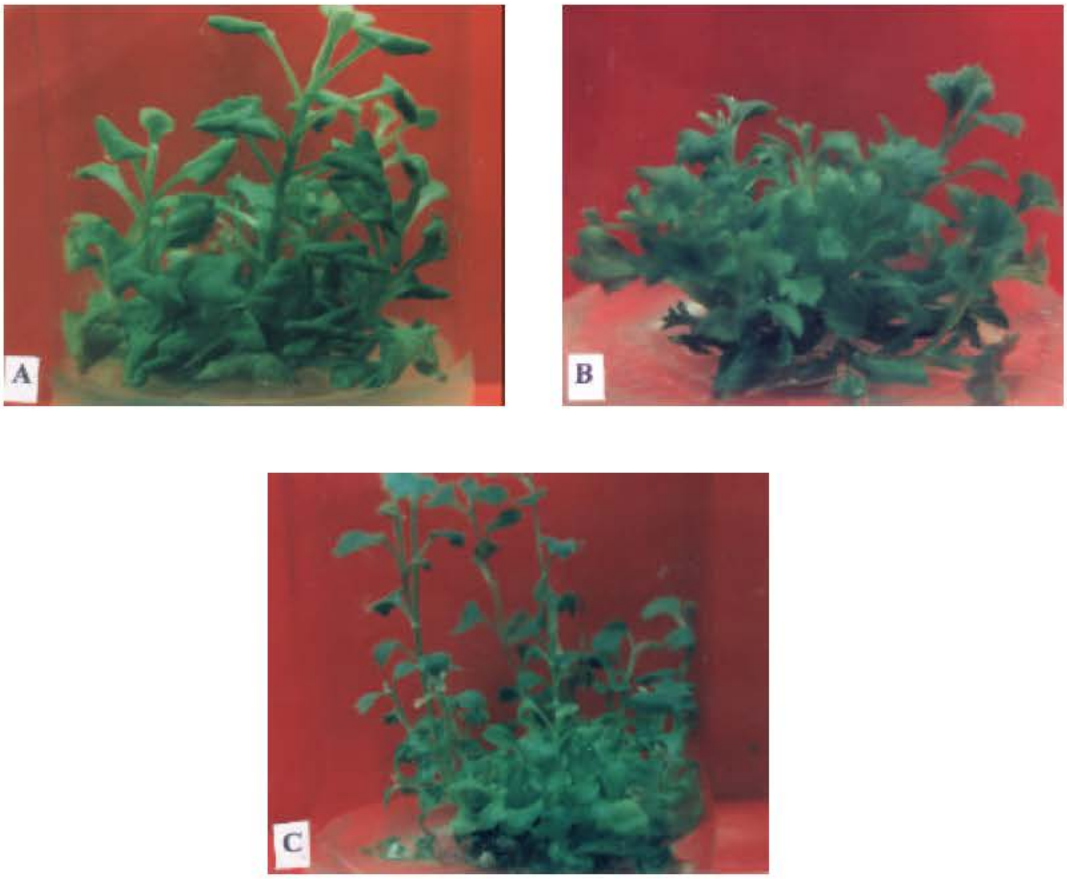

Fig 1: Shoot Multiplication from nodal explant as affected by Sucrose,Agar and pH of *C. morifolium*

A. Development of multiple shoots on MS medium containing BAP+ Sucrose 30 gm l^{-1} from the nodal explant
B. Growth and shoot multiplication from nodal explant on MS medium containing BAP+ 6 gm l^{-1} agar
C. Formation and multiplication of shoots on MS medium with BAP+ pH 5.5-6.0 from the nodal explant of *C.morifolium*

Concentrations of agar in the media can effect the culture growth and development of shoots. Nodal explant were cultured on MS medium having 0.2 mg l BAP at five different strength of agar viz.2, 4, 6, 8 and 10 gm l^{-1} to standardise the optimum agar strength for maximum growth and development of the axillary shoots. Data on shoot proliferation and shoot elongation were recorded after 6-7 weeks of culture as presented in Table 2.The percentage of explant showing

Table 1: Effects of Sucrose on proliferation and growth of axillary shoot from nodal segments of *in vitro* proliferated shoots. Data were recorded after 6-7 weeks of culture on MS medium containing either 0.2 mg l^{-1} of BAP.

Sucrose concentration gm l^{-1}	% of explant responded	No. of usable shoot/culture	No. of node per shoot	Average length of shoot (cm).
0	-	-	-	-
2	67	4.1±0.8	3.9±0.1	5.1±0.7
4	79	5.2±0.7	4.9±0.2	5.2±0.8
6	100	5.4±0.6	5.1±0.8	5.6±0.4
8	94	5.3±0.7	5.0±0.2	5.2±0.1
10	59	4.0±1.0	3.6±0.1	4.5±0.5

Table 2: Effects of Agar on induction and development of axillary shoot from nodal segments of *in vitro* proliferated shoots. Data were recorded after 6-7 weeks of culture on MS medium containing either 0.2mg l^{-1} of BAP.

Agar concentration gm l^{-1}	% of explant responded	No. of usable shoot/culture	No. of node per shoot	Average length of shoot (cm).
0	-	-	-	-
10	40	3.9±0.1	3.3±0.7	4.8±0.2
20	54	4.0±0.2	3.4±0.6	4.1 ±0.1
30	100	6.0±0.5	6.5±0.3	5.7±0.3
40	84	5.5±0.1	5.1±0.8	5.1±0.1
50	33	3.3±1.5	3.4±0.6	4.0±0.5

Table 3: Effects of pH on growth and proliferation of axillary shoot from nodal segments of *in vitro* proliferated shoots. Data were recorded after 6-7 weeks of culture on MS medium containing either 0.2 mg l^{-1} of BAP.

pH level of the medium	% of explant responded	No. of usable shoot/culture	No. of node per shoot	Average length of shoot (cm).
4.5	49	5.0±0.1	4.0±0.5	5.0±0.3
5.0	74	4.7±0.3	4.1±0.3	5.2±0.1
5.5	100	5.8±0.1	4.9±0.1	5.9±0.3
6.0	100	5.7±0.2	5.0±0.2	6.0±0.2
6.5	44	4.6±0.4	3.8±0.2	4.0±0.6

proliferation was lowest in medium having the highest concentration of agar (10gm l^{-1}) and that was only 33%. Whereas, the highest proliferation response of the explant was observed on medium having 6 gm l^{-1} of agar and the frequency was 100%. Number of usable shoots culture^{-1}, number of nodes shoots^{-1} and average length of shoots per culture were also highest on the media that contained 6 gm l^{-1} agar and they were 6.0±0.5, 6.5±.0.3 and 5.7±0.3 cm (Fig. 1B).

The early sprouting and rapid growth of shoots as were noticed on medium with lowest agar concentrations (2-4 gm l^{-1}) could be distributed to the easy availability of nutrient elements like Ca, Mg, K and Mn in the soft-gel medium (Debergh, 1983). ***In vitro*** growth abnormalities like fasciated shoots and vitrified leaves have also been observed in guava cultures grown with lower concentrations of agar (Amin and Jaiswal, 1989). This growth anomalies are apprehended to be the changes in the matric potential of media water under lower gelling agent concentrations. Whereas, the highest proliferation response of the explants was observed on medium having 6 gm l^{-1} agar (BDH) and the frequency was 100%. Contrary to this, reduced growth and less number

of shoots on media gelled with 10 gm l^{-1} agar could be due to restricted diffusion of macro-nutrients (Romberger and Tabor, 1971) or reduced availability of organic matters and water (Stoltz, 1971; Skirvin, 1981 and Debergh, 1983). It is evident from the results of present investigation and those of other (Debergh, 1983 and Amin and jaiswal, 1989) that concentrations of agar in the media can effect the culture growth in many ways. Therefore, the level of agar in the medium should be such that it minimizes the water loss and allows the good diffusion of nutrient elements.

In vitro multiple shoot development depends upon some other factors rather than cytokinin, auxins and gibberellins. The pH of the culture medium is an important factor for the ***in vitro*** proliferation and healthy culture growth. Nodal segments taken from ***invitro*** culture that grew on medium containing 0.2 mg l^{-1} BAP were used in the present study. Nodal segments were cultured on MS medium adjusted to five different levels of pH viz.4.5, 5.0, 5.7, 6.0 and 6.5 (Table 3) but supplemented with only one concentration of cytokinin (0.2 mg l^{-1} BAP). Among these pH levels, the highest percentage of explant showing proliferation was observed on the media adjusted to pH 5.5 and 6.0.and that was 100%.The second highest percentage of explant showing proliferation was observed on media having 5.0 pH and it was 74%. The lowest frequency of explant showing proliferation was observed on the media where pH was adjusted to 4.5 and 6.5 where the proliferation frequency ranged from 44-49%. Number of usable shoot culture^{-1} was highest in medium having pH 5.5 and the value was 5.8±0.1 (Fig. 1C) and lowest in medium having 6.5 pH.

From the present investigation it was revealed that both lower (4.5) and higher (6.5) pH levels hindered multiple shoot proliferation. Comparatively less acidic pH (6.5) gave harder gel which might have adverse effects on regeneration and proliferation of shoots. ***In vitro*** proliferation of ***Azadirachta indica*** (Gautam ***et al.***, 1993), ***Plantago ovata*** (Barna and Walklu,1988) and ***Smilax zeylanica*** (Jha ***et al.***,1987) shoots was increased significantly when the pH the culture media was adjusted at 5.8 before autoclaving.

Plant regeneration through tissue culture technique would be a noble alternative for improving the quality and faster production of Chrysanthemum. ***In vitro*** culture techniques permit the shoot induction and multiplication under aseptic condition with reduced space requirements because of the small size of explant. It has been demonstrated that sucrose, agar and pH on the medium play an important role on ***in vitro*** growth and development of shoots. Therefore, the present report showed that in MS medium containing BAP, sucrose 30 gm l^{-1}, agar 6 gm l^{-1} and pH 5.5-6.0 proved more effective on shoot multiplication.

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