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Standarization of growth regulators for rapid shoot proliferation in chrysanthemum morifolium

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Chrysanthemum morifoIium is a vegetatively propagated perennial ornamental plant. The shoot proliferation medium comprised of different concentrations of BAP and NAA in different combinations. The best proliferation with well differentiated micro shoots was achieved when the cultures were transferred to MS medium fortified with 3.0 mg/l BAP and NAA 0.01 mg/l. The treatments recorded 4.0 to 4.43 micro shoots per explants after 15 to 20 days of first sub culture.

Key words : Explant, Proliferation, Micro shoots, Growth regulator.

INTRODUCTION

Chrysanthemum morifolium is a semi hardy herbaceous, perennial flowering plant belongs to family Asteracae. It is one of the most important commercially flower crops of the world, which is extensively used as pot plant, decorative green plant and cut flower production .In the global export market ,the plant material of a specific variety must be propagated in a very short period. It is propagated both through seeds and vegetatively. Promotion of elongation in micro shoots makes them ready for induction of *in vitro* adventitious rooting (Bhat, 1990).

Sterilized explants of Chrysanthemum cultivars supplemented with various concentrations of growth regulators such as BAP and NAA are used and rooting was initiated after 10-12 days of inoculation (Kumari and Varghese,2003).For rapid propagation of Chrysanthemum surface sterilization treatments on establishment of axillary bud must be cultured on MS medium supplemented with BAP (3.0 mg/l), NAA (0.01 mg/l) and GA_3 (0.5 mg/l). In view of the above facts, the present investigation was carried with standardization of growth regulators for rapid initiation *in vitro* culture.

MATERIALS AND METHODS

Plant material:

Plant of Chrysanthemum was selected as experimental material obtained from National Botanical Survey of India, Allahabad.

Culture medium:

Murashige and Skoog's medium (1992) was used. The pH of the solution was adjusted to 5.7-5.8 using either 0.1 N HCL or 0.1 N KOH. For solidification of the medium , agar powder (0.8% w/v) was added and autoclaved for 15 to 20 min. at 15 psi at 121.0 ^{0}C. The explants were inoculated on the culture medium (15ml) in culture tubes and incubated in culture room.

Explants:

Axillary buds (Fig. 1) from the middle portion of current season flowering shoots were selected and cut during cooler parts of the day. Cut shoots were transported in moist condition to the laboratory and axillary buds were isolated with a sterilized secatuer.

Surface sterilization of explants:

The collected explants were washed with a solution containing 3- 4 drops of liquid detergent teepal. Thereafter, the detergent was completely drained out from the explants by 3-4 washing with vigorous shaking by hand. The explants were then cultured on MS medium supplemented with 3.0 mg BAP and 0.01 mg NAA.

Culture conditions:

Cultures were incubated at 25 ± 2^{0}C under cool fluorescent light (1500-2000 lux) with 16h/8h light / dark cycle. Each treatment consisted of minimum 10 explants and all experiments were repeated 3 times.

Standardization of growth regulators:

Growth regulators such as BAP and NAA were used in combinations. To find out the optimum combination for growth regulator(s) and multiple shoot formation, the sprouted shoots were cultured on the medium with

different growth regulator combinations. (Table 1)

RESULTS AND DISCUSSION

Sub-culturing of sprouted explants (Fig. 1 and 2) on MS medium supplemented with various concentrations of different growth regulators such as BAP and NAA indicated significant response in respect of proliferation and growth of cultures (Table 2)

Average shoot proliferation:

Use of BAP in combination with NAA significantly improved the proliferation rate over the control (Table 2). Data presented in Table 2 show that 3.0 mg/l BAP in combination with 0.1 mg/l NAA (T-6) resulted in highest shoot proliferation (4.43 shoot lets/ explants) after 15 days of first sub culturing as compared to control and the other combinations of BAP and NAA (Fig. 3).

Callus induction:

Visual observations of cultures indicated that the higher concentration of NAA induced callus formation (Table 2). Dark green cultures with normal growth were recorded in T-6 as compared to other treatments.

General growth of shootlets:

Excellent growth with dark green leaves was

Fig. 1 : Axillary bud explant

Fig. 2 : Sprouted explant

Table 1: Growth regulator combinations

Sr. No.	Treatment no.	Concentrations (mg/l)		
		BAP	+	NAA
1.	T_0	0.0	+	0.0
2.	T_1	1.0	+	0.01
3.	T_2	1.0	+	0.1
4.	T_3	2.0	+	0.01
5.	T_4	2.0	+	0.1
6.	T_5	3.0	+	0.01
7.	T_6	3.0	+	0.1

Table 2 : Effect of different concentrations of BAP and NAA supplemented to MS medium on shoot proliferation in *Chrysanthemum morifolium* after 15 days of first subculture

Treatment no.	Treatment (BAP+NAA)	Average No. of micro shoots/explant (mg/l)	Callus formation	Culture appearance
T-0	0.0+0.0	1.25	-	Poor
T-1	1.0+0.01	1.82	-	Poor
T-2	1.0+0.1	2.25	-	Poor
T-3	2.0+0.01	2.57	-	Poor
T-4	2.0+0.1	3.10	+	Good
T-5	3.0+0.01	3.27	+	Good
T-6	3.0+0.1	4.43	++	Excellent

+ = Callus formation; ++ = More callus formation; - = No callus formation

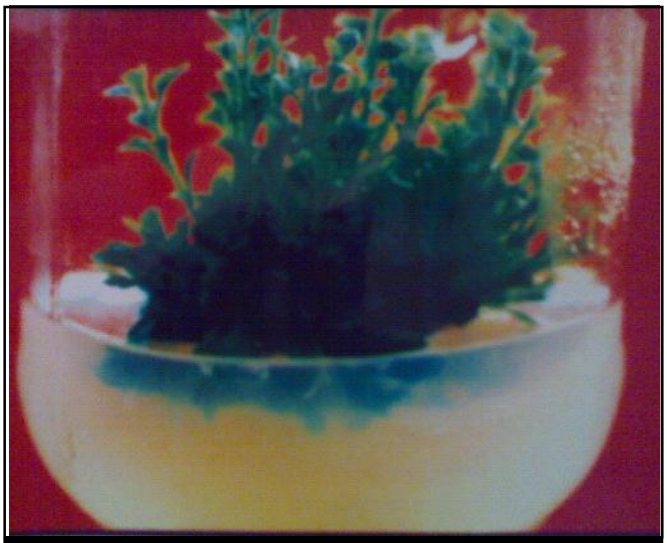

Fig. 3 : Multiple shoot formation

observed in the cultures growing on MS medium supplemented with 3.0 mg/l BAP in combination with 0.1 mg/l NAA (T-6). Sufficient numbers of good quality micro shoots were obtained after 3-4 sub cultures in this medium.

The shoot proliferation medium comprised of different concentrations of BAP and NAA in different combinations. The best proliferation with well differentiated micro shoots was achieved when the cultures were transferred to MS medium fortified with 3.0 mg/l BAP and NAA 0.1 mg/l (Fig. 3). The treatments recorded 4.0 to 4.43 micro shoots per explants after 15 to 20 days of first sub culture. The shoot proliferation in tissue culture was largely due to the action of BAP. Optimum dose of BAP enhanced axillary branching and multiple shoot formation. An increase in NAA concentration beyond an optimal concentration induced more callus formation and retarded the axillary bud sprouting. In general, the method, which avoids callus formation and stimulates axillary branching, is considered to be ideal. The presents results lends support from the previous work done in the shoot proliferation of *Chrysanthemum* (Lu *et al.*, 1990; Chakrabarty *et al.*, 1999; Annadana *et al.*, 2000; Kumari and Varghese, 2003) who had regenerated the different explants of Chrysanthemum using various concentrations and combinations of BAP and NAA *in vitro*. It is well established that optimal ratio of cytokinin:auxin causes the dormant meristematic zone existing in the axillary node regions to show shoot development.

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