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Standardization of auxin concentration for root induction in *Chrysanthemum morifolium*

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ABSTRACT

Chrysanthemum morifoIium is a vegetative propagated perennial ornamental plant. The Root initiation medium comprised of different concentrations in various combinations. The best initiation with well differentiated micro shoots was achieved when the cultures were transferred to MS medium fortified with the various concentrations of auxins. Out of the different treatments tried 0.5 mg/l NAA induced maximum rooting (88.66%), followed by control 79.00%) as compared to 1.0 mg/l NAA (76.66).

Keywords: Explants, Initiation, Micro shoots, NAA.

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].

The micro shoots regenerated from callus observed to be very tiny by Kumari and Varghese [2003]. They transferred these micro shoots to MS medium containing GA_3 (5-10 mg/l) a sufficient amount of elongation could be observed in the shoots. Rooting was initiated on same medium after 10-12 days. In view of the above facts the present investigation were carried with standardization of auxin concentrations for root initiation and hardening of plantlets to field conditions.

MATERIALS AND METHODS

Plant Material

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

Culture Medium

Murashige and Skoog's medium [1961] 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 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, 0.01 mg NAA and 0,05 GA_3 per liter.

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 auxins and their Concentrations
For obtaining successful and quicker root initiation, auxin i.e. NAA in different concentrations was added to the MS medium ½ strength). A constant dose of sucrose i.e., 60g /l was supplied to all the treatments as given in Table 1.

Acclimatization stage
Aseptically, the microplants were transferred to autoclaved glass bottles wih polyethylene screw lid containing agropeat supplemented with half strength of MS liquid medium devoid of calcium, organic, growth regulators and sucrose components. The pH of the medium was adjusted to 5.7-5.8. The cultures were kept in culture rooms for one week. The relative humidity in side the glass bottle was gradually reduced by unscrewing and finally, removing the lid.

Transfer of plantlets to field conditions
The hardened plantlets were transferred to the earthen pots (12'' size) filled with FYM: sand:garden siol (2:1/2:1/2) and supplemented with one full tea spoon of bone meal and 20 g neem-cake per pot and kept under field conditions.

RESULTS AND DISCUSSION

Root Induction
Auxin concentration: Although, half strength MS medium devoid of any auxin induced 68.49% rooting of microshoots in 12.67 days, however, the quality of roots observed was inferior and unsuitable for successful hardening of plantlets. For accelerating the *in vitro* rooting process and improving the quality of roots as well shoots, different levels of NAA were added in the MS medium (1/2 strength) and data collected in respect of pecent rooting, duration required for root initiation, number of roots/ shoot, length of longest root, quality of roots along with color, etc. are presented in Table 2. A perusal of data given in Table 1 reveals the superiority of NAA (0.05 mg/l) over the higher concentration in all respects (Figure 1).

Percent rooting
The effect of auxins on *in vitro* rooting was found to be significant among the different treatments and control. Out of the different treatments tried 0.5 mg/l NAA induced maximum rooting (88.66%), followed by control 79.00%) as compared to 1.0 mg/l NAA (76.66).

Duration required for root initiation
Perusal of data presented in Table 2 it reveals that root initiation was recorded in the micro-shoots treated with 0.5 mg/l NAA (6.78) followed by 1.0 mg/l NAA (8.56days) compared to control days.

Number of roots
As indicated that microshoots proliferated on MS medium fortified with 0.5 mg/l NAA (11.77/shoot) produced maximum primary roots followed by 1.0 mg/l NAA (7.17/shoot) as compared to (5.73/shoot) and ighest survival of plantlets (86.89%) was recorded after 40 days of transplanting in glass jars for hardening (Figure 2 and Figure 3).

For successful root induction onto elongated shoots, strength of basal medium salts, sucrose level and supplementation of auxins are considered to be important. It is well known that rooting in certain plant species may occur ideally when the overall salt strength of the medium is reduced. In some cases, the salt strength reduction may eliminate the need for auxin(s) for rooting. The results of the present findings on induction of rooting with out auxin (in control microshoots) are in conformity with the previous work done by Fugii and Shimizu [1990] who reported that good rooting in the *in vitro* raised shoots of Chrysanthemum could be achieved on harmone free MS medium.

Although, a high percentage was recorded when the shoots were placed on half strength of MS with 60 g/l sucrose devoid of auxin, but the quality of roots was in general very poor. The roots were invariably very long, thin unbranched and turning black in colour, which not only affect *ex vitro* survival but also the overall performance of the *in vitro* raised plantlets.

The rooting of microshoots could be significantly improved after addition of auxin into the rooting medium. The highest rooting (88.66%) and more number of roots (11.77) in shortest duration (6.78 days) were obtained after placing the shoots half strength MS medium supplemented with 0.5 mg/l NAA and 60 g/l sucrose. Like any morphogenetic process, rooting is an energy intensive process and thus a higher level of carbon source is required. Thus, high sugar level along with low auxin were very effective. These results in conformity with those of Chakrabarty et al. [1999].

The preparation of hardening –off and planting out of the microplants starts from shoot elongation and rooting stages of plant propagation by tissue culture [Bhojwani and Razdan, 1983]. During short enongation stage, the addition of gibberellic acid in the medium resulted in rapid shoot growth in terms of length and thickness, and formation of well- developed leaves, which made them sturdy and ready for root induction followed by acclimatization. Furthermore, reduction in salt levels in the MS medium particularly macronutrients including potassium during *in vitro* rhizogenesis, utilizes and prevents the accumulation of salt complex by guard cells in the stomata. Therefore, the plantlets on transfer to the soil show high water contents in leaves due to minimum water loss through stomata [Prasad, 1995].

Furthermore, the *in vitro* derived plantlets have another characteristic feather i.e. the epicuticular waxy layer is poorly developed. This leads to uncontrolled foliar water loss when the plants are taken out from the culture vessels. However, when the plants are kept at high humidity conditions, they synthesize more epicuticular wax, which enhances the survival success during acclimatization [Short et al., 1981].

For obtaining the high success during the plantlets acclimatization the two strategies were employed in the present investigation. The plantlets transferred to glass jars with polypropylene lids each filled with sterile agropeat moistened with half strength MS medium (devoid of growth regulators, calcium, organics and sucrose) recorded 86.89% survival. The plantlets acclimatized in glass jars received less open space but appropriate relative humidity. Gradual loosening the lids of the glass jars at regular interval enabled the moisture to escape. The *ex vitro* plantlet mortality may be doe to the fact that microshoots developed *in vitro* have several anatomical abnormalities like poor cuticle development, less palisade cells, more air space, poor vascular bundles etc. Besides, the *in vitro* plants have , poorly developed epicuticular waxes, more stomata per unit area and raised guard cells with wide open opening, which resut in more transpiration losses and less survival of plantlet [Prasad, 1995].

Table 1: Different treatment combinations

S. No.	Treatment No.	Auxin	Concentrations (mg/l)
1	T_0	Control	-
2	T_1	NAA	0.5
3	T_2	NAA	1.0

Table 2: Effect of various auxins and their concentrations on *in vitro* induction of rooting in Chrysanthemum on MS medium* (Half strength)

Treatment No.	Auxin (mg/l)	Rooting (%)	Duration for root initiation (days)	No. of primary roots/shoot
T-0	Control	79.00 (62.72)	19.37	5.73
T-1	NAA (0.5)	88.66 (70.36)	6.78	11.77
T-2	NAA (1.0)	76.78 61.21)	8.56	7.17

** Supplimented with 60 g/l sucrose and Values in parenthesis indicate arc sin transformed.*

Figure 1: *In vitro* rooting

Figure 2: Hardening of microshoots

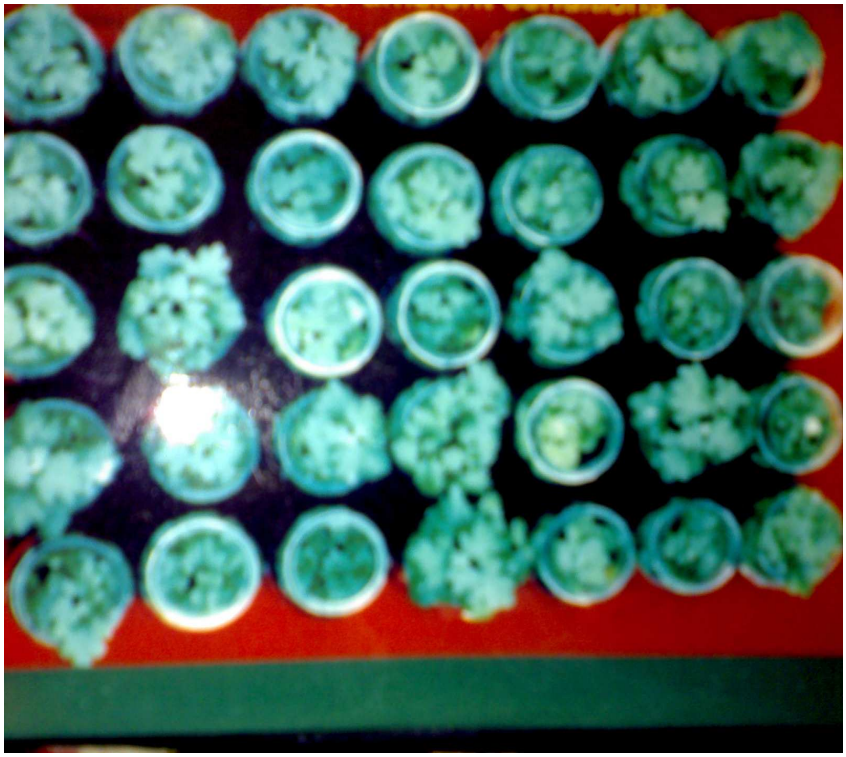

Figure 3: Plants ready to transfer under ambient conditions.

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