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Plant Regeneration Studies in Chrysanthemum (*Chrysanthemum morifolium* L.)

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Abstract:

The present investigation entitled "*Plant r*egeneration studies in Chrysanthemum (*Chrysanthemum morifolium* L.)" was carried out with objective for Regeneration of chrysanthemum plant. The explants (nodal segment) were treated with different concentrations of auxins (IAA, IBA) and cytokinins (BAP) alone or in combinations. The highest results were observed on MS medium containing 1.0 mg/L BAP + 0.1 mg/L IAA, shown 90% shoot initiation and 5.5 ± 0.51 average length of shoot per explants. Nodal segments of chrysanthemum were cultured in MS media with different concentration of BAP. The highest results were observed on MS medium containing 1.0 mg/L BAP, shown 93% shoot proliferation, 5.7 ± 0.22 cm average length of shoot per explants and 4.4 ± 0.88 nodes per explants. The regenerated shootlets were rooted on MS medium and ½ MS medium with different concentration of IBA. The highest results were observed on ½ MS medium containing 0.2 mg/L IBA shown 90% microcuttings rooted. 9 ± 0.19 cm average length of roots per explants and 11.8 ± 0.75 number per explant.

Keywords: Chrysanthemum, auxins, cytokinins, Nodal segments, microcuttings

Introduction:

Chrysanthemum is commonly known as Autumn Queen. It belongs to the family *Compositeae* (*Asteraceae*). It is highly attractive and charming short day plant, which behaves both as an annual as well as perennial flowering herb. The plant height ranges from 1-3 feet. The leaves are alternate and toothed, roots are adventitious and the stem is woody solid. The flowers bloom in early winter with a wide range of color, shape and sizes. The flower color ranges from white and cream through the shades of yellow, pink, bronze, red, deep purple and green.

Chrysanthemum can be grown on all types of soil, but light, rich, fertile and well-drained soil is the best for its cultivation. It is widely distributed over Europe, Asia, Africa and America. Among the 200 known species of chrysanthemum, several are suitable for the rock garden or herbaceous border. Most of the present day florets *Chrysanthemum morifolium* have resulted from inter crossing between *C. sinensis, C. indicum* and *C.ornatum*. It has about 275 different varieties grown in the different parts of the world for the beautification and decoration purposes.

Chrysanthemum cultivation by seeds is very rare and poorly nourished plants are known to seed better than well nourished ones, because plants arising from seeds are not true-to-type and the genetic heredity of the plants is altered. Chrysanthemum is propagated vegitatively either through rooted (suckers) or un-rooted cuttings. This conventional process of shoot cutting is very slow. Clonal propagation through *in vitro* culture can enhance multiplication many folds. Regeneration through in-vitro culture has become now a viable alternative to the conventional propagation methods. Large-scale micropropagation laboratories are providing millions of plants for the commercial ornamental market and the agricultural, clonally-propagated crop market. Due to high popularity and demand for chrysanthemum it becomes one of the first commercial targets for micropropagation and thus tissue culture can be utilized for the large-scale production of Chrysanthemum. Micropropagation using axillary shoots proliferation from nodal and shoot tip culture is the most desirable and safe as micropropagules to minimize genetic variation. It is possible now to obtain a large number of plants from one explant through *in-vitro*. Therefore, the present study was attempted to determine a suitable protocol and to find out the effect of different growth regulators and their combinations on shoot formation, multiplication and rooting of chrysanthemum.

Material And Methods

Material:

Plant material: The explant material (*Chrysanthemum morifolium L.)* was collected from 6 months old plants at the Sunrise Agro Industries, Wakad, Pune- 411057.

Methods

Sterilization of Glassware: The glassware such as cultural Bottles, measuring cylinders and the other equipment like forceps, cutting paper and blade holder were washing in tap water using detergent. Before being dried in oven they were rinsed in distilled water, wrapped in aluminum foil and subsequently autoclaved at 121^{o} C at 15 spi pressure for 20 min.

Preparation of stock solutions: Separate stock solutions of macronutrients, micronutrients, potassium iodide, iron, glycine and various vitamins were prepared by dissolving each chemical separately in small quantity of double distilled water and making up the required volume with double distilled water

Media preparation: According to the available literature on *in vitro* propagation of c*hrysanthemum morifolium L.* plants, the Murashige and Skoog's (1962) medium is the most commonly used growing medium for chrysanthemum

Initiation stage

Surface sterilization: Explants were cut into optimum size (1-1.5cm) Explants were washed under running tap water for 10 min. Treated with 1% (v/v) Savlon for 5 min. Treated with 0.1% bavistin for 15 min. Explants were washed under running tap water 4-5 times. Explants were treated with tween-80 for 15 min. Treated with of 70% ethanol for 30 sec. in LAF. Then treated with 0.1% $Hgcl_2$ for 03 min in LAF. Explants were thoroughly washed with sterile D.W. (for 5-6 times)

Inoculation of explants: After surface sterilization, explants were transferred to large sterile glass plate having sterile cardboard paper on it, with the help of sterile forceps under strict aseptic conditions. Then explants were cut into very small pieces (about 1-2 cm) with sterile scalpel blade and inoculated in culture bottles aseptically.

The initiation medium was 5 types.

1. MS medium
2. MS medium with 1.0 mg/L BAP + 0.1 mg/L IAA.
3. MS medium with 1.0 mg/L BAP + 0.2 mg/L IAA.
4. MS medium with 2.0 mg/L BAP + 0.1 mg/L IAA.
5. MS medium with 2.0 mg/L BAP +0.2 mg/L IAA.

After vertically inoculating the explants in culture bottle the mouth of bottle is quick flamed and bottles are tightly capped and properly sealed with klin film to avoid entry of external air. After proper labeling clearly mentioning media, date of inoculation etc. the bottles was transferred to growth room. Data were recorded after five weeks in terms of average length (cm) of explant & survival percentage.

Culture incubation condition: The inoculated culture bottles were incubated at 16 hr. daily light with intensified 1000 Lux at 25 ± 1^{0}C temperature.

Shoot Multiplication: After 5 weeks of initiation the initiated culture shifted to a medium that was for shoot multiplication. The shoot multiplication medium was 4 types.

1. MS medium
2. MS medium with 0.5 mg/L BAP.
3. MS medium with 1.0 mg/L BAP
4. MS medium with 2.0 mg/L BAP.

Initiated cultures were transferred to autoclaved medium within bottles in laminar air flow hood only. Every possible care has been taken to prevent any further contamination. Shoot proliferation was determined after five weeks of culture. The data were recorded number of shoots formed per explant and average shoot length (cm) & survival percentage.

Rooting: For rooting of chrysanthemum shoots, half strength MS media supplemented with 0.2 mg/l IBA and charcoal, full strength MS media supplemented with different concentrations of IBA (0.2, 0.5 mg/l) and charcoal. The percentage of survival, number of roots formed per shoot and average root length (cm) were determined after 6 weeks of culture on the rooting medium.

1. MS medium with 0.2mg/l IBA
2. MS medium with 0.5 mg/L IBA
3. 1/2MS medium with 0.2mg/l IBA

Results

Initiation: The result revealed that 1.0 mg/l BAP + 0.1 mg/l IAA showed it's superiority among all the other treatments. Average length of shoot (5.5 ± 0.51 cm) & percentage of survival rate was 90 % recorded in 1.0 mg/l BAP + 0.1 mg/l IAA.

Table 1: Effect of different conc. of BAP & IAA on chrysanthemum initiation

Sr. no.	Treatment	% of survival	Average length of shoots
1.	Control	60%	2.2 ± 0.83
2.	MS+1.0mg/l BAP+0.1mg/l IAA	90%	5.5 ± 0.51
3.	MS+1.0mg/l BAP+0.2 mg/l IAA	75%	3.6 ± 0.48
4.	MS+2.0mg/l BAP+0.1mg/l IAA	85%	4.9 ± 0.43
5.	MS+2.0mg/l BAP+0.2mg/l IAA	80%	4.0 ± 0.24

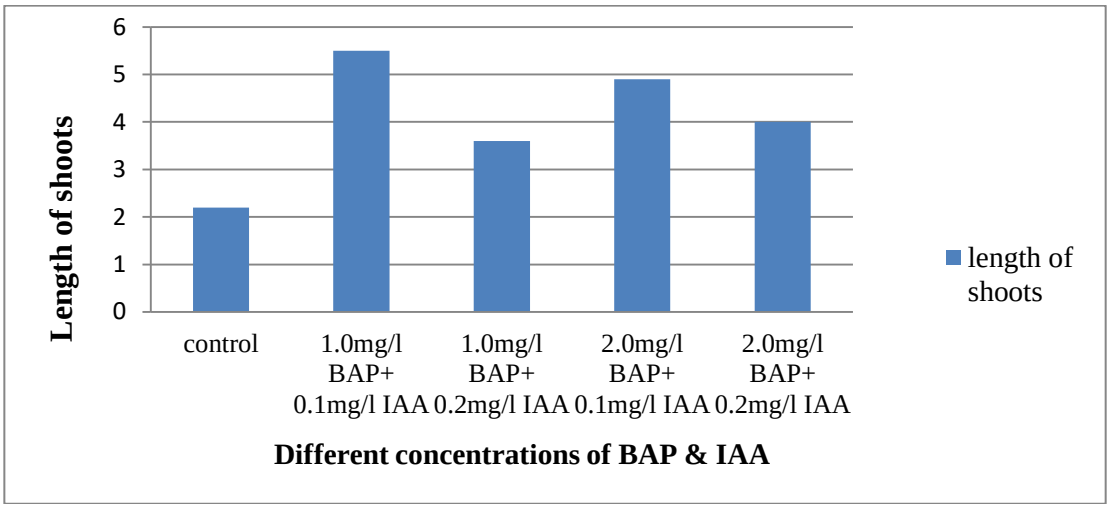

Fig 1: Effect of diff. conc. of BAP & IAA on chrysanthemum initiation.

Multiplication: The result revealed that BAP (0.1mg/l) showed it's superiority amongst all the other treatments. Average length of shoots (5.7 ± 0.22 cm) and Average no. of shoots (4.4 ± 0.88 cm) & survival rate was 93% were recorded in 0.1mg/l BAP.

Table 2: Effect of diff. conc. of BAP on multiplication:

Sr. no.	Treatment	% of Survival	Average length of shoot	No. of nodes /explant
1.	Control	40	2.1 ±0.2863	1.4 ±1.02
2.	MS+0.5mg/l BAP	80	4.8±0.4123	4.1 ±0.78
3.	MS+1.0mg/l BAP	93	5.7 ±0.2150	4.4 ±0.88
4.	MS+2.0mg/l BAP	66.66	4.4 ±0.3098	3.5 ±0.55

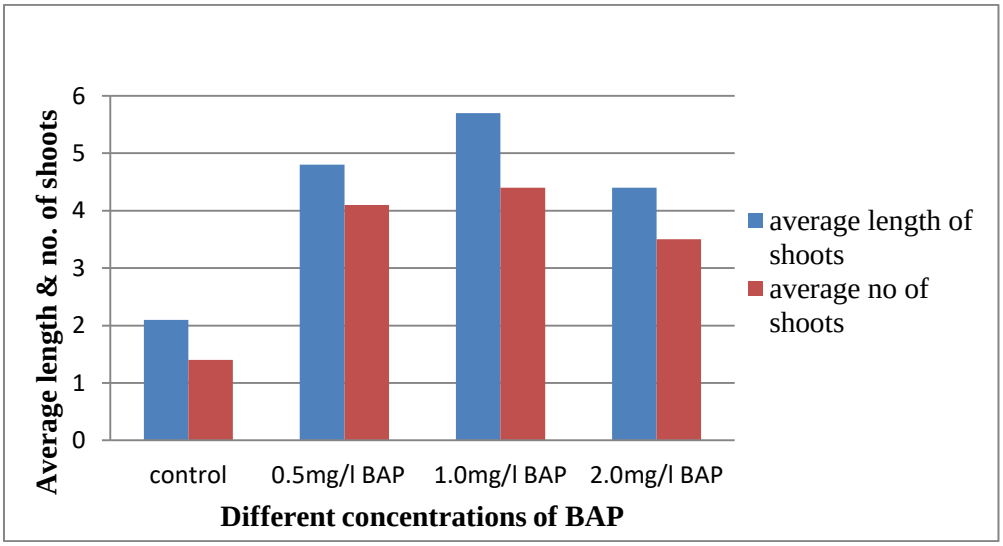

Fig 2: Effect of diff. conc. of BAP on multiplication

Rooting of shoots: The result showed superiority of ½ MS +0.2mg/l IBA as compared to all the other treatment as it produce significantly maximum no. of roots per explant (11.8 ±0.75) and largest roots(9 ± 0.19 cm) & survival rate was 90% were recorded in ½ MS +0.2mg/l IBA. IBA is considered as the most efficient auxins in root induction and development.

Table 3: Effect of different conc. of IBA on root formation

Sr.no.	Treatment	% of survival	Average length of roots	No. of root developed/explant
1	MS+0.2mg/l IBA	80%	7.2±0.3114	6.8±0.84
2	MS+0.5mg/l IBA	70%	8.3±0.5149	7.8±0.75
3	1/2MS+0.2mg/l IBA	90%	9±0.1870	11.8±0.75

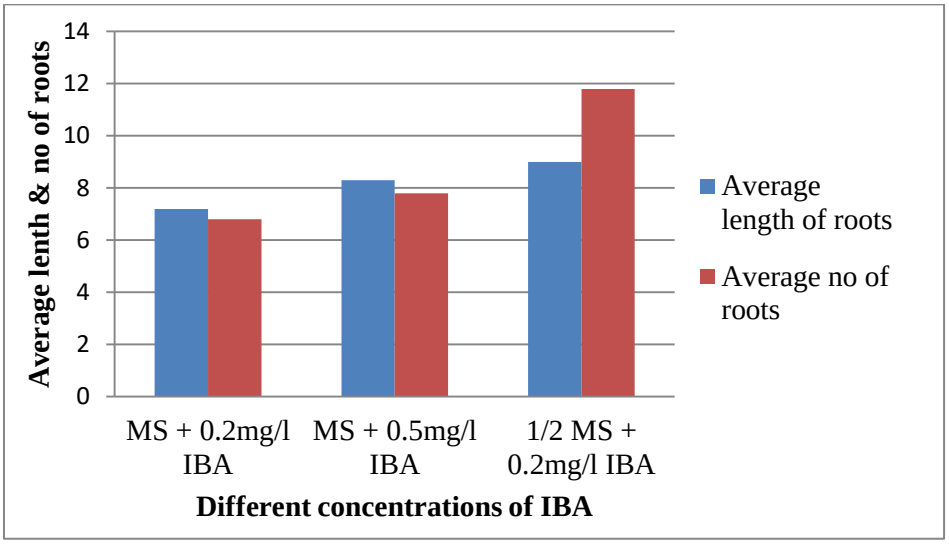

Fig 3: Effect of diff. conc. of IBA on root formation.

Summary And Conclusion

Summary: Present studies were carried out to find out an efficient protocol for *in vitro* regeneration of chrysanthemum plantlets for the efficient mass production of chrysanthemum plantlets for commercial purposes. Nodal segments used for the regeneration of chrysanthemum plantlets using tissue culture techniques. Explant establishment, shoot initiation, shoot proliferation, and rooting were carried out. The nodal segments were cultured in MS media fortified with different concentrations of auxins and cytokinins alone and their combinations for the shoot proliferation, to induce rooting of the micro shoot raised from nodal segments.

MS media along with 4 different concentrations of auxins were used. The most promising result for shoot proliferation was recorded at 1.0 mg/l BAP, followed by 0.5, control and 2.0 mg/l BAP, respectively. Maximum number of shoots (4.4±0.88) per explant, longest shoot (5.7±0.22cm), were recorded in MS media supplemented with 1.0 mg/l BAP. Whereas, control treatment showed the least response for shoot proliferation. The best response ware recorded in (1.0 mg/l BAP + 0.1 mg/l IAA), followed by (2.0 mg/l BAP + 0.1 mg/l IAA) and (2.0mg/l BAP + 0.2 mg/l IAA), respectively, in all the parameters. Longest shoots (5.5±0.51cm) per explant were recorded in (1.0 mg/l BAP + 0.1 mg/l IAA). Whereas, the lowest response towards the shoot proliferation was observed in control, for all the parameters.

To induce rooting, the micro-shoots raised from nodal segments of chrysanthemum were cultured in rooting media containing different concentrations of auxins. The data showed that (½ MS + 0.2 mg/l IBA), showed the best response for rooting of micro-shoots with 11.8±0.75 roots per plantlet and 9±0.19 cm long roots, respectively. The least response was recorded in (MS +0.2 mg/l IBA) for all the parameters, while the other treatments showed intermediate results.

Conclusion:

Regeneration of chrysanthemum plantlets through *in vitro* culture was obtained by using nodal segment explants. These explants were treated with different concentrations of auxins (IAA, IBA) and cytokinins (BAP) alone or in combinations. Nodal segments of chrysanthemum as the best response towards the shoot proliferation was observed when the nodal segments of chrysanthemum were cultured in MS media with 1.0 mg/l BAP as compared with all the other hormonal concentrations when used alone respectively. Half strength MS media supplemented with0.2 mg/l IBA was the best rooting media, as it excelled all the other treatments for all the rooting parameters.

Photogallary:

A: Explant Preparation

B: Initiation stage (2nd week)

C: Multiplication stage (2nd week)

D: Multiplication stage (5th week)

E: Rooting stage.(5th week)

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