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Effect of BAP with IAA growth hormones on *In vitro* regeneration in chrysanthemum (*Dendranthema grandiflora* T.) cv. Marigold

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Abstract
An efficient protocol for *in vitro* regeneration of Chrysanthemum (*Dendranthema grandiflora* T.) cv. Marigold was developed. In the present study shoot, tips and nodal segments were used as explants. Nodal explants responded better when compared to shoot tips. Explants were cultured on Murashige and Skoog (MS) medium with different concentrations of 6-benzylaminopurine (BAP) with Indole acetic acid (IAA). MS medium supplemented with BAP 1.0 mg L^{-1} + IAA 0.1 mg L^{-1} gave early shoot proliferation at 11.00 days after culture, maximum number of shoots per explant of 4.40, 8.01 and 10.36 and maximum shoot length of 3.43, 5.51. and 6.24 cm were recorded at 30, 60 and 90 days after shoot initiation. Satisfactory rooting response was obtained in half strength MS medium supplemented with 0.2 mg L^{-1} indole butyric acid (IBA) took as early as 5.20 days after culture, and produced maximum number of roots per. Shoots of 4.49, 6.10 and 8.21 and root length of 3.85, 5.54 and 6.42 cm at 15, 30 and 45 days after root initiation.

Keywords: Chrysanthemum, *In vitro*, shoot tips, nodal segment, cytokinin, auxin

Introduction
Chrysanthemum (*Dendranthema grandiflora* T.) belongs to family Asteraceae and it is a second most important flower crop after rose, globally it has a high value (Su *et al*., 2019) [8]. It is mainly grown in the southern part of the country and supplying loose flowers to the market for making garlands, decoration and for worship purpose. It is mainly grown in the states of Bihar, Gujarat, Karnataka (Bengaluru, Kolar, Hassan, Tumkur etc.) Madhya Pradesh, Maharashtra, Rajasthan and Tamil Nadu. On account of its origin and commercial production in Asia it is called as, Queen of East or Glory of East and sometimes winter queen as the flowers are available during winter. In chrysanthemum, the Marigold is an introduced cultivar which is very popular in Southern India and is being cultivated by the local farmers. This cultivar has high demand due to its bright yellow colour, ray florets orientation and especially high shelf life alongside with high rate of production (Ghosh *et al*., 2018) [1]. Availability of planting material through conventional cultivation of this crop is very less. Therefore it is difficult to mitigate the gradual increase in demand of quality planting material through vegetative means of propagation, which is time consuming, low multiplication rate and availability of planting material depends on season. To avoid this, micro propagation is a boon in such cases to augment the multiplication rate faster. *In vitro* culture is a greatly acceptable and dependable method of propagation for chrysanthemum (*Dendranthemum grandiflora* T.).

Material and Methods
The investigation was carried out at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru-560065, during 2019-20.

Selection of Explants
Chrysanthemum plants were maintained in the greenhouse at Plant Tissue Culture Laboratory, Department of Horticulture, GKVK, Bangalore. The explants were collected from 5 to 6 months old plants. Approximately 1 cm of shoot tip and nodal cuttings of 1 to 1.5cm with 1 or 2 nodes was isolated for culturing in the prepared medium.

Sterilization
Explants were rinsed in running tap water for 20 minutes and washed with soap solution for 5 minutes and then transferred to 100 to 200 ml of distilled water.

The separated nodes and shoot tips were washed with sterile water for 2 times with 3 minutes per wash, then the explants were kept in antiphenol solution (ascorbic acid + citric acid solution) for 45 minutes. After this, the explants were transferred to 0.2 per cent (w/v) Bavistin solution with 1 to 2 drops (v/v) of wetting agent Tween 20 (10 minutes for nodes and 5 minutes for shoot tips). The explants were given 3 sterile water wash (5 minutes per wash). After this, the explants were surface sterilized with 0.1 per cent (v/v) Mercuric chloride 2 to 3 minutes. Then explants were washed 3 times with sterile water for 5 minutes in each wash. These explants were transferred to 0.2 per cent (w/v) streptomycin solution for a period of 5 minutes.

Shooting

The explants were placed on medium consisting of MS salts (Murashige and Skoog, 1962) [7] supplemented with 3 per cent (w/v) sucrose and the media were solidified by 0.6 per cent agar having different concentrations of BAP (0.5, 1.0, 1.5, and 2.0 mg L^{-1}) and IAA (0.1, 0.2, 0.3 and 0.4 mg L^{-1}) combinations. The culture bottles were kept in growth room having temperature of 24 ± 2 ^{0}C. Light intensity of 2000 lux was provided using white fluorescence tubes for eight hours of light and 16 hours dark period.

The data was recorded for different parameters *viz.*, number of days for shoot initiation (number of days from the date of the culturing to the date of emergence of shoot), number of shoots per explants (average number of shoot produced per explant was recorded on 30, 60, 90 days interval after shoot initiation) and length of shoots (Length of the shoot was recorded on 30, 60, 90 days interval after shoot initiation and expressed as centimeter).

Rooting

The regenerated shoots were placed in the half strength MS medium supplemented with IBA and IAA both at a concentration of 0.1, 0.2, 0.3, 0.4 and 0.5 mgL^{-1} to induce roots. The data was recorded for different parameters *viz*., number of days for root initiation (number of days from the date of the culturing to the date of emergence of root), number of roots per shoot (average number of root produced per shoot was recorded on 15, 30, 45 days interval after root initiation) and length of roots (Length of the root was recorded on 15, 30, 45 days interval after root initiation and expressed as centimeter).

Statistical analysis

The experiments were laid out in completely randomized design (CRD). In all the experiments ten replications were taken to record the data. The complete data was analyzed using SPSS software.

Results and Discussion

The shoot tip explants did not responded positively when they were cultured on MS medium without any growth regulator and also on MS medium with growth regulators. The shoot tips turned brown and remained as same. They did not produce any further growth even after 7 weeks of culture.

Effect of different concentration of BAP with IAA on shoot induction from nodal segment

The effects of different concentration and in combination of BAP and IAA on number of shoots per explant were presented in the Table 1. Addition of cytokinin in combination with auxin in the.MS medium increased the shoot growth. There was a significant difference noticed between the combinations of BAP and IAA on number of days for shoot initiation, number of shoots per explant and shoot length. The MS medium with BAP 1.0 mgL^{-1} + IAA 0.1 mgL^{-1} found as the better medium. It gave early shoot initiation (11.00 days) as compared to all other combinations. Maximum number of shoots per explant (4.41, 8.01 and 10.36) and maximum shoot length (3.43, 5.51. and 6.24 cm) was observed in the same medium at 30, 60 and 90 DAS respectively. BAP 2.0 mgL^{-1} + IAA 0.1 mgL^{-1} was on par with BAP 1.0 mgL^{-1} + IAA 0.1 mgL^{-1} as it showed shoot initiation in 12.20 days, produced 4.34, 7.72 and 10.14 number of shoots per explant 30, 60 and 90 DAS respectively and 3.36, 5.35 and 5.94 cm shoot length at 30, 60 and 90 DAS respectively.

30 DAS 60 DAS 90 DAS

Plate 1: Shoot proliferation from nodes in MS medium contains BAP 1.0 mgL^{-1} with IAA 0.1 mgL^{-1} at a) 30 DAS b) 60 DAS and c) 90 DAS

Table 1: Effect of different concentration of BAP with IAA on shoot induction from nodal segment

Treatments	Days for shoot initiation	Number of shoots per explant			Shoot length		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	24.20	1.07	2.09	3.57	0.90	1.26	2.16
T_2	16.10	3.34	6.40	7.20	1.78	3.57	4.61
T_3	16.40	3.24	6.14	6.82	1.56	3.16	4.45
T_4	20.30	1.90	3.96	4.77	0.98	1.87	3.61
T_5	22.20	1.55	3.35	4.43	0.98	1.79	2.70
T_6	11.00	4.41	8.01	10.36	3.43	5.51	6.24
T_7	13.10	4.20	7.66	9.68	3.17	5.11	6.03

T_8	15.50	3.73	6.80	7.82	2.22	4.39	5.23
T_9	15.60	3.55	6.56	7.56	1.89	3.74	4.78
T_{10}	14.10	3.92	7.22	8.47	2.44	4.73	5.55
T_{11}	15.10	3.83	7.00	8.12	2.27	4.30	5.37
T_{12}	18.10	3.11	5.83	6.57	1.23	2.77	4.36
T_{13}	18.30	2.92	5.65	6.35	1.20	2.55	4.24
T_{14}	12.20	4.34	7.72	10.14	3.36	5.35	5.94
T_{15}	13.60	4.10	7.34	9.25	2.96	5.07	5.85
T_{16}	18.90	2.58	5.11	5.83	1.18	2.38	4.09
T_{17}	20.10	2.36	4.74	5.56	1.08	2.09	3.81
F-test	*	*	*	*	*	*	*
S.Em±	0.407	0.123	0.105	0.103	0.092	0.107	0.095
CD (1%)	1.500	0.455	0.386	0.381	0.338	0.395	0.315

*Significant at 1% ; S.Em - Standard Error of Mean; DAS - Days after shoot initiation ; CD - Critical difference ; T_1 - Basal medium (Control) ; T_2 - BAP 0.5 mgL^{-1} + IAA 0.1 mgL^{-1}; T_3 - BAP 0.5 mgL^{-1} + IAA 0.2 mgL^{-1}; T_4 - BAP 0.5 mgL^{-1} + IAA 0.3 mgL^{-1}; T_5 - BAP 0.5 mgL^{-1} + IAA 0.4 mgL^{-1}; T_6 - BAP 1.0 mgL^{-1} + IAA 0.1 mgL^{-1}; T_7 - BAP 1.0 mgL^{-1} + IAA 0.2 mgL^{-1} ; T_8 - BAP 1.0 mgL^{-1} + IAA 0.3 mgL^{-1}; T_9 - BAP 1.0 mgL^{-1} + IAA 0.4 mgL^{-1} ; T_{10} - BAP 1.5 mgL^{-1} + IAA 0.1mgL^{-1}; T_{11} - BAP 1.5 mgL^{-1} + IAA 0.2mgL^{-1}; T_{12} - BAP 1.5 mgL^{-1} + IAA 0.3mgL^{-1}; T_{13} - BAP 1.5 mgL^{-1} + IAA 0.4mgL^{-1}; T_{14} - BAP 2.0 mgL^{-1} + IAA 0.1mgL^{-1}; T_{15} - BAP 2.0 mgL^{-1} + IAA 0.2mgL^{-1}; T_{16} - BAP 2.0 mgL^{-1} + IAA 0.3mgL^{-1}; T_{17} - BAP 2.0 mgL^{-1} + IAA 0.4mg L^{-1}

Cytokinins along with auxins play a vital role in shoot regeneration in chrysanthemum (Karim *et al.,* 2003) [4]. This might be because of BAP belongs to cytokinin group it has a role in cell division and accelerates the development of apical meristem and it have a tendency towards shoot development and thus its influence on shoot development was not affected by lower concentrations of IAA.

These observations are in corresponding with Waseem *et al*. (2009) [9], they reported that, highest shoot length in MS medium with BAP 1.0 mgL-1 + IAA 0.1 mgL-1. Intermediate level of BAP and low level of IAA concentration was also suggested by Karim *et al*. (2003) [4]; Zafarullah *et al*. (2013) [13] and Waseem *et al*. (2011) [10, 11]. Many researchers reported the supporting results of combinations of BAP + IAA on chrysanthemum regeneration: Hoque *et al*. (1998) [3] and Yesmin *et al.* (2014) [12].

Effect of different concentration of IBA and IAA on rooting from the micro shoots regenerated from nodal segments

Effect of different concentration of IBA and IAA on root regeneration is depicted in the Table 2. Among the different concentrations of auxins (IBA and IAA) used, half strength MS medium fortified with IBA 0.2 mgL^{-1} showed minimum duration (5.20 days) for initiation of roots, production of maximum number of roots per. shoot (4.49, 6.10 and 8.21) and root length (3.85, 5.54 and 6.42 cm) at 15, 30 and 45 days after root initiation. IBA 0.1 mgL^{-1} was on par with IBA 0.2 mgL^{-1} for number of days for root initiation (5.80 days). IBA 0.2 mgL^{-1} was followed by IBA 0.1 mgL^{-1} for number of root per shoot (4.10, 5.81 and 7.16) and root length (3.45, 5.22 and 5.95cm) at 15, 30 and 45 days of root initiation respectively.

The lower concentration of IAA showed more influence on root initiation than the higher concentration. The results revealed the supremacy of IBA over IAA in root induction. This might be due to the fact that IAA photo-oxidized rapidly than IBA in tissue culture.

So IAA degraded soon after initial root induction in the rooting medium. The IBA even at lower concentration remained active for longer period, which positively affected the root length. These finding are more or less similar with earlier reports of Yesmin *et al*. (2014) who reported 12.27 number of roots with 6.65 cm of root length in IBA 0.2 mgL^{-1}. Waseem *et al*. (2009) [9] stated that, 9.00 cm root length in IBA 0.2 mgL^{-1} in 5.00 days. Several earlier workers Waseem *et al*. (2011) [10, 11]; Keresa *et al.* (2012) [5] and Khan *et al*. (1994) [6] reported maximum root length in chrysanthemum by using 0.2 mgL^{-1}IBA in half MS.

Table 2: Effect of different concentration of IBA and IAA on rooting from the micro shoots regenerated from nodal segments.

Treatments	Days for shoot initiation	Number of shoots per explant			Shoot length		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	12.10	1.09	2.24	4.13	1.26	2.64	3.52
T_2	5.80	4.10	5.81	7.16	3.45	5.22	5.95
T_3	5.20	4.49	6.10	8.21	3.85	5.54	6.42
T_4	6.90	3.11	5.04	6.31	2.54	4.07	5.11
T_5	8.60	2.05	3.20	5.31	1.82	3.18	4.36
T_6	7.50	2.99	4.61	6.13	2.28	4.02	4.96
T_7	7.70	2.64	3.77	5.97	2.08	3.93	4.75
T_8	6.40	3.41	5.28	6.35	2.77	4.56	5.23
T_9	8.30	2.35	3.56	5.61	1.91	3.84	4.57
T_{10}	8.90	1.94	2.91	4.86	1.34	3.03	4.10
T_{11}	9.10	1.45	2.74	4.88	1.53	3.53	4.28
F-test	*	*	*	*	*	*	*
S.Em±	0.281	0.084	0.075	0.065	0.047	0.050	0.048
CD (1%)	1.045	0.313	0.285	0.240	0.175	0.184	0.179

*Significant at 1% S.Em - Standard Error of Mean DAS - Days after shoot initiation CD - Critical difference
T_1 - Basal medium (Control) T_2 - IBA 0.1 mgL^{-1}
T_3- IBA 0.2 mgL^{-1} T_4-IBA 0.3 mgL^{-1}
T_5- IBA 0.4 mgL^{-1} T_6- IBA 0.5 mgL^{-1}
T_7- IAA 0.1 mgL^{-1} T_8- IAA 0.2 mgL^{-1}
T_9- IAA 0.3 mgL^{-1} T_{10}- IAA 0.4 mgL^{-1};
T_{11}- IAA 0.5 mgL^{-1}

Plate 3: Root regeneration in MS medium supplemented with IAA and IBA

Conclusion

Present study reveals that, combination of BAP 1.0 mgL^{-1} + IAA 0.1 mgL^{-1} showed better response for shoot regeneration and the IBA 0.2 mgL^{-1} was better for root regeneration in the production of chrysanthemum plants through *in vitro* culture. The established protocol further utilized for production of virus free, true to type plants and for large scale multiplication of desired types to fulfill the demand of quality planting material of Chrysanthemum (*Dendranthema grandiflora* T.) cv. Marigold.

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