

To Standardize the Optimum Concentration of Auxins (NAA and IBA) for Better Rooting of Shoots Under *In Vitro* Conditions

T Navya swetha^{1*}, A Girwani², A Manohar Rao³ and P Saidaiah⁴

¹Floriculture and Landscaping Architecture, Sri Konda Laxman Telangana State Horticultural University, Budwel, Hyderabad, Telangana

²Floriculture and Research Station, Sri Konda Laxman Telangana State Horticultural University, Budwel, Hyderabad, Telangana

³Department of Horticulture, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana

⁴Genetics and Plant Breeding, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Budwel, Hyderabad, Telangana

***Corresponding Author:** T Navya swetha, Floriculture and Landscaping Architecture, Sri Konda Laxman Telangana State Horticultural University, Budwel, Hyderabad, Telangana.

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Abstract

The present investigation was carried out at "AGRI-BIOTECH FOUNDATION" in PJTSAU campus and College of Horticulture, Rajendranagar, Hyderabad during the year 2015 - 2016. The present study was conducted under four experiments for regeneration of capitulum explants in Murashige and Skoog medium supplemented with different hormonal concentrations with an objective to determine the best concentration of growth regulators for culture establishment, shoots multiplication, rooting of in vitro shoots and further to standardize suitable hardening media for establishment of gerbera plants. In the third experiment for standardization of basal media for in vitro rooting, seven treatments were formulated with MS basal medium (control), MS medium + IBA, MS medium + NAA at different concentrations. Highest per cent rooting (83.33%), earlier rooting (19.00) with maximum root length (54.66 mm) was obtained in MS medium supplemented with 2 mg/L IBA.

Keywords: NAA; IBA; Rooting; Shoots

Introduction

Two major developments which made shoot culture feasible were the development of improved media for plant tissue culture [1] and discovery of the cytokinins as a class of growth regulators [2] and Skoog, *et al.* [3] with an ability to release lateral buds from dormancy [4]. These developments were not immediately applied to shoot culture and some years elapsed before it was appreciated that multiple shoots could be induced to form by appropriate growth regulator treatments [5]. Micropropagation work in Gerbera was initiated by shoot tip culture for rapid multiplication of elite varieties as early as 1974 by Murashige, *et al.* and in 1985 by Huang and Chu. The capitulum explants are also being used for micro propagation with the advantage of easier sterile isolation in vitro and non-destructive nature as only inflorescences are used and no shoots are lost from the plant [6].

Material and Methods

The experiment was taken up under the guidance of "AGRI BIOTECH FOUNDATION" Professor Jayashankar Telangana State Agricultural University Campus, Rajendranagar, Hyderabad. Capitulum explants of Gerbera about (1.0 - 1.5 cm) diameter were washed under running tap water there by rinsing with 0.1% tween-20 for 15 minutes and further soaked in 0.1% bavistin solution for 20 minutes and later washed with sterile distilled water for 2 - 3 times. The explants were surface sterilized with 0.1% HgCl₂ solution for about 7 minutes then finally rinsed with distilled water to remove any traces of HgCl₂. MS medium supplemented with BAP (1, 3, and 5 mg/L) individually and in combination (0.1 mg/L) of IAA, NAA respectively was used for culture establishment, the sterilized explants were inoculated in jar bottles containing media.

BAP (1, 2 and 3 mg/L) in combination with 0.1 and 0.5 mg/L NAA respectively used for multiple shoots, the regenerated capitulum transferred to the above media. IBA and NAA (0.5, 1 and 2 mg/L) used for rooting the micro shoots. A temperature of $25 \pm 20^\circ\text{C}$ and illuminated for 16 hours of light at 3000 lux and 8 hour dark per day maintaining a relative humidity of 70% consider for growth room. The data was analysed by analysis of variance test as suggested by Panse and Sukhatme (1967).

Results and Discussion

In the third experiment, seven treatments were formulated with MS basal medium (control), MS medium + IBA, MS medium + NAA at different concentrations. The micro shoots of more than 1.0 cm in height were taken out after five weeks of inoculation and placed in the jar bottles containing media and observations were recorded on number of days taken root initiation, rooting percentage, number of roots per shoot and root length. Among seven treatments, early root initiation (19 days) was observed on MS medium supplemented with 2 mg/L IBA (T_4) followed by 1 mg/L IBA (T_3) which was on par with 2 mg/L NAA (T_7) with 21.33 and 22.33 days respectively. Induction of roots at the base of *in vitro* grown shoots is essential and indispensable step to establish tissue culture derived plantlets in the soil. In the present study, MS medium supplemented with higher concentration of IBA at 2 mg/L (T_4) was found to be better for root initiation. The results are in agreement with Bhargava, *et al.* [7] who reported earlier root initiation on half strength MS medium supplemented with 2 mg/L IBA using capitulum as explant in gerbera.

The results on rooting percentage (Table 2) after five weeks of inoculation differed significantly among various treatments and revealed that 2 mg/L of IBA (T_4) has recorded significantly maximum percentage (83.33) of rooting followed by 2 mg/L NAA (T_7) and 1 mg/L IBA (T_3) with 70% and 60.00% respectively. In the present study, MS medium supplemented with higher concentration 2 mg/L IBA (T_4) found to be better for rooting percentage. This result is similar to that of Kabir, *et al.* [8] obtained maximum percent of rooting at higher concentration of IBA (2.5 mg/L BAP).

Maximum number of roots (7.66) were produced in shoots cultured on MS medium supplemented 0.5 mg/L IBA (T_2) followed by 1 mg/L IBA (T_3) which was on par with 2 mg/L IBA (T_4) with 6.00 and 5.33 number of roots respectively. In the present study, MS medium supplemented with low concentration 0.5 mg/L IBA (T_2) found to be optimum concentration for obtaining more number of roots per shoot. This results are in conformity with the report of Kharrazi, *et al.* [9] who obtained maximum number of roots per shoot on MS medium supplemented with low concentration of IBA in carnation using shoot explants.

The root length was recorded after five weeks of inoculation and differed significantly among various treatments. The results (Table 4) revealed that 2 mg/L of IBA (T_4) has recorded significantly maximum root length (54.66 mm) followed by (T_7) 2mg/L of NAA with (46.00 mm) root length. It was observed that concentration of auxin strongly influenced the quality of the root system at the end of the rooting period. In the present study, MS medium supplemented with higher concentration 2 mg/L IBA (T_4) found to be better for obtaining maximum root length. The results were similar to that of Bhargava, *et al.* [7] who reported maximum root length on half MS medium supplemented with 2 mg/L IBA and Priyakumari and Sheela [10] obtained maximum root length on MS medium supplemented with 2 mg/L IBA.

Treatments	Mean
T_1 - Control (MS medium)	68.33 (8.32)
T_2 - MS medium + 0.5mg/L IBA	23.33 (4.93)
T_3 - MS medium + 1.0mg/L IBA	21.33 (4.72)
T_4 - MS medium + 2.0mg/L IBA	19.00 (4.47)
T_5 - MS medium + 0.5mg/L NAA	25.66 (5.16)
T_6 - MS medium + 1.0mg/L NAA	24.00 (4.99)
T_7 - MS medium + 2.0mg/L NAA	22.33 (4.82)
SEm±1	0.99 (0.08)
CD @ 5%	3.03 (0.26)

Table 1: Number of days for root initiation.

The values in parentheses are Square root transformation.

Treatments	Mean
T_1 - Control (MS medium)	10.00 (18.42)
T_2 - MS medium + 0.5mg/L IBA	50.00 (44.98)
T_3 - MS medium + 1.0mg/L IBA	60.00 (50.74)
T_4 - MS medium + 2.0mg/L IBA	83.33 (66.11)
T_5 - MS medium + 0.5mg/L NAA	40.00 (39.21)
T_6 - MS medium + 1.0mg/L NAA	50.00 (44.98)
T_7 - MS medium + 2.0mg/L NAA	70.00 (56.76)
Sem ± 1	1.26 (1.02)
CD @ 5%	3.85 (3.13)

Table 2: Rooting Percentage (%).

The values in parentheses are Angular transformation.

Treatments	Mean
T ₁ - Control (MS medium)	1.00 (1.41)
T ₂ - MS medium + 0.5mg/L IBA	7.66 (2.94)
T ₃ - MS medium + 1.0mg/L IBA	6.00 (2.64)
T ₄ - MS medium + 2.0mg/L IBA	5.33 (2.51)
T ₅ - MS medium + 0.5mg/L NAA	4.33 (2.30)
T ₆ - MS medium + 1.0mg/L NAA	3.00 (2.00)
T ₇ - MS medium + 2.0mg/L NAA	2.00 (1.73)
Sem ± 1	0.30 (0.05)
CD @ 5%	0.94 (0.18)

Table 3: Number of roots per shoot.

The values in parentheses are Square root transformation.

Treatments	Mean
T ₁ - Control (MS medium)	8.33 (3.04)
T ₂ - MS medium + 0.5mg/L IBA	30.66 (5.62)
T ₃ - MS medium + 1.0mg/L IBA	36.33 (6.10)
T ₄ - MS medium + 2.0mg/L IBA	54.66 (7.46)
T ₅ - MS medium + 0.5mg/L NAA	21.00 (4.68)
T ₆ - MS medium + 1.0mg/L NAA	32.33 (5.77)
T ₇ - MS medium + 2.0mg/L NAA	46.00 (6.85)
SEm±1	1.32 (0.11)
CD @ 5%	4.04 (0.35)

Table 4: Root length (mm).

The values in parentheses are Square root transformation.

Conclusion

Under this study, the number of roots generated per shoot was recorded in shoots derived from capitulum explant of gerbera. Among the different treatments formulated MS medium supplemented with 2 mg/L IBA (T₄) recorded minimum number of days (19.00 days) for root initiation with maximum rooting percentage (83.33%) and root length (54.66 mm). While, MS medium supplemented with 0.5 mg/L IBA (T₂) produced more number of roots (7.66) per shoot. Maximum number of days for root initiation (68.33 days), minimum rooting percentage (10.00%) provided with less number of roots per shoot (1.00) and minimum root length (8.33 mm) was observed with control (T₁) MS basal medium that is devoid of hormones.

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