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Role and efficacy of rooting hormones in *in vitro* rooting of gerbera: A scientific exploration

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Abstract
In the present paper, a procedure for rooting of *in vitro* plantlets raised from capitulum culture of gerbera is described. Subsequent to *in vitro* regeneration and multiplication, the plantlets are transferred to a rooting medium, initiating an examination of their rooting behaviour and pertinent parameters. Full strength MS media supplemented with 3% sucrose, 0.4% gellan gum and 1000 ppm NAA has resulted in 99.6% of rooting. Earliest root initiation (9.20 days) and maximum root length (4.71 cm) were also recorded in treatment T_5 i.e., full strength MS media supplemented with 3% sucrose, 0.4% gellan gum and 1000 ppm NAA. After 30 days the rooted plantlets are transferred to jiffy plugs for primary hardening and acclimatisation.

Keywords: Gerbera, *in vitro* rooting, IAA, NAA, root initiation

Introduction
Gerbera, a prominent cut flower, holds the fifth position among the world's top ten cut flowers. The genus Gerbera, comprising around forty species was named in tribute to Traugott Gerber, a German naturalist who explored Russia in 1743. Of these species, Gerbera jamesonii stands as the lone cultivated variety. Belonging to the Asteraceae family, Gerbera can be propagated through sexual and asexual methods. Commercial cultivars are predominantly propagated vegetatively to maintain uniformity, genetic purity, and high-quality flower production Clump division is the prevalent technique for vegetative propagation, although cuttings can also be utilized. Nevertheless, the slow pace of multiplication via these methods hinders commercial feasibility. In modern times, the introduction of new varieties necessitates a consistent supply of planting material. Utilizing meristem and non-meristem cultures for plant propagation enables rapid and extensive production in limited space. This approach facilitates substantial expansion and ensures disease-free, robust planting material.

Conducting an experiment on *in vitro* rooting of Gerbera is essential to address the complexities and challenges associated with this crucial stage of plant propagation. Achieving successful root development is pivotal for subsequent growth and establishment of healthy plants in natural environments. By systematically investigating factors such as number of days for root initiation, number of roots per microshoot and rooting percent, the experiment aims to optimize the rooting process, improve transplant survival rates, and contribute to the advancement of micropropagation methods for Gerbera. This research enhances our understanding factors affecting *in vitro* rooting but also provides valuable insights that can benefit the horticultural industry by facilitating the production of high-quality Gerbera plants for ornamental purposes.

The research conducted by Shagufta *et al.* (2012) [13] involved transferring gerbera shoots from apical meristem and vegetative bud explants to MS medium supplemented with NAA concentrations spanning 1.0 to 10 mg/L. The optimal rooting response was recorded at the highest concentration of 10 mg/L NAA. Conversely, a decline in NAA concentration led to reduced root induction, particularly demonstrating poor outcomes at 1.0 mg/L. Son *et al.* (2011) [15] conducted a study where they isolated individual gerbera shoots from a multiple shoot complex derived from a flower bud explant. These isolated shoots were then placed on MS medium with varying concentrations of NAA (0.5, 1, and 2 mg/L). According to their findings, the medium containing 2 mg/L NAA yielded the highest results in terms of percent rooting, initiation of roots, and the number of roots.

Vijayalakshmi *et al*. (2019) [16] For root regeneration MS media supplemented with 2 mg per litre IAA was recorded as the best rooting media as regenerated roots early (10.87) with maximum number of roots (4.73) and maximum root length (3.47 cm).

Materials and Methods

The present investigation on role and efficacy of rooting hormones in *in vitro* rooting of gerbera: a scientific exploration was carried out in Plant Tissue Culture laboratory, Division of Floriculture and Medicinal Crops, ICAR-Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru during 2022-2023.

All chemicals necessary for preparing the media were of analytical grade and were obtained from Hi-media. For a carbon source, sucrose was acquired from Hi-media Limited. Throughout the research, Biojel's Gellan Gum was used as a gelling agent. The glassware utilized in the experiments, including culture bottles, tubes, beakers, pipettes, and funnels, were purchased from M/s. Borosil India Limited in Mumbai. To ensure cleanliness, the glassware was soaked in a potassium dichromate solution for six hours, followed by thorough washing with tap water to eliminate any residual dichromate. After an overnight soak in a 0.1% Teepol detergent solution, the glassware was meticulously washed with tap water and then rinsed twice with double distilled water. Subsequently, the glassware was dried at 100 °C in a hot air oven and stored securely until use.

To prepare the stock solutions of salts, the necessary chemicals were dissolved in autoclaved double distilled water. For plant growth hormones like auxins and cytokinins, stock solutions were created by dissolving them in a small amount of NaOH or HCl and then diluting with autoclaved double distilled water to achieve the final desired volume. These solutions were stored in reagent bottles and kept in a refrigerator at 4 °C for future use. The required amounts of stock solutions were combined as per requirements. Sucrose, dissolved in distilled water at a concentration of 30 g/l, was introduced into the nutrient solution, and the volume was adjusted accordingly. Growth regulators were incorporated based on the specific treatments. The pH was fine-tuned to fall between 5.7 and 5.8 using 1N HCl or 1N NaOH as necessary. Following heating, gellan gum was added at a concentration of 4g/l. The resulting medium, after boiling, was dispensed into culture bottles at a rate of 50 ml per bottle. These bottles, containing the medium, underwent autoclaving at a temperature of 121 °C and pressure of 15 pounds per square inch for 15 minutes. Subsequently, the medium was allowed to solidify by cooling.

Microshoots ranging from 1 to 3 cm in length were excised from the culture medium and subsequently transferred to Murashige and Skoog (MS) medium supplemented with varying concentrations of IAA, NAA or a combination of both IAA and NAA at a concentration of 500ppm, 1000 ppm, 1500 ppm @ 1.0 mg L-1. This medium also contained 3% (w/v) sucrose that acts as carbon source for the plantlets. Notably, three microshoots were cultured collectively within a single glass jar; all subjected to identical culture conditions as earlier detailed. Following duration of 4 weeks, several parameters were evaluated, including the number of days required for root initiation, the percentage of shoots that successfully initiated roots, the count of roots formed per individual shoot, and the length of the developed roots.

Experiment details

Design	:	Completely Randomized Design
Treatments	:	10
Replications	:	04
Plantlet per replication	:	5 *in-vitro* micro shoots

Table 1: Concentrations of rooting hormones added to full strength MS media for rooting

Treatments	IAA(ppm)	NAA(ppm)
T_0(Control)	-	-
T_1	500	-
T_2	1000	-
T_3	1500	-
T_4	-	500
T_5	-	1000
T_6	-	1500
T_7	250	250
T_8	500	500
T_9	750	750

T_0 (Control): Full strength MS media devoid of rooting hormones

The multiplied microshoots are separated into individual microshoot and the basal portion is slightly trimmed while retaining the leaves on the top. Before transferring the microshoot to rooting media the microshoots should be devoid of any residue of the media from the subculture. The data recorded was analyzed by using OPSTAT.

Results and Discussion

The findings derived from observing how diverse hormonal compositions impacted the inception of root initiation in microshoots cultured *in vitro* have been discussed below along with analysis of data summarized in Table 2

Number of days for root initiation: The outcomes extracted from Table 4.4 showcased that the supplementation of MS medium with 1000 ppm NAA facilitated an early initiation of root primordia, with a noteworthy initiation period of 9.20 days. Similar findings were observed in the case of MS medium fortified with 1000 ppm IAA, requiring 10.93 days for root initiation. In contrast, shoots cultivated on a media devoid of rooting hormones displayed a meager rooting rate (33.4%) and underwent the longest initiation duration of 22.28 days.

Number of roots per shoot: Maximum number of roots per shoot was observed in treatment T_5 (NAA @ 1000 ppm) with 4.98 shoots followed by T_2 (IAA @ 1000 ppm) treatment with 4.86 shoots. Minimum number of roots per shoot was recorded in T_0 (control) with 1.67 roots per shoot

Root length

Maximum root length was recorded in treatment T_5 (NAA @ 1000 ppm) with length of 4.71 cm followed by T_2 (IAA @ 1000 ppm) with length of 4.28 cm. Minimum root length was recorded in T_0 (control) with 0.57 cm

Rooting %

Maximum rooting percentage was recorded in treatment T_5 (NAA @ 1000ppm) with 99.6 percent and it is statistically on par with treatment T_2 (IAA @ 1000 ppm) of 97.2 percent. The minimum rooting percentage was recorded in T_0 (control) with 33.4 percent.

Table 2: Effect of different rooting hormones on initiation of roots from *in vitro* raised microshoots

Treatments	Hormones (ppm)		Number of days for root initiation	Number of roots per shoot	Root length (cm)	Rooting (%)
	IAA	NAA				
T_0 (Control)	-	-	22.28	1.67	0.57	33.4
T_1	500	-	12.07	3.59	2.52	71.80
T_2	1000	-	10.93	4.86	4.28	97.2
T_3	1500	-	13.53	3.70	2.77	74
T_4	-	500	13.87	3.06	3.36	61.20
T_5	-	1000	9.20	4.98	5.15	99.6
T_6	-	1500	12.05	3.78	2.64	75.60
T_7	250	250	12.13	3.67	3.33	73.40
T_8	500	500	11.25	4.14	4.71	82.80
T_9	750	750	13.33	4.10	3.02	82.00
S.E m+			0.70	0.36	0.23	7.29
CD @ 1%			2.05	1.06	0.65	21.66

In all treatments basal media is full strength MS media

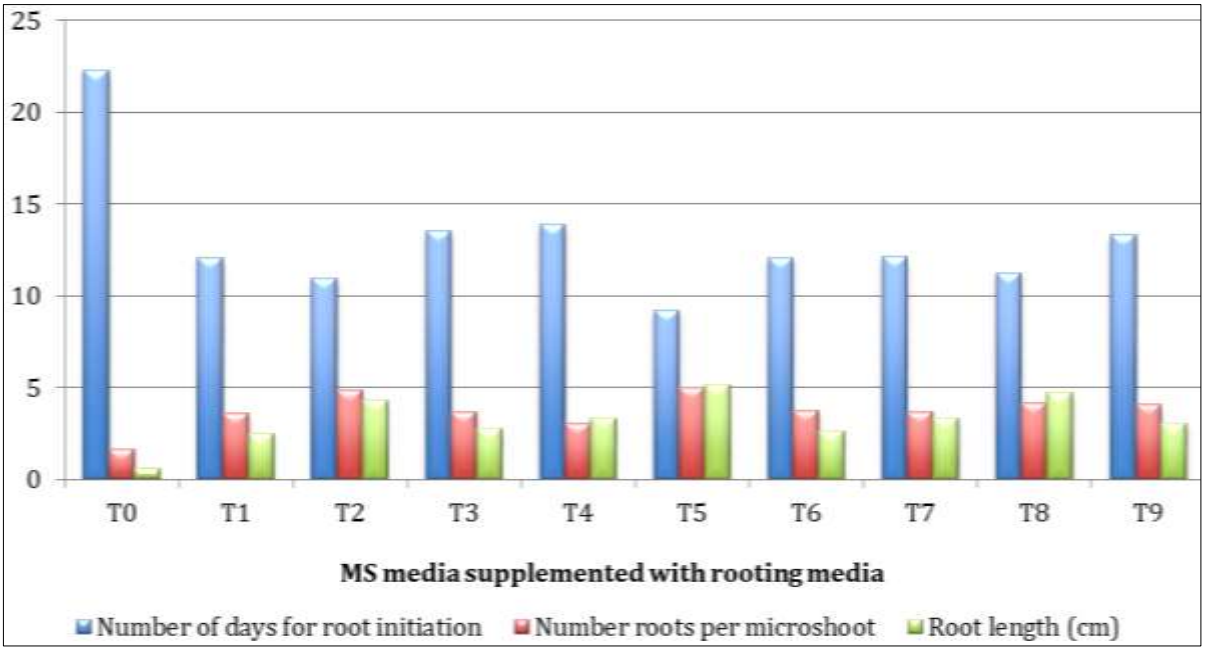

Fig 1: Graphical representation of growth regulators on *in vitro* rooting

The outcomes achieved align closely with the findings of with Naz *et al*. (2012) [9], he optimal rooting response was observed when shoots were transitioned for rooting on a MS medium supplemented with 1 mg/l NAA. Similarly, Ali *et al*. (2012) [9] observed the most favourable *in vitro* rooting response in Carnation when the MS medium was enriched with 1.0 mg/l NAA. Moreover, Nazari *et al*. (2014) [10] noted that the MS medium enriched with 0.5 mg/l and 1 mg/l NAA demonstrated the greatest root length (3.45 cm), as well as fresh (1.24 g) and dry weight (0.13 g) early rooting.

The intricate world of plant root development has witnessed a breakthrough with the role of auxins coming to the forefront. As detailed by Celenza *et al*. (1995) [2], these plant hormones play a pivotal role in provoking dedifferentiation of pericycle and parenchyma cells, marking the initiation of initial cell division processes. Building on this, Laskowski *et al*. (1995) [15] underscored the close association between heightened auxin accumulation in root tissues and a remarkable upsurge in the emergence of adventitious roots. A captivating discovery has also been made concerning the impact of auxin treatment on myelin basic protein (MBP) kinase activity. This surge in activity acts as a catalyst for activating mitogen-activated protein kinase (MAPK), thus triggers the cascade of mitotic events. Consequently, the dedifferentiation of xylematic or parenchyma cells unfolds, granting them meristematic prowess, ultimately leading to heightened cell division and, in turn, a notable increase in adventitious roots (Mockaitis and Howell, 2000) [6].

Plate 1: a) Rooting observed in T_5 treatment i.e., MS media supplemented with 1000 ppm of NAA b) Rooting observed in T_3 treatment i.e., MS media supplemented with 1000 ppm of IAA

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