The Pharma Innovation Journal 2023; 12(12): 2340-2344

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 2340-2344

www.thepharmajournal.com
Received: 15-09-2023
Accepted: 29-10-2023

KL Nikhila
P.G Scholar, Department of Floriculture and Landscape Architecture, Dr. YSR Horticultural University, College of Horticulture, Anantharajupeta, Annamayya, Andhra Pradesh, India

C Aswath
Principal Scientist and Head, Division of Flower and Medicinal crops, ICAR-IIHR, Bengaluru, Karnataka, India

PT Srinivas
Professor and Head, Department of Floriculture and Landscape Architecture, Dr. YSR Horticultural University, College of Horticulture, Anantharajupeta, Annamayya, Andhra Pradesh, India

P Nandeesha
Senior Scientist, Division of Basic Sciences, ICAR-IIHR, Bengaluru, India

Corresponding Author:
KL Nikhila
P.G Scholar, Department of Floriculture and Landscape Architecture, Dr. YSR Horticultural University, College of Horticulture, Anantharajupeta, Annamayya, Andhra Pradesh, India

Rooting dynamics of gerbera in jiffy bags: A hormonal perspective

KL Nikhila, C Aswath, PT Srinivas and P Nandeesha

Abstract
The present investigation entitled "Rooting dynamics of gerbera in jiffy bags: a hormonal perspective" was conducted at Plant Tissue Culture Laboratory, Division of Flower and Medicinal crops, ICAR-IIHR, Bengaluru in a Completely Randomized Design. During the study, capitulum is used as explant for regeneration and subsequent to *in vitro* regeneration and multiplication; the plantlets were transferred to a jiffy plugs, initiating an examination of their rooting behaviour and pertinent parameters. Jiffy plugs, a compressed cocopeat pellets with a pH around 5.3 is used a major substrate to initiate *in vivo* rooting. Treatment T_2 i.e., hormonal treatment with 1000 ppm IAA has resulted in 100% of rooting with earliest root initiation (8.80 days). After 30 days the rooted plantlets are transferred to naturally ventilated polyhouse for cultivation.

Keywords: Gerbera, *in vivo* rooting, Jiffy plugs, auxin

Introduction
The outstanding cut flower, Gerbera, ranks fifth in the world and among the top ten cut flowers (Parthasarathy and Nagaraju, 1999) [10]. The genus Gerbera, which includes approximately forty species (Das and Singh, 1989) [5], was named after Traugott Gerber, a German naturalist who explored Russia in 1743. Of these species, Gerbera jamesonii is the only cultivated variety. Gerbera, belonging to the Asteraceae family, can be propagated sexually and asexually. Commercial cultivars are mostly propagated vegetatively to maintain uniformity, genetic purity and high quality flower production. The most common vegetative propagation technique is clump division, although cuttings can also be used. However, the slow speed of multiplication using these methods hinders commercial feasibility. The use of meristem and non-meristem cultures for plant propagation allows rapid and large-scale production in limited space. Today, the introduction of new varieties requires a constant supply of planting material so that tissue culture can be used for large-scale plant production in a short time. This approach greatly facilitates expansion and ensures disease-free, resistant plant material (Aswath *et al.*, 2002; Murashige *et al.*, 1974) [1, 8].
Conducting a Gerbera rooting experiment *in vivo* is necessary to address the complexities and challenges involved in this important phase of plant propagation. Successful root development is the key to subsequent growth and establishment of healthy plants in the wild. By systematically investigating factors such as days to root formation, number of roots per micro shoot and rooting percentage, the experiment aims to optimize the rooting process, improve graft survival and advance Gerbera micro propagation methods. This study improves our understanding of the factors influencing glass root, but also provides valuable knowledge that can benefit the horticultural industry by facilitating the production of high quality Gerbera plants for ornamental purposes.

Materials and Methods
The present investigation "Rooting Dynamics of Gerbera In Jiggy Bags: A Hormonal Persepctive" 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 of the analytical-grade chemicals including sucrose, which is a source of carbon needed for media preparation, were purchased from Hi-media Limited India. Throughout the research project, Gellan Gum-which was purchased from Biojel-was utilised as a gelling agent. Glassware such as funnels, pipettes, beakers, culture bottles, and tubes were all purchased from M/s. Borosil India Limited in Mumbai and used throughout the experiment.

Glassware was soaked in potassium dichromate solution for six hours, then thoroughly cleaned with tap water to ensure that no dichromate solution remained. The glasses were then immersed in a detergent solution (Teepol 0.1%) for an additional night, and finally rinsed twice with double distilled water. After being dried at 100°C in a hot air oven, the glassware was stored away in dust-free locations until needed. In autoclaved double distilled water, the required quantity of chemicals was dissolved to create all of the salt stock solutions. In order to generate stock solutions of plant growth hormones like auxins and cytokinins, a little amount of NaOH or HCl was added, and autoclaved double distilled water was then added to make the final volume. To be used later, the solutions were preserved in reagent bottles and refrigerated at 40C. The needed amount of the stock solution was drawn, then mixed. To the nutritional solution, 30 g/l of dissolved sucrose in distilled water was added, and the volume was adjusted. Based on the treatments, the growth regulators were added. A solution of 1N HCl or 1N NaOH was added whenever necessary to bring the pH down to 5.7 to 5.8. Following a heating of the medium, 4g/l of gellan gum was added. Once it had boiled, 50 millilitres of the medium were poured into each culture bottle. After autoclaving for 15 minutes at 121 degrees Celsius and 15 pounds per square inch of pressure, the media-filled bottles were solidified by cooling.

Micro shoots ranging from 1 to 3 cm in length were excised from the culture medium and subsequently transferred to jiffy plugs and before transferring the micro shoots were given a quick dip treatment of 0.1% carbendazim followed by varying concentrations of IAA, NAA or a combination of both IAA and NAA at a concentration of 500 ppm, 1000 ppm, 1500 ppm @ 1.0 mg L-1. Following duration of 5 weeks, parameters recorded including the number of days required for root initiation, the percentage of shoots that successfully initiated roots, the number of roots formed per individual shoot, and the length of the developed roots.

- Design: Completely Randomized Design
- Treatments: 10
- Replications: 03
- Plantlet per replication:5 *in-vitro* micro shoots
- Treatment details

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

Observations recorded

- Number of roots per shoot recorded after four weeks, from three randomly selected plants, was calculated as the average of total number of truly formed roots per shoot.
- Root length (cm) was taken as the average length of the roots from three randomly selected shoots.
- Rooting percentage was calculated as the ratio of total shoots forming roots out of the total shoots cultured.
- Observations were recorded after 30 days

Statistical analysis

The above experiments were laid out in completely randomized design (CRD). The data recorded was analyzed by using OPSTAT (Sheoren *et al.*, 1998) [20]. The mean values of data were subjected to analysis of variance as described by Panse and Sukhatme (2000) [9].

Results and Discussion

The accomplishment of tissue culture's objectives relies on the smooth integration of *in vitro* generated plants with their natural habitat. In the controlled environment of *in vitro* settings, the plantlets function as heterotrophic entities. Hence, a gradual transition towards autotrophy becomes imperative. The adoption of pre-hardening and hardening protocols facilitates the adaptation of *in- vitro* nurtured plantlets to the *ex vitro* environment, as described by Beura (1998) [2] and Sapthari *et al.* (2017) [11].

In vivo rooting refers to the process of a plant developing roots naturally in its typical growing environment, such as in soil or another able substrate outside of a controlled laboratory setting. When a plant is propagated using *in vivo* rooting, it is transferred from a controlled environment (such as a tissue culture laboratory) to a natural environment where it can grow and establish itself. In this new environment, the plant develops roots from the root primordia that were initiated during the tissue culture phase or from any existing nodes on the plant.

Compressed peat forms the foundation of jiffy plugs, accompanied by lime and a specialized fertilizer featuring minimal ammonium content. This fertilizer blend serves the purpose of stimulating plant growth. Enclosed within a delicate, biodegradable nylon mesh, these pellets boast a pH level hovering around 5.3. The nylon casing facilitates uninhibited root expansion and establishes a trustworthy substrate that is prepped for storage, transit, and utilization. With each plug priced at a modest INR 1.50, jiffy plugs emerge as a cost-efficient choice for the extensive hardening of gerbera plants cultivated *in vitro*.

Table 1: Initiation of roots from micro shoots and their establishment in jiffy using different rooting hormone combination

Treatments	Hormones (ppm)		Number of days for root initiation	Number of roots per shoot	Root Length (cm)	Rooting (%)
	IAA	NAA				
T_0 (Control)	-	-	14.13	2.51	5.24	51
T_1	500	-	10.93	3.13	6.91	64
T_2	1000	-	8.80	5.00	7.73	100
T_3	1500	-	10.87	4.46	7.57	86.6
T_4	-	500	12.13	4.40	6.86	66.4
T_5	-	1000	9.93	5.00	7.38	100
T_6	-	1500	10.00	3.31	7.44	78.4
T_7	250	250	11.73	3.79	6.96	81.9
T_8	500	500	9.20	4.48	**7.77**	87.1
T_9	750	750	11.33	3.54	6.93	72.8
S.E m+			1.24	0.30	0.44	1.18
CD @ 1%			3.67	0.89	1.29	3.50

Observations were recorded 30 days after transferring to jiffy plugs

A) *In vitro* rooted plantlets transferred to jiffy plugs for primary hardening
B) Growth recorded in plantlet 30 days after transferring to jiffy
C) Development of secondary roots in primary hardened gerbera plantlet

Plate 1: Hardening in *in vivo* conditions using jiffy plugs

In this experiment, the microshoots multiplied up to two subcultures in multiplication media are transferred to *in vivo* conditions and quick dip hormone treatment was given to microshoots and tested for their rooting nature and establishment in jiffy plugs. The results obtained with respect to *in vivo* rooting are presented in the Table 1.

Number of days for root initiation

When *in vitro* multiplied plantlets are given a quick dip rooting treatment with various rooting hormones, earliest root primordia initiation was recorded in T_2 treatment at 8.80 days followed by T_5 treatment at 9.20 days. Maximum number of days taken for initiation of root primordia was in control at 14.13 days.

The use of high auxin concentrations is a common practice in plant tissue culture to induce and enhance root formation. IAA at high concentrations induces the formation of adventitious roots. Adventitious roots develop from non-root tissues, and a high concentration of IAA promotes the differentiation of cells into root primordia in areas where roots wouldn't typically form. This can lead to the early initiation of roots in plant tissues. High concentrations of IAA and NAA influence the expression of genes related to rooting. These auxins activate or suppress specific genes involved in the root development pathway, leading to the initiation and promotion of root growth in the cultured tissues.

The results obtained are similar to that of Son *et al.* (2011) [13] separated individual shoots of gerbera from a multiple shoot complex obtained from flower bud explant and cultured on MS medium containing various level of NAA (0.5, 1 and 2 mg/L) and reported that percent rooting, root initiation and number of roots were best in medium supplemented with 1 mg/L NAA.

Number of roots per shoot

The results described here illustrate the potential of plant regulators on number of roots per microshoot regenerated from the capitulum explant of gerbera. There was significant difference with regard to number of roots produced among treatments of IBA and NAA after 30 days of transferring to the jiffy plugs. Maximum number of roots per shoot was observed in treatment T_2 with 5 roots per shoots followed by T_5 treatment with 5 roots per shoot. Minimum number of roots per shoot was recorded in T_0 (control) with 2.51 roots per shoot.

The possible reason of how high concentration of auxin IAA and NAA at 1000 ppm respectively resulted in production of more number of roots per microshoots is high auxin concentrations often lead to the formation of callus, an undifferentiated mass of cells. As callus differentiates, the application of high auxin concentrations can guide the development of roots within the callus tissue, resulting in the formation of a root system and also Auxins at high concentrations promote cell division and elongation, particularly in the cambial and pericycle regions. This enhanced cell division leads to the formation of root initials, and subsequent elongation results in the emergence of more number of roots from the explant tissues.

Root length

Maximum root length was recorded in treatment $T_8$with length of 7.77 cm which is statistically on par with T_2 with 7.73 cm followed by T_5 with length of 7.38 cm. Minimum root length was recorded in T_0 (control) with 5.24 cm.

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, equal concentrations of IAA and NAA at 500 ppm (T_8) found to be better for obtaining maximum root length due to IAA and NAA both bind to specific auxin receptors in plant cells, triggering signal transduction pathways that regulate gene expression. Equal concentrations of these auxins may activate similar or overlapping sets of genes, influencing processes related to root development and elongation.

Rooting %

Maximum rooting percentage was recorded in treatment T_2 with 100 percent and it is statistically on par with treatment T_5 with 100 percent followed by T_8 with 87.1 percent. The minimum rooting percentage was recorded in T_0 (control) with 51 percent

The results obtained are akin to that of Bhavana *et al.* (2018) [4] when *in vitro* rooted plantlets are transferred to jiffy plugs for primary hardening. At the end of 30 days, the highest percentage (75%) of the plantlets from liquid medium were successfully hardened than solid medium derived plants (52%). After 60 days of secondary hardening, 71.3% of plants derived from the liquid medium survived and 43% of plants from solid medium were successfully transferred to field. The results also aligned with Bhargava *et al.* (2013) [3] separated micro-shoots of gerbera capitulum explant from the multiplication medium and transferred individual shoots on different concentrations of auxins, reported that earliest root induction with maximum rooting, longest roots, more number of roots with good root growth were recorded in 1 mg/L IAA.

Hardening of plantlets in jiffy plugs

Hardening is the process of conditioning the *in vitro* plantlets before acclimatization to the *ex vitro* environment. In the *in vitro* conditions, for metabolism, plants need not make sugar from CO_2 and water (unlike in *ex vitro* conditions) because sugar as an energy source is readily available in the culture medium. Also, the temperature of the *in vitro* culture medium is maintained for optimal plant growth. Even the humidity within the culture chamber is strictly controlled, unlike in outside environment where it could be very fluctuating but with acclimatization, the *in vitro* plants/plantlets should begin autotrophic metabolism. They should also get used to the harsh environment with higher temperature and lower humidity. This difference in environmental condition causes stress (high evaporation) on the plantlets and leads to their death on the nursery. Hence, hardening as a technique is very important in helping plantlets adapt to the *ex vitro* environment (Sapthari *et al.* 2017) [11].

Gellan gum-based medium was removed from the rooted plantlets by washing them with distilled water. These plantlets were dipped in 0.1% carbendazim solution for about 2-3 minutes and transplanted in poly bags containing different hardening media. The potted plants were kept inside a small glasshouse and watered regularly with sprinkler for a week. Further, they were watered twice a day with the help of a water cane. A temperature of 26 ± 2 0 C and humidity of 85 - 90% were maintained inside the glasshouse. Then they were transferred to greenhouse after two weeks for further acclimatization. Therefore, acclimatization is a crucial stage in which not only the number of shoots obtained from the

explants but also on the morphological quality and vigour of these plantlets (Martre *et al.* 2001) [7] that decides the success of micro propagation (Martinez-Estrada *et al.* 2019) [6].

Rooted plantlets were planted in jiffy plugs to study their survival and growth. During this process, survival percentage of plantlets has been recorded and presented in Table 1. The results described here illustrate the potential of hardening media on survival percentage of plantlets in capitulum explant of gerbera. The results on survival percentage of plantlets (Table 1) observed after four weeks of transferring rooted *in vitro* plantlets to hardening media differed significantly among the various treatments. Among, the ten treatments, maximum survival percentage of plantlets (97.66%) was observed in plantlets treated with IAA at 1000 pmm (T_2). However, control (T_1) devoid of rooting treatment has recorded minimum survival percentage of plantlets (22.33%) in jiffy plugs. In the present study, use of T_2 IAA at 1000 ppm found to be ideal for obtaining maximum survival percentage of plants might be due to the higher root formation helping in greater absorption of nutrients, water uptake, regulating evapotranspiration by maintaining plantlets under high humidity (80-90%) for the first 10-15 days inside glass house. The results obtained are similar to that of Aswath and Choudhary, 2002 [1] b. Agar was carefully washed from the regenerated plantlets with well-established root system before transfer to pots (15 cm) filled with a mixture of coco peat and compost (1:1, v/v). Gerbera Plantlets were maintained under high relative humidity (90%) for 3 weeks. Acclimatized plants were kept under a natural photoperiod condition at a temperature of 25±20 C. Survival rate of plantlets was almost 100 percent when plantlets after root development were transferred to plastic pots filled with coco peat, red soil, and sand in a 3:1:1 ratio.

Summary and Conclusions

In this experiment, the *in vitro* rooted plantlets are transferred to *in vivo* conditions i.e., jiffy plugs and observations were recorded for their establishment in number of roots per shoot, root length and rooting percentage were recorded in microshoots derived from capitulum explants of gerbera in *in vivo* conditions. Among the different treatments NAA @ 1000 ppm (T_5) and IAA 1000 ppm (T_2) has resulted in maximum number of roots per shoot i.e., 5 roots per microshoot and maximum rooting percentage of 100 is obtained both in 1000ppm NAA(T_5) and 1000 ppm IAA(T_2) in comparison to that of control with 51 percent in *in vivo* conditions. Root length was recorded maximum in IAA + NAA @ 1000 ppm (T_8).

References

1. Aswath CR, Choudhary ML. Rapid plant regeneration from Gerbera (*Gerbera jamesonii Bolus*.) callus cultures. Acta Botany Croatia. 2002;61:125-134.
2. Beura S. *In vitro* multiplication, Agrobacterium mediated transformation and post-harvest handling of spikes in Gladiolus. Ph.D. Thesis. Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India; 1998.
3. Bhargava B, Dilta BS, Gupta YC. Studies on micropropagation of Gerbera (*Gerbera jamesonii Bolus*). Indian Journal of Applied Research. 2013;3:8-11.
4. Bhavana GP, Satyan KB, Aswath C. Indirect somatic embryogenesis and SEM studies for *in vitro* propagation of *Anthurium* crossed lines. Bioscience Biotechnology Research Communications. 2018;11(1):31-40.
5. Das P, Singh PKS. *Gerbera*. In: Commercial Flowers (Bose TK, Yadav LP, Eds.). Naya Prakash, Calcutta; 1989. p. 601-622.
6. Martínez-Estrada E, Islas-Luna B, Pérez-Sato JA, Bello-Bello JJ. Temporary immersion improves *in vitro* multiplication and acclimatization of *Anthurium andreanum* Lind. Scientia Horticulturae. 2019;249:185-191.
7. Martre P, Lacan D, Just D, Teisson C. Physiological effects of temporary immersion on *Hevea brasiliensis* callus. Plant Cell, Tissue and Organ Culture. 2001;67(1):25-35.
8. Murashige T, Serpa M, Jones JB. Clonal multiplication of *Gerbera* through tissue culture. Horticultural Science. 1974;9(3):175-180.
9. Panse VG, Sukhatme PV. Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research, New Delhi, India; 2000.
10. Parthasarathy VA, Nagaraju V. *In vitro* propagation in *Gerbera jamesonii Bolus*. Indian Journal of Horticulture. 1999;56:82-85.
11. Sapthari RT, Sinta MM, Budiani A. *In vitro* propagation of *Anthurium andraeanum* cv. Nitta through organogenesis. Agrivita, Journal of Agricultural Science. 2017;39(2):192-200.
12. Sheoran OP, Tonk DS, Kaushik LS, Hasija RC, Pannu RS. Statistical software package for agricultural research workers. In: Recent Advances in Information Theory, Statistics and Computer Application (Hooda DS, Hasija RC, Eds.). Department of Mathematics Statistics, CCS HAU, Hisar; 1998. p. 139-143.
13. Son NV, Mokashi AN, Hegde RV, Patil VS, Lingaraju S. Response of Gerbera (*Gerbera jamesonii Bolus*) varieties to micropropagation. Karnataka Journal of Agriculture Science. 2011;24:354-357.