IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

INDUCTION OF MULTIPLE SHOOTS IN *GERBERA JAMESONII* USING ZrO_2 NANOPARTICLES

Mohammed Farooq†, Javeria Uzma1†, Thatikayala Dayakar and Praveen Mamidala*
Department of Biotechnology, Telangana University, Nizamabad, Telangana, India-503322
†Contributed equally

Abstract:
In the field of agriculture and plant development against biotic and abiotic stress, nanoparticles have become exceptional tools. Nevertheless, little research has been done on how they can enhance multiplication of shoots during in vitro regeneration of explants. In the current study, ZrO_2 nanoparticles were produced, characterized, and applied to induce *in vitro* multiple shoots in *Gerbera jamesonii*. The effects of Zirconium dioxide nanoparticles (ZrO_2 NPs) in three concentrations (1.0, 5.0 and 10.0 mg/L) in combination with MS medium were investigated to unveil their role in induction of multiple shoots under *in vitro conditions*. Explants from 3-day-old capitulum yielded maximum number of shoots (21.97±1.041 shoots/explant) using Murashige and Skoog (MS) basal medium supplemented with 1.0 mg/L of 6-benzyladenine (BA) and 1-naphthal acetic acid (NAA) (0.1 mg/L) and ZrO_2 nanoparticles (10 mg/L). Further studies on elucidation of ZrO_2 Nanoparticle at cellular level on *in vitro* regeneration of *Gerbera jamesonii* may better help in developing improved *in vitro* regeneration protocols several other ornamental plants.

Key words: ZrO_2 nanoparticles, *in vitro* regeneration, *Gerbera jamesonii*, multiple shoot induction.

I. Introduction:

Small matter particles with a diameter of one to one hundred nanometers are known as nanoparticles (NPs). Because of their small size, NPs have special physicochemical characteristics. These features, which are different at the micrometric scale from those of their counterparts in materials, frequently guarantee novel uses for materials that already exist (Nalci et al 2019). The NPs are thought to have the potential to be both insecticides and growth enhancers in agriculture (Landa et al 2021) On the other hand, the role of ZrO_2 NPs remains largely unexplored in plant cell and tissue culture applications. The recent findings of research on the interactions between NPs and plants are not only important from a scientific standpoint, but they can also be applied in horticultural settings to boost plant productivity and/or aid in the development of novel cultivar breeding initiatives (Tymoszuk et al 2019; 2020). Micropropagation is a clonal propagation method of plants performed under aseptic and fully controlled laboratory conditions. Inside the culture vessels, plants are grown on synthetic media that contain nutrient formulations of salts and vitamins, sugar, and growth regulator (Cavallaro et al 2022). Under these circumstances, differentiated somatic cells have the potential to dedifferentiate and then redifferentiate into shoots, roots, or embryos by chance. While somatic embryogenesis refers to the creation of adventitious (somatic) embryos, adventitious organogenesis describes the formation of adventitious shoots (caulogenesis) and roots (rhizogenesis). In tissue culture, accidental organogenesis can be generated with a very high degree of effectiveness, making it one of the fundamental techniques for vegetative plant multiplication and a helpful instrument for plant breeding (De Klerk et al 2009).

In vitro cultures have been used in modern horticulture for a number of reasons, including as the large-scale production of pathogen-free plants, the preservation of germplasm, or the synthesis of secondary metabolites (Award et al 2020) Additionally, appropriate and objective growth conditions are provided by plant cell and tissue culture systems for researching the independent effects of an elicitor or inhibitor, or other nanoparticles, on micropropagation course and efficiency (Mazaheri-Tirani et al 2020). The ZrO_2 nanoparticles are considered as a bio-safe material for plants and are used in plant production to stimulate seed germination and plant growth, as well as in plant protection for disease suppression, due to their antimicrobial activity (Singh et al 2018). Nonetheless, both positive and negative effects on the in vitro and in vivo plant growth were reported. These depended on the physicochemical properties of ZrO_2 NPs and acted in a dose-dependent manner (Singh et al 2018). The NPs are suggested to provide plants with near-exact concentrations of required.

However, determining the deficiency/sufficiency/toxicity thresholds of ZrO NPs in culture media is still under investigation (Mazaheri-Tirani et al 2020). The addition of ZnO NPs (50–150 mg·L−1) to the culture medium stimulated callus proliferation, shoot multiplication, and rooting in date palm (*Phoenix dactylifera* L.) (Award et al 2020, Al-Mayahi 2021). Their outstanding biomedical uses in dental care and drug delivery, as well as their intriguing biological characteristics, such as their anti-cancer, anti-microbial, and antioxidant activity, have further encouraged researchers to investigate their physicochemical properties using various synthetic pathways (Chitoria et al 2023). In tomato plants, foliar spraying ZrO2 NPs (0.20 g/L) resulted in the greatest increase in plant growth metrics, as well as the greatest reduction in disease indices and the highest levels of proline, chlorophyll, carotenoids, and defense enzyme activity. The ZrO_2 NPs shown antibacterial properties *in vitro*, with varying deleterious effects on the microorganisms being studied. A principal component analysis revealed that the most efficient method for raising plant growth parameters, proline, carotenoid, and chlorophyll contents, as well as the activities of defense enzymes and the highest decrease of disease indices, was foliar spraying with 0.20 gL−1 ZrO_2 NPs (Parveen et al 2022). In the current study we have explored the impact of ZrO_2 NPs in inducing multiple shoots during in vitro regeneration of *Gerbera jamesonii* cv. Bolus.

II. Materials and methods:

Flower buds (capitulum) aged one week from the plantlets of *Gerbera jamesonii* cv. Bolus, Basic variety (pink colored flower) were collected from the green house and used as explants after cutting them into appropriate size. For surface sterilization, dust particles from the capitula were removed by thoroughly cleaning them in running tap water. Following this, the explants were completely immersed in a solution containing 1.5% (w/v) Tween 20 for 10-12 min after which they were washed with distilled water (DW) for five times. Post washing, the capitula are subjected to fungicide treatment wherein 1.0% (w/v) bavistin solution, a fungicide was used to flood the explants for 15 minutes and washed five times with DW. Now the explants were shifted into sterile laminar air flow hood and treated with NaOCl (10%) (v/v) for 2min, 6min, and 9 min and then rinsed with DW for five times. Explants were then washed with 0.1% HgCl2 for different time periods (2, 3, 5 and 7 min) and rinsed 5 times in DW. The sterilized explants were allowed to dry on a sterile tissue paper and cut into pieces of approximately 1.0x1.0 cm square. These are now inoculated in culture tubes (150* 25mm) containing 15 mL of MS medium (SIM) plugged with non-absorbent cotton wrapped in layers of muslin cloth. For regeneration and organogenesis studies, Murashige and Skoog's (1962) medium (MS) were prepared with 3% sucrose and 0.8% (w/v) agar (HiMedia, India). The pH of the medium was adjusted to 5.8 with 0.1 N NaOH prior to adding agar and autoclaved at 121°C for 15 min.

The capitulum explants were inoculated on MS medium fortified with varying concentrations of indole-3- acetic acid (IAA 0.1, 0.2 and 0.5 mg/L) and Benzyladenine (BA 0.5, 1.0, 2.0 and 3.0 mg/L) individually (HiMedia, India). and with combination of BA and IAA or NAA (10.0, 5.0 and 1.0 mg/L ZrO2) MS medium without plant growth regulators (PGRs) served as control. Inoculated explants were maintained in culture room under standard growth conditions (white fluorescent light emitting 64 mE / m2 /s with 16 h photoperiod) or in dark conditions at 25 ±2 °C for 20 days. The twenty days' time duration was optimized after performing preliminary experiments for different time periods from 10 days to 30 days. Shoot initiation was observed after 20 days of explant inoculation on medium, after successful multiple shoot initiation, elongation and multiplication, elongated shoots from clumps of multiple shoots (5.0 cm) with six to seven leaves were detached and shifted to MS medium (half and full strength).

III. Results and Discussion:

Response of multiple shooting with ZrO_2 Nanoparticles:

MS media fortified for shoot regeneration medium (SRM) – 1 mg/L BA + 0.1 mg/L IAA with 10 mg/L ZrO_2 NPs yielded multiple shoots (83% response) with 21.764 ± 1.146 number of shoots and 5.96 ± 0.131 (cm) length of shoots in combination of 1 mg/L BA + 0.1 mg/L NAA SRM and 21.96 ± 1.041 number of shoots and 5.69 ± 0.068 (cm) length of shoots in combination of 1 mg/L BAP + 0.1 mg/L NAA and 10 mg/L ZrO_2 Nanoparticles.

MS basal medium with BA or NAA in high concentrations (BA 3mg/L and NAA 0.5 mg/L) did not give good number of the induction of multiple shoots only 65% of response was observed of the explants, BA at a concentration of 1mg/l along with IAA 0.5 with 5mg/L ZrO_2 nanoparticles was resulting in 25% response in (Table 1) induced multiple shoots in explants in 20 days, but number of multiple shoots was higher with 1 mg/l BA, 0.1 mg/l NAA and 10.0 mg/ml ZrO_2 Nanoparticles resulting 86% response. than with 1 mg/l BA, 0.1 mg/L IAA with10.0 mg/ml ZrO_2 Nanoparticles 83% response.

ZrO_2 NPs mg/L	BA mg/L	IAA mg/L	NAA mg/L	Percentage response (%)	No. of shoots/explants (Mean ± SE)	# Shoot length (cm) (Mean ± SE)
0	0.5	0.1	-	35	9.833 ± 0.342^{a}	0
10	0.5	0.1	-	45	10.933 ± 0.4920^{a}	3.1 ± 0.13^{a}
10	1	0.1	-	83	21.764 ± 1.146^{b}	5.69 ± 0.131^{b}
10	1	0.2	-	80	18.933 ± 0.6790^{c}	4.42 ± 0.162^{c}
5	1	0.5	-	25	9.6670 ± 0.566^{d}	3.74 ± 0.163^{cd}
5	2	0.1	-	78	19.667 ± 0.809^{e}	4.4 ± 0.144^{e}
5	2	0.2	-	72	$16.8 \pm 0 .857^{bdf}$	4.14 ± 0.0745^{bf}
1	3	0.1	-	25	6.8 ± 0.554^{g}	2.07 ± 0.13^{g}
1	3	0.2	-	20	6.067 ± 0.539^{ah}	1.93 ± 0.158^{fh}
1	0.5	-	0.1	45	14.6670 ± 0.54^{a}	4.34 ± 0.238^{a}
10	1	-	0.1	86	21.9 ± 1.041^{b}	5.69 ± 0.0686^{b}
10	1	-	0.2	73	15.8 ± 0.5620^{bc}	3.52 ± 0.162^{ac}
5	1	-	0.5	65	11.8670 ± 0.608^{cd}	3.91 ± 0.176^{d}
5	2	-	0.1	82	19.8 ± 0.712^{e}	4.56 ± 0.127^{e}
5	2	-	0.2	75	18.8 ± 0.439^{af}	5.13 ± 0.154^{bf}
1	3	-	0.1	10	9.933 ± 0.597^{g}	2.18 ± 0.152^{g}
1	3	-	0.1	20	9.067 ± 0.753^{ah}	1.93 ± 0.1610^{dh}

Table 1: *In vitro* multiplication and shoot elongation of pre-cultured *Gerbera* explants on regeneration medium (RM: MS +BA+IAA or MS + BA + NAA + ZrO_2 NPs) S-shoot R-root; values are mean ± SE of 3 replicates; different letters in each column indicates statistically significant difference ($p<0.05$) according to Holm-Sidak method as determined by one way ANOVA.

This is may be because of ZrO_2 Nanoparticles with optimum concentration of (1 mg/l BA, 0.1 mg/L IAA) are involved in metabolic adjustments to induce the multiple shoots as nanoparticles were reported to increase the nutrients uptake by plants (Hongting et al 2022) during *in vitro conditions*. Our results are in line with earlier

studies (Ibrahim 2019) wherein the application of Cu-NPs (5 µM) increased the percentage of explants producing somatic embryos (36–84%) and the average number of regenerated plantlets/explant (from 7.4 to 18.7) compared to $CuSO_4 \cdot 5H_2O$ (5 µM) (Ibrahim et al 2019; Nalci et al 2019). The ZrO2 nanoparticles were proven to play a vital role in disease resistance as well (Parveen et al 2022). A principal component analysis revealed in the recent study that the most efficient method for raising plant growth parameters, proline, carotenoid, and chlorophyll contents, as well as the activities of defense enzymes and the highest decrease of disease indices, was foliar spraying with 0.20 g/L ZrO_2 nanoparticles (Parveen et al 2022).

IV. Conclusions:

From the experiments we can conclude that the multiple shoots in high numbers was observed with the supplementation of of ZrO_2 Nanoparticles (10 mg/L) in the medium in combination with (BA)1.0 mg/L and 1.0 mg/L IAA. Further, the ZrO_2 Nanoparticles (10 mg/L) with BA (1.0 mg/L) and NAA (1.0 mg/L) was most effective to obtain high multiple shoots. Experiments are underway to evaluate the cellular mechanisms of uptake of ZrO_2 nanoparticles so as to discover the exact role of nanoparticles in inducing the multiple shoots during *in vitro* plant regeneration.

Conflict of interest: The authors have no conflicts of interest to declare.

Author Contributions: MP conceived the presented idea wherein MF, UJ and DT developed the protocols and performed the wet lab experiments. MP, MF, UJ and DT verified the biochemical/analytical methods and MP supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Acknowledgements:
We thank the Head, Department of Biotechnology, Telangana University for supporting all the facilities to carry out the tissue culture experiments during the current investigations.

References:

1. **Al-Mayahi, A.M.W** (2021). The effect of humic acid (HA) and zinc oxide nanoparticles (ZnO-NPS) on in vitro regeneration of date palm (*Phoenix dactylifera* L.) cv. Quntar. Plant Cell Tissue Organ Cult.145: 445–456.
2. **Award, K.M.; Al-Mayahi, A.M.W.; Mahdi, M.A.; Al-Asadi, A.S.M.; Abass, M.H.** (2020) In vitro assessment of ZnO nanoparticles on *Phoenix dactylifera L.* micropropagation. Sci. J. King Faisal Univ. (Basic Appl. Sci.) 21: 1441.
3. **Cavallaro, V.; Pellegrino, A.; Muleo, R.; Forgione, I** (2022). Light and plant growth regulators on in vitro proliferation. Plants 11: 844.
4. **Chitoria AK, Mir A, Shah MA** (2023). A review of ZrO_2 nanoparticles applications and recent advancements. Ceramics International. 7:7.
5. **De Klerk, G.J.** (2009). Adventitious Organogenesis In Encyclopedia of Industrial Biotechnology; Flickinger, M.C., Ed.; John Wiley & Sons, Inc.: New York, NY, USA, 2009; pp. 1–16.
6. **Ibrahim AS, Ali GA, Hassanein A, Attia AM, Marzouk ER.** (2022). Toxicity and uptake of CuO nanoparticles: evaluation of an emerging nanofertilizer on wheat (*Triticum aestivum* L.) plant. Sustainability. 14 (9):4914.
7. **Hongting J, Zhi Guo, Guodong Wang, Xin Wang, and Hongjiang Liu** (2022). "Effect of ZnO and CuO nanoparticles on the growth, nutrient absorption, and potential health risk of the seasonal vegetable *Medicago polymorpha* L." PeerJ 10: e14038.
8. **Landa, P** (2021). Positive effects of metallic nanoparticles on plants: Overview of involved mechanisms. Plant Physiol. Biochem. 161: 12-24.

9. **Mazaheri-Tirani, M.; Dayani, S** (2020). *In vitro* effect of zinc oxide nanoparticles on *Nicotiana tabacum* callus compared to ZnO micro particles and zinc sulfate ($ZnSO_4$). Plant Cell Tissue Organ Cult. 140: 279–289.
10. **Nalci, O.B.; Nadaroglu, H.; Pour, A.H.; Gungor, A.A.; Haliloglu, K** (2019). Effects of ZnO, CuO and γ-Fe3O4 nanoparticles on mature embryo culture of wheat (*Triticum aestivum* L.). Plant Cell Tissue Organ Cult.136: 269–277.
11. **Parveen, Aiman, and Zaki A. Siddiqui** (2022). "Zirconium dioxide nanoparticles affect growth, photosynthetic pigments, defense enzymes activities and mitigate severity of bacterial and fungal diseases of tomato." Gesunde Pflanzen 74 (3): 615-628.
12. **Singh, A.; Singh, N.B.; Afzal, S.; Singh, T.; Hussain, I** (2018). Zinc oxide nanoparticles: A review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants. J. Mater. Sci. 53: 185–201.
13. **Tymoszuk, A.; Miler, N** (2019). Silver and gold nanoparticles impact on in vitro adventitious organogenesis in Chrysanthemum, Gerbera and Cape Primrose. Sci. Hortic. 257: 108766.
14. **Tymoszuk, A.; Kulus, D** (2020). Silver nanoparticles induce genetic, biochemical, and phenotype variation in chrysanthemum. Plant Cell Tissue Organ Cult. 143: 331–344.