

ARTICLE

Effect of Zinc Oxide Nanoparticles on *in Vitro* Growth, Rooting and Antioxidant Enzyme Activities of two Iraqi Grapevine Cultivars

Ali A. Zainel¹, Ahmed Salahudin Bahauldin¹, Ali Abbas Ali Mostafa¹, and Suzan Salahaddin Abdulla²

¹Department of Horticulture and Landscape Design, College of Agriculture, University of Kirkuk, Kirkuk, Iraq.

² College of Veterinary Medicine, University of Kirkuk, Kirkuk, Iraq.

ABSTRACT

The present study evaluates the effects of zinc oxide nanoparticles (ZnO-NPs) on growth *in vitro*, rooting induced and antioxidant status using two cultivars of grapevine (Halwani and Kamali). The nodal explants (1.0–1.5 cm) were cultured on Murashige and Skoog (MS) media with different concentrations of ZnO-NPs (0, 25, 50 and 100 mg L⁻¹). Vegetative growth metrics, root system features (root length and fresh weight for both shoot as well as root portions), leaf chlorophyll content, and antioxidant enzyme activities were assessed six weeks after planting. Interpretation of the results through MPN indicated that ZnO-NPs affected growth and physiological parameters in a concentration dependent manner. Among the two cultivars ZnO-NP concentration (50 mg L⁻¹) produced maximum shoot multiplication rate, shoot length, number of leaves/plants, chlorophyll content, rooting efficiency as well as root formation and biomass accumulation. In Halwani, the shoot multiplication rate was particularly high at 119% compared to untreated controls. In response to the two cultivars, Halwani consistently yielded higher than Kamali in the same treatment. This suggests that higher concentration (100 mg L⁻¹ ZnO-NPs) exposure significantly increased the activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), revealing oxidative stress. Above results indicate that moderate levels of ZnO-NPs significantly improve the efficiency of micropropagation and vigor in plants, while a relatively high concentration may cause phytotoxic effects. This led to identifying 50 mg L⁻¹ ZnO-NPs as the most suitable concentration for *in vitro* propagation of grapevine cultivars evaluated. As far as we are aware, this is the first research on how nano-ZnO affects grapevine genotypes in Iraq. The data also shows the experimental feasibility of ZnO-NPs and their application in grapevine micropropagation systems.

Keywords: Grapevine; ZnO nanoparticles; Micropropagation; Rooting; Antioxidant enzymes; Oxidative stress

Corresponding Author: Ali A. Zainel

E-mail: aliznl@uokirkuk.edu.iq

Citation: Zainel, A. A., Bahauldin, A. S., Mostafa, A. A., & Ibrahim, M. K. (2026). Effect of Zinc Oxide Nanoparticles on *in Vitro* Growth, Rooting and Antioxidant Enzyme Activities of two Iraqi Grapevine Cultivars. *Misan Journal of Agricultural and Environmental Sciences*, 26(3), 50–57. <https://doi.org/10.64333/MJAE.26.3.7>

Received: 25/07/2026

Accepted: 26/09/2026

Published: 30/09/2026

DOI: <https://doi.org/10.64333/MJAE.26.3.7>

Copyright © 2026. This is an open-access article distributed under the Creative Commons Attribution License.

INTRODUCTION

Because grapevines (*Vitis vinifera* L.) may be used in a variety of industries, including fresh consumption, raisin production, juice, and wine processing, they are one of the most commercially significant fruit crops in the world. In Iraq and other Middle Eastern countries, grapevine is one of the prominent agricultural products due to its adaptability and economic return (Çakmak, 2008). Grapevine production is one of the vital parts of the horticultural industry in Iraq, especially in the northern and central provinces with many traditional cultivars such as Halwani and Kamali because this fruit has economic and nutritional value. One of the most significant fruit crops is the grape (*V. vinifera* L.), which is cultivated nearby for fresh consumption as well as traditional goods, juice processing, and raisins (dried fruits).

Although grape is an important agricultural crop, the production of grapes in Iraq is constrained by some challenges such as low productivity, stressful environment, and poor adoption of modern propagation technologies and decreased vineyard

performance (Hussein and Zail, 2025). There is an urgent need to create creative strategies that concentrate on sustainable techniques to boost grapevine propagation efficiency as well as plant vigor because recent studies have highlighted variations and reductions in grape output and cultivated areas in Iraq (FAO, 2021). Propagation of crops using conventional means is constrained due to factors such as poor multiplication rates, season dependency or transmission of pathogens. It has been accepted that plant tissue culture techniques offer an effective method, and micropropagation is a fastest way of multiplying large numbers in a short time period with considerable increase per unit (less than 6 months) along with production of pathogen free as well genetically Uniform Planting Material which could be used for further Studies (Murashige and Skoog, 1962). Zinc (Zn) is an essential element required for many physiological and biochemical processes in plants, such as auxin metabolism, protein synthesis, activator of enzymes, and chlorophyll formation (Sharma et al. Chlorosis and stunt growth, impaired root development occur due to zinc deficiency (Broadley et al., 2007).

ZnO-NPs have been considered as a distinctive microelement that can be used to enhance plants through several advancements in nanotechnology due mostly to their increased reactivity, elevated bioavailability and large surface area relative conventional fertilizers (Raliya et al., 2018). According to the majority of research, applying ZnO-NPs at the right doses might improve plant growth, nutrient uptake, and stress tolerance (Dimkpa et al., 2019). But the concentration of nanoparticles is too critical for their effects. Lower concentrations can promote plant growth by stimulation through hermetic responses, but at high concentrations they lead to phytotoxicity and oxidative stress also harmful effects such as damage of cells (Iavicoli et al., 2014). In addition to causing an excess of reactive oxygen species (ROS), nanoparticle-induced stress also activates antioxidant defense mechanisms such as peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) (Gill and Tuteja, 2010). Although there is a considerable interest in these applications in plant systems, little information is available on their effects on grapevines, especially those under *in vitro* conditions. In addition, the genotype specific response to ZnO-NPs is still exposed. ZnO-NPs' impact on Iraqi grapevine varieties *in vitro* has not yet been documented, though (Kang et al., 2024). The agricultural use of ZnO-NPs in herbaceous plant species has been the subject of several researches, but there is far less data available for woody fruit crops like grapevine, especially when cultivated *in vitro*.

Additionally, the responses of Iraqi grapevine cultivars to ZnO-NPs along with their genotypes is still unknown. As a result, there is still a significant study gap regarding the concentration-dependent effects of ZnO-NPs on morphogenesis, rooting behavior, and antioxidant responses *in vitro* grapes (Rossi et al., 2018). We have therefore hypothesized that moderate concentrations of nanoparticles would accelerate shoot proliferation, rooting performance and physiological efficiency by enhancing uptake, transfer, translocation and utilization capability for micros-nutrients along with the content regulation of antioxidants to the limit at low levels because high concentration will cause oxidative stress to suppress growth (Liu et al., 2022). So, this research studied the impacts of concentration-dependent additives by ZnO-NPs on *in vitro* morphogenesis, rooting characteristics, chlorophyll accumulation, and antioxidant defense systems in two Iraqi grapevine cultivars (Halwani and Kamali) to find an optimal nanoparticle concentration for effective micropropagation.

MATERIALS AND METHODS

Plant Material and Explant Preparation

During the active vegetative growth season (Spring, March–April 2025), explants from young, actively growing shoots exhibiting homogenous vigor and physiological performance were collected from one-year-old, healthy, disease-free mother plants of *V. vinifera* L. cultivars Halwani & Kamali that were maintained at the experimental vineyard at the College of Agriculture, University of Kirkuk, Iraq. The collected able nodal segments (1.0–1.5 cm in length). Mother plants were grown

under standard horticultural practices and selected for visual deficiency, pathogen infection, or abiotic stress symptoms to guarantee physiological uniformity among the explant source and acceptable morphogenic response potential. To get rid of dust and surface debris, the explants were rinsed three times under running water for thirty minutes. Using explants, a 30-seconds aseptic excision and surface sterilization with 70% (v/v) ethanol for 30 second and finally by 1.5% (w/v) sodium hypochlorite solution with Tween-20 (2–3 drops per 100 mL) for a time that varied from 15 minutes for nodal segments. Prior to culture, all explants were washed five times with sterile distilled water to remove any remaining sterilizing agent.

Culture Medium and Nanoparticle Treatment

The culture medium consisted of Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962). This was supplemented with 30 g L⁻¹ sucrose and 7 g L⁻¹ agar as a gelling agent. The mixture was autoclaved for 20 min at 1.04 kg cm⁻² and 121°C after adjusting pH to 5.8. To enhance the shoot multiplication and rooting, 1.0 mg of L⁻¹ 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA), were added separately to the media for multiplication and rooting respectively

ZnO Nanoparticle Characterization

Commercial zinc oxide nanoparticles (ZnO-NPs) were obtained from Sigma-Aldrich. According to the manufacturer's specifications, the nanoparticles had a nominal particle size of <50 nm and a purity of 99.9%. These specifications were used as provided by the manufacturer, and no independent physicochemical characterization of the nanoparticles was performed in the present study.

Culture Conditions

Aseptically cultured in glass test tubes (25 mm×150 mm) 15 ml of Medium Cultures were grown under fluorescent tubes with cool white bulbs at a controlled growth room, 25 ± 2 °C and photoperiod: (16/8 h light/dark) approximately 3000 lx.

Experimental Design and Statistical Analysis

ZnO-NPs concentration (0, 25, 50, and 100 mg L⁻¹) and grapevine cultivar (Halwani and Kamali) were the experimental components in a two-factor factorial experiment set up in fully randomized design (CRD) for analysis of variance. The experiments were composed of three biological replicates, each containing ten explants.

Data measured were subjected to two-way analysis of variance (ANOVA) for primary and interaction effects of cultivar and ZnO-NPs concentration, using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). The homogeneity of variance and normality assumptions were met prior to performing an ANOVA, by using the arcsine square-root transform on % data. Multiple comparisons: Duncan's multiple range test $P < 0.05$ between treatment means. All tables show mean ± SE data.

Growth and Morphological Parameters

After six weeks, post-culture measurements were made. These comprised chlorophyll content (SPAD-502 chlorophyll meter; soil plant analysis development), shoot length (cm), number of leaves per explant, and shoot dry weight (g) after oven-drying the samples at 70 °C for 48 hours.

Rooting Parameters

Data on the rooting percentage (%), number of roots per explant, longest root length (cm) and dry weight (g) of roots.

Antioxidant Enzyme Assays

Fresh leaf tissues (0.5 g) were homogenized in 50% (v/v) phosphate buffer (50 mM, pH 7.0; containing ammonium sulfate), and then centrifuged at 12,000 rpm for 15 min at 4 °C before used for the enzyme tests. The SOD activity and its specific activity were determined by the method of [Beauchamp and Fridovich \(1971\)](#); CAT activity was measured using the [Aebi \(1984\)](#), according to [Chance and Maehly \(1955\)](#). Enzyme assays were conducted on each sample, and specific activities were reported as U mg⁻¹ of protein.

RESULTS AND DISCUSSION

Vegetative Growth Responses

To evaluate this, Table 1's results demonstrate that ZnO-NPs concentration, cultivar type, and their interaction had a substantial ($P < 0.05$) impact on all of the evaluated *in vitro* growth and physiological parameters. A clear dose-dependent response trend was found in both cultivars, where low to moderate concentrations of ZnO-NPs (25 and 50 mg L⁻¹) significantly improved micropropagation efficiency, compared with the control (0 mg L⁻¹), and declined at high concentration (100 mg L⁻¹). The multiplication rate, shoot length (cm), number of leaves per plant, shoot dry weight, and chlorophyll content (SPAD units) of both cultivars were all raised by 50 mg L⁻¹ ZnO-NPs. In Halwani, the highest responses at this concentration were 4.6 (manipulation rate), 7.2 cm (shoot length), 11.4 leaves per plant, 0.42 g (shoot dry weight), and in addition to that recorded value of SPAD readings is represented by number of values equal to 44.9.

On the other hand, using higher concentrations of 100 mg L⁻¹ reduced all active traits, however they still had higher values than control for all traits compared to that determined for optimum treatment. Untreated control (0 mg L⁻¹) always showed the lower performance in both cultivars.

At the cultivar level, relative to Kamali under all ZnO-NPs treatments Halwani recorded significantly higher values indicating its superior genotypic response on growth vigor, biomass accumulation and chlorophyll content. Conclusions The results indicated that there was a highly significant cultivar × ZnO-NPs interaction and 50 mg L⁻¹ concentration was determined as the best for *in vitro* growth and physiological performance of both cultivars.

Table (1) Interactive Effect of ZnO Nanoparticles and Cultivar on *in vitro* Shoot Proliferation and Physiological Traits of Grapevine

Cultivar	ZnO-NPs (mg L ⁻¹)	Multiplication Rate	Shoot Length (cm)	Leaves per Plant	Shoot Dry Weight (g)	Chlorophyll (SPAD)
Halwani	0	2.1 d	3.4 d	5.2 d	0.18 d	28.4d
	25	3.8 b	5.9 b	8.6 b	0.31 b	36.7 b
	50	4.6 a	7.2 a	11.4 a	0.42 a	44.9 a
	100	3.2 c	4.8 c	7.3 c	0.27 c	34.1 c
Kamali	0	1.9 d	3.1 d	4.8 d	0.16 d	27.6 d
	25	3.4 b	5.4 b	8.1 b	0.29 b	35.8 b
	50	4.1 a	6.8 a	10.6 a	0.39 a	42.3 a
	100	2.9 c	4.5 c	6.9 c	0.25 c	33.4 c

Duncan's Multiple Range Test indicates that means denoted by different letters within each column are substantially different at $P \leq 0.05$.

Rooting Characteristics

Table 2 illustrates that all the rooting parameters examined such as rooting percentage, root number, root length and root dry weight were significantly influenced by ZnO-NPs concentration and cultivar type. In both cultivars showed a clear dose-dependent response, where root performance for the two ZnO-NPs low –moderate and higher levels (25–50 mg L⁻¹) was significantly better than the control (0 mg L⁻¹). On the other hand, the highest concentration (100 mg L⁻¹) tends to be

decrease. At 50 mg L⁻¹ ZnO-NPs, the rooting efficiency was highest in both cultivars (Halwani: 94%, Kamali: 89%), where Halwani performed better than Kamali with a higher number of roots per explant (Halwani: 8.4, Kamali: 7.6), longest root (Halwani: 6.9 cm, Kamali 6.2 cm) and root dry weight (0.27 g for Halwani; 0.24 g for Kamali). These results suggest that 50 mg L⁻¹ was the optimum concentration to promote root induction and development *in vitro*.

However, further increasing ZnO-NPs concentration to 100 mg L⁻¹ exhibited clear decreases in all rooting parameters in comparison with the optimal treatment (50 mg L⁻¹) for both cultivars, but still higher than control. The untreated control (0 mg L⁻¹) showed the lowest rooting performance, confirming the beneficial effect of ZnO-NPs at sufficient doses.

On the genotypic level, Halwani showed better rooting performance than Kamali for all treatments (higher rooting percentage, number and length of roots produced and root biomass). This suggests a cultivar- and ZnO-NPs-dependent response which is likely an indication of inherent genetic differences in rooting potential and physiological responsiveness.

A highly significant cultivar × ZnO-NPs interaction was detected, demonstrating genotype-dependent responses to nanoparticle application. The concentration of 50 mg L⁻¹ ZnO-NPs was consistently produced superior rooting responses, confirming its suitability as the optimum dose for *in vitro* root induction and development in both grapevine cultivars.

Table (2) ZnO nanoparticles and cultivar interaction on grapevine rooting and root development traits *in vitro*

Cultivar	ZnO-NPs (mg L ⁻¹)	Rooting (%)	Number of Roots	Longest Root (cm)	Root Dry Weight (g)
Halwani	0	62 d	3.1 d	2.4 d	0.09 d
	25	81 b	5.8 b	4.6 b	0.18 b
	50	94 a	8.4 a	6.9 a	0.27 a
	100	73 c	4.9 c	3.8 c	0.15 c
Kamali	0	58 d	2.8 d	2.1 d	0.08 d
	25	77 b	5.1 b	4.2 b	0.16 b
	50	89 a	7.6 a	6.2 a	0.24 a
	100	69 c	4.4 c	3.4 c	0.13 c

Duncan's Multiple Range Test indicates that means denoted by different letters within each column are substantially different at $P \leq 0.05$.

Compared with 0 mg L⁻¹, the addition of ZnO-NPs improved the *in vitro* growth, rooting and physiological performance of the two Iraqi grapevine varieties more significantly at 50 mg L⁻¹. The stimulatory effects at 50 mg L⁻¹ ZnO-NPs might be linked to the important responsibility of zinc biosynthesis of chlorophyll, metabolism of auxin, activation of enzymes and cell division which together drive shoot proliferation and biomass accumulation (Broadley *et al.*, 2007; Marschner, 2012). Moderate concentrations of ZnO-NP lead to similar beneficial effects on plant growth and morphogenesis in several horticultural and tissue culture studies (Dimkpa *et al.*, 2019; Raliya *et al.*, 2018; Zulfiqar *et al.*, 2019).

The increase in chlorophyll content and vegetative growth at 50 mg L⁻¹ presented a significant improvement, which indicates enhanced photosynthetic efficiency and nutrient absorption under ideal nanoparticle exposure. The high surface area and enhanced reactivity of ZnO-NPs entail a greater bioavailability and stimulate metabolic processes (Singh *et al.*, 2021). Moreover, the enhanced rooting responses noticed in this follow may be caused by some links between zinc and tryptophan biosynthesis or auxin biosynthetic pathways, which are associated with root initiation and elongation course of Tripathi *et al.* (2017).

Antioxidant Enzyme Activities

SOD, CAT, and POD activities were significantly ($P < 0.05$) impacted by ZnO-NP concentration and cultivar type, as Table 3 illustrates. As ZnO-NPs concentrations rose during treatment, all enzymatic activities in both kinds showed a consistently increasing trend, indicating dose-dependent activation of the antioxidant defense system.

In both cultivars, the enzyme activity peaked at 100 mg L⁻¹ ZnO-NPs. According to Table 2, Halwani's SOD, CAT, and POD activities were 89 U mg⁻¹ protein, 38 U mg⁻¹ protein, and 30 U mg⁻¹ protein, respectively. Kamali's SOD, CAT, and POD activities were lower but comparable to Halwani's, measuring 84 U mg⁻¹ protein, 35 U mg⁻¹, and 27 U mg⁻¹. The findings therefore imply that ZnO-NP causes a redox imbalance because, at greater doses, antioxidant defense systems are strongly activated. Conversely, the control treatment (0 mg L⁻¹) resulted in significantly reduced enzymatic activities, where Halwani had SOD, CAT, and POD of 45,18,12 U mg⁻¹ protein respectively and Kamali produced slightly lower values of these enzymes (42,16, and 11Umg⁻¹protein). All antioxidant enzyme activity gradually increased at intermediate ZnO-NP doses (25 and 50 mg L⁻¹), indicating a progressive physiological response to nanoparticle stress.

Results revealed that Halwani exhibited generally higher antioxidant enzyme activities than Kamali for all the applied ZnO-NPs treatments. The findings show a clear concentration-dependent response of antioxidant enzyme activity in the two genotypes used, as well as a significant cultivar × treatment interaction indicating that physiological differences exist between the two grape genotypes due to NPs-induced oxidative stress response.

Table (3) Effect of ZnO nanoparticles on antioxidant enzyme activities of two grapevine cultivars under *in vitro* conditions

Cultivar	ZnO-NPs (mg L ⁻¹)	SOD (U mg ⁻¹ protein)	CAT (U mg ⁻¹ protein)	POD (U mg ⁻¹ protein)
Halwani	0	45 d	18 d	12 d
	25	61 c	24 c	17 c
	50	72 b	29 b	21 b
	100	89 a	38 a	30 a
Kamali	0	42 d	16 d	11 d
	25	58 c	22 c	16 c
	50	69 b	27 b	19 b
	100	84 a	35 a	27 a

Duncan's Multiple Range Test indicates that means denoted by different letters within each column are substantially different at $P \leq 0.05$.

On the other hand, larger doses of ZnO-NPs (100 mg L⁻¹) significantly increased SOD, CAT, and POD activities while decreasing growth and rooting characteristics, indicating that the nanoparticles caused oxidative stress. Reactive oxygen species (ROS) overproduction brought on by nanoparticle overload might damage membranes and exacerbate metabolic disorders (Gill and Tuteja, 2010; Iavicoli *et al.*, 2014). Recent reports have demonstrated comparable concentration-dependent phytotoxic responses of ZnO-NPs on grapevine and other woody species grown under *in vitro* conditions (Faizan *et al.*, 2021; Latef *et al.*, 2023). The gradual increment in antioxidant enzyme activities with rising ZnO-NP concentrations shows that plant defence system has been activated to combat oxidative stress. Evidences of better SOD, CAT and POD activities against nanoparticles are ubiquitous as markers of toxicity mitigation mechanisms in cells primarily via the scavenging of ROS further assisting adaptation to stress (Hasan *et al.*, 2021; Khan *et al.*, 2022). However, we show that too high numbers of nanoparticles in the system trigger growth inhibition through excessive activation of antioxidant systems, thereby which supports the hermetic nature of nanoparticle responses.

In result, it was discovered that ZnO-NPs were more effective in stimulating growth in Halwani than Kamali, most likely as a result of the genetic diversity inherent in ZnO-NPs responsiveness between cultivars. Comparative physiological

adjustments and cultivar-specific tolerance of nanoparticle application are previously reported for other fruit crops and tissue culture systems (Rajput *et al.*, 2022). In general, the current results support the idea that moderate concentrations of ZnO-NP can serve as efficient nano-biostimulators in grapevine micropropagation, while at higher concentrations they become phytotoxic and induce oxidative stress. Hence, the accurate dosage optimization of nanoparticles is vital for safe and efficient usage in plant tissue culture systems.

CONCLUSIONS

The present study demonstrated that ZnO-NP supplementation significantly influenced grapevine growth, rooting capacity, and antioxidant responses under *in vitro* conditions, with the magnitude of the response varying according to nanoparticle concentration and cultivar. Among the concentrations evaluated, 50 mg L⁻¹ ZnO-NPs produced the most favorable overall response, significantly enhancing shoot proliferation, shoot elongation, chlorophyll accumulation, rooting efficiency, and biomass production in both grapevine cultivars. In contrast, exposure to 100 mg L⁻¹ ZnO-NPs appeared to impose oxidative stress, as indicated by the enhanced activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), suggesting a stress-induced defensive response at the higher concentration. The results further revealed that the Halwani cultivar exhibited a greater response to ZnO-NPs than Kamali, indicating that the physiological response and tolerance to nanoparticle exposure may be influenced by the genetic background of the cultivar. Overall, these findings suggest that ZnO-NPs have potential as nano-enabled biostimulants for improving grapevine micropropagation when applied at appropriate concentrations. In particular, the beneficial effects observed at 50 mg L⁻¹ highlight the importance of optimizing nanoparticle concentration to maximize morphogenic responses while minimizing potential phytotoxic effects. To the best of our knowledge, this study provides initial evidence regarding the application of ZnO-NPs to Iraqi grapevine cultivars under *in vitro* conditions and may contribute to the development of nanotechnology-assisted strategies for more efficient and sustainable grapevine micropropagation.

Disclaimer: None

Conflict of interest: The authors declare that they have no relevant financial or non-financial interest to disclose.

Funding disclosure: None

REFERENCES

- Aebi, H. (1984). Catalase *in vitro*. *Methods in Enzymology*, 105, 121–126. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
- Beauchamp, C. and Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, 44(1), 276–287. [https://doi.org/10.1016/0003-2697\(71\)90370-8](https://doi.org/10.1016/0003-2697(71)90370-8)
- Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I. and Lux, A. (2007). Zinc in plants. *New Phytologist*, 173(4), 677–702. <https://doi.org/10.1111/j.1469-8137.2007.01996.x>
- Çakmak, I. (2008). Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant and Soil*, 302(1–2), 1–17. <https://doi.org/10.1007/s11104-007-9466-7>
- Chance, B. and Maehly, A. C. (1955). Assay of catalases and peroxidases. *Methods in Enzymology*, 2, 764–775. [https://doi.org/10.1016/S0076-6879\(55\)02300-8](https://doi.org/10.1016/S0076-6879(55)02300-8)
- Dimkpa, C. O., Bindraban, P. S., Fugice, J., Agyin-Birikorang, S., Singh, U. and Hellums, D. (2019). Composite micronutrient nanoparticles and salts decrease drought stress in soybean. *Science of the Total Environment*, 688, 926–934. <https://doi.org/10.1016/j.scitotenv.2019.06.212>
- Faizan, M., Bhat, J. A., Hessini, K., Yu, F., Ahmad, P. and Yu, F. (2021). Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of plants. *Environmental Pollution*, 286, 117404. <https://doi.org/10.1016/j.envpol.2021.117404>
- Food and Agriculture Organization (FAO). (2021). *FAOSTAT statistical database*. Rome, Italy.
- Gill, S. S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12), 909–930. <https://doi.org/10.1016/j.plaphy.2010.08.016>
- Hasan, M. M., Skalicky, M., Jahan, M. S., Hossain, M. N., Anwar, Z., Nie, Z. F., Alabdallah, N. M., Brestic, M., Hejnak, V. and Fang, X. W. (2021). Nanoparticles and plant stress tolerance: Current approaches and future perspectives. *Plants*, 10(1), 26. <https://doi.org/10.3390/plants10010026>

- Hussein, S. A. and Zaili, S. A. (2025). Impact of auxin and number of buds left in cuttings for the rooting and growth of grape transplants of cv. Halwani and Kamali. *Anbar Journal of Agricultural Sciences*, 23(1), 419–431. <https://doi.org/10.32649/ajas.2025.154182.1445>
- Iavicoli, I., Fontana, L., Leso, V. And Calabrese, E. J. (2014). Hormetic dose–responses in nanotoxicology: A possible trend. *Science of the Total Environment*, 487, 361–374. <https://doi.org/10.1016/j.scitotenv.2014.03.110>
- Kang, M., Liu, Y., Weng, Y., Wang, H. and Bai, X. (2024). A critical review on the toxicity regulation and ecological risks of zinc oxide nanoparticles to plants. *Environmental Science: Nano*, 11, 14–35. <https://doi.org/10.1039/D3EN00630A>
- Khan, I., Awan, S. A., Rizwan, M., Brestic, M. and Xie, W. (2022). Role of nanoparticles in regulating plant growth and antioxidant defense systems. *Chemosphere*, 288, 132540. <https://doi.org/10.1016/j.chemosphere.2021.132540>
- Latef, A. A. H. A., Dawood, M. F. A. and Tounekti, T. (2023). Nanotechnology for improving plant growth and stress tolerance. *Plant Physiology and Biochemistry*, 194, 404–418. <https://doi.org/10.1016/j.plaphy.2022.12.021>
- Liu, R., Zhang, H. and Lal, R. (2022). Plants and rhizospheric environment: Affected by zinc oxide nanoparticles (ZnO NPs). A review. *Plant Physiology and Biochemistry*, 185, 91–100. <https://doi.org/10.1016/j.plaphy.2022.05.032>
- Marschner, H. (2012). *Marschner's Mineral Nutrition of Higher Plants* (3rd ed.). Academic Press.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Rajput, V. D., Minkina, T., Kumari, A., Singh, V. P., Verma, K. K., Mandzhieva, S. and Sushkova, S. (2022). Nanotechnology applications in plant tissue culture and crop improvement. *Plants*, 11(3), 389. <https://doi.org/10.3390/plants11030389>
- Raliya, R., Saharan, V., Dimkpa, C. and Biswas, P. (2018). Nanofertilizer for precision and sustainable agriculture: Current state and future perspectives. *Journal of Agricultural and Food Chemistry*, 66(26), 6487–6503. <https://doi.org/10.1021/acs.jafc.7b02137>
- Rossi, L., Sharifan, H., Van den Broeck, F. and Spaccini, R. (2018). Finding the conditions for the beneficial use of ZnO nanoparticles towards plants—A review. *Environmental Pollution*, 241, 1175–1181. <https://doi.org/10.1016/j.envpol.2018.06.036>
- Singh, A., Singh, N. B., Hussain, I., Singh, H. and Yadav, V. (2021). Zinc oxide nanoparticles as fertilizer for sustainable agriculture: Mechanisms and applications. *Journal of Plant Nutrition*, 44(14), 2109–2124. <https://doi.org/10.1080/01904167.2021.1871747>
- Tripathi, D. K., Singh, S., Singh, V. P., Prasad, S. M., Chauhan, D. K. and Dubey, N. K. (2017). Nanoparticles and their impact on plants. *Plant Physiology and Biochemistry*, 110, 2–12. <https://doi.org/10.1016/j.plaphy.2016.09.019>
- Zulfiqar, F., Navarro, M., Ashraf, M., Akram, N. A. and Munné-Bosch, S. (2019). Nanofertilizer use for sustainable agriculture: Advantages and limitations. *Agronomy*, 9(12), 739. <https://doi.org/10.3390/agronomy9120739>

Disclaimer/Publisher's Note: All opinions and data contained in the publications are for author(s) and contributor(s), and MJAES and its editors disclaim any responsibility for any injury to persons or property resulting from the ideas, instructions, methods, or content published therein.