Different Reactions of Olive Explants in Response to Zinc Oxide Nanoparticles and Zinc Sulfate under *in vitro* Conditions

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ABSTRACT

Olive shoots have a hard-rooting nature that causes significant problems associated with olive micro-propagation under *in vitro* conditions. Several factors may contribute to alleviating this problem, in cluding zinc and its derivatives. The current research involved the application of zinc oxide nanoparticles and zinc sulfate on olive explants 'Caillette', 'Beldi' and 'Dezfoli' cultivars. FT-IR spectroscopy, UV-Vis spectroscopy, FESEM, EDS, and DLS techniques characterized the zinc oxide nanoparticles. Then, micro cuttings containing two buds were cultured on a half-strength MS medium containing 1.5 mg L^{-1} of BAP hormone and 1 mg L^{-1} of GA3 hormone. The treatments led to olive shoots, 3 cm in length that were transferred to a 1/2 MS medium containing 3 mg L^{-1} of IBA. The olive shoots were treated with two types of zinc compounds, including zinc sulfate (0.0, 2.43, and 4.86 mg L^{-1}) and zinc oxide nanoparticles (0.0, 2.43, 4.86, and 7.29 mg L^{-1}). The results showed that zinc oxide nanoparticles induced callus growth in response to all concentrations but prevented root growth. Alternatively, zinc sulfate at all concentrations induced root and callus growth, although to a smaller extent than nanoparticles. The 'Caillette' cultivar had the highest rooting percentage and heaviest fresh and dry root weight. The 'Beldi' cultivar had the lowest of these values. Several factors potentially contributed to these results, such as hormonal (auxin biosynthesis), biochemical (enzyme and other proteins), and molecular factors (bZIP TFs), which changed by zinc application. A complementary bioinformati cs study was conducted as well.

Abbreviations: 6-Benzylaminopurine (BAP), Dynamic Light Scattering (DLS), Energy Dispersive Spectroscopy (EDS), Field-Emission Scanning Electron Microscopy (FESEM), Fourier-transform infrared spectroscopy (FT-IR spectroscopy), Gibberellic acid (GA3), Indole-3-butyric acid (IBA), half-strength Murashige and Skoog medium (1/2 MS), Transcript ion factors (TFs), Ultraviolet–visible spectroscopy (UV-Vis spectroscopy), Zinc oxide nanoparticles (Zn NPs)

Introduction

As a valuable species in the Oleaceae family, olives (*Olea europaea* L.) can be described as permanent evergreen woody trees. This plant species is one of the most important sources of healthy edible oil (Zhou et al., 2020). Despite its cultivation on a wide scale for economical fruit production, orchardists have traditionally planted olive trees on large areas of low-yielding land where other crops cannot be produced economically. This placement is due to its ability to adapt to adverse environmental conditions like drought and high temperatures (Brito et al., 2019). The most common traditional method for propagating olive trees is rooting leafy stem cuttings under a mist system. However, rooting ability varies depending on genotypes, season, and health status of stock plants and rootstocks.

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Another problem is associated with hard-to-root cultivars. Grafting is another technique for clonal propagation of olive trees, but has limitations that make it more expensive and complex because it requires nurseries with controlled environments and skilled staff. To overcome these problems, *in vitro* micropropagation stands as an alternative to facilitate the vegetative propagation of olive trees (Bayraktar et al., 2020). In tissue culture conditions, several problems may hamper the production of olive plantlets, including apical dominance, low rate of shoot growth, and difficulty in root production that requires several repeated sub-cultures. Sub-culturing olive micro shoots reportedly made them more juvenile and enhanced their rooting ability (Rugini, 1984; Binet et al., 2007). Researchers are continuously searching for different methods to speed up the production of adventitious roots in olive shoots and improve their quality. In terms of adventitious roots, different olive cultivars produce them differently (Porfírio et al., 2016). Auxins play an important role in root induction and growth of olive cuttings. Other hormones, such as abscisic acid, cytokinins, and GA3 negatively influence root induction and prevent adventitious root formation (Zhao et al., 2022). On cuttings, IAA (auxin) is reportedly responsible for adventitious root formation. Olive cuttings can also be stimulated to produce adventitious roots by endogenous and exogenous auxin. While the free auxin concentration increases at the bottom of the cutting, adventitious roots are more likely to be induced. The increase in endogenous IAA concentration in the olive 'Nabali' cultivar, from 11.54 µg g^{-1} to 48.77 µg g^{-1}, caused an increase in the rooting percentage of the cuttings from 5.7% to 55.6%. (Ayoub et al., 2006). Endogenous auxin proliferates adventitious primordial cells in roots. Proper auxin concentrations can stimulate cellular division in the vascular cambium, resulting in primordial cell formation (Haissig et al., 1972). Among the essential and trace elements for healthy growth and crop production in plants, zinc plays an important role in the biosynthesis of indole acetic acid (IAA) hormones (Castillo-González et al., 2018). Zinc is essential for the biosynthesis of the IAA hormone through involvement in tryptophan production. It is also essential in methionine synthesis, super-oxidase dismutase enzyme, and carbonic anhydrase enzyme in chloroplast activity (Hassan et al., 2020; Hsieh et al., 2013). In addition to maintaining cellular homeostasis, zinc plays another role in plants (Chevallet et al., 2017). Its use in nano form has greatly developed in recent years because of its unique properties. Unlike bulk materials, nanoparticles have different physical and chemical properties than atomic or molecular assemblies with sizes between 1-100 nm. Many countries use zinc oxide (ZnO) nanoparticles on an industrial scale in electronic, textile, pharmaceutical, cosmetics, catalysts, ceramics, sensors, and other applications (Gharbavi et al., 2023). In addition to their applications in dentistry, the gas, rubber, and oil industries, zinc-based compounds are used as fungicides and fertilizers in agriculture (Gharbavi et al., 2022). A hyper-accumulating plant like the olive can quickly absorb zinc particles and transfer them to its various organs to provide or store zinc. At high concentrations, however, they can be toxic (Al-Habahbeh et al., 2021). In addition to their chemical composition, nanoparticles can cause toxicity by releasing ions in high concentrations, causing molecular tension and stimulation caused by their surface, size, and shape (Harish et al., 2022). Zinc oxide nanoparticles have positive effects on seed germination and plant growth parameters. However, in some cases, these nanoparticles showed negative effects, probably depending on the concentration, size, and nanoparticle synthesis (Tondey et al., 2022). In this research, zinc oxide nanoparticles are studied *in vitro* for their ability to induce callus and root formation in olive micro cuttings. Before applying them to the plants, the nanoparticles were synthesized and characterized using DLS, FT-IR, UV-Vis, SEM, and EDS techniques.

Materials and methods
Synthesis of zinc oxide

After dissolving 0.2 M zinc acetate dehydrate in methanol at room temperature, ZnO nanoparticles were prepared by ultrasonically mixing this solution at 25 °C for two hours. The mixture was clear and transparent without any observable precipitate or turbidity. In the next step, we added 0.02 M of NaOH (0.1 N NaOH) to the solution and stirred it ultrasonically for 60 minutes to dissolve the NaOH. After vortexing the mixture a few times, it remained undisturbed until white precipitates accumulated at its bottom. The precipitates were filtered and washed with excess methanol after the precipitation to remove the starting material. A hot plate provided a condition for drying the precipitate for 15 minutes at 80 °C (Hasnidawani et al., 2016).

Physicochemical characterizations of ZnO nanoparticles

Several techniques enabled the characterization of the physical and chemical properties of ZnO nanoparticles. DLS (Malvern NanoZS model) involved measuring the hydrodynamic diameter of ZnO nanoparticles, their polydispersity index, and charge surface (zeta potential). Two hundred µL per sample was diluted into deionized water until the absorbance at 633 nm reached 0.09 ± 0.02 units. The chemical structure of ZnO nanoparticles was determined using FT-IR (Bruker, Tensor 27). The nanoparticles were mixed and mechanically ground with potassium bromide (KBr) at a unique weight ratio (1:10) to create pellets at 10-ton pressure. In addition, the UV-SPECORD 210 PLUS Spectrophotometer (Analytik Jena) operated between 200 and 800 nm with a spectral resolution of 1 nm. The ZnO nanoparticle size and morphology were estimated using scanning electron microscopy (SEM; MIRA TESCAN, Czech Republic). The elemental distribution of the prepared ZnO nanoparticles appeared in estimation using the EDS technique. At a scale of 100,000 magnification, we covered the samples with platinum and observed the changes at 15 kV (Gharbavi et al., 2021; Felenji et al., 2022).

Explant preparation

Microcuttings (nodal segments) were three-centimeter-long pieces of shoots. Each microcutting had two healthy buds taken from green branches of mature olive trees in the olive orchard of the Shahid Chamran University, Faculty of Agriculture, Ahvaz. Mature olive trees were from three cultivars, i.e., 'Caillette,' 'Beldi,' and 'Dezfuli' (Fig. 1). After remaining for 30 min in a mixture of citric acid and ascorbic acid, the explants were rinsed with sterile water to prevent browning. Surface disinfection involved immersing the explants in 70% alcohol for 30 seconds, followed by immersion in 2.5% sodium hypochlorite and one drop of Tween 20 per 100 mL for 5 min. Finally, the explants were washed three times with distilled water.
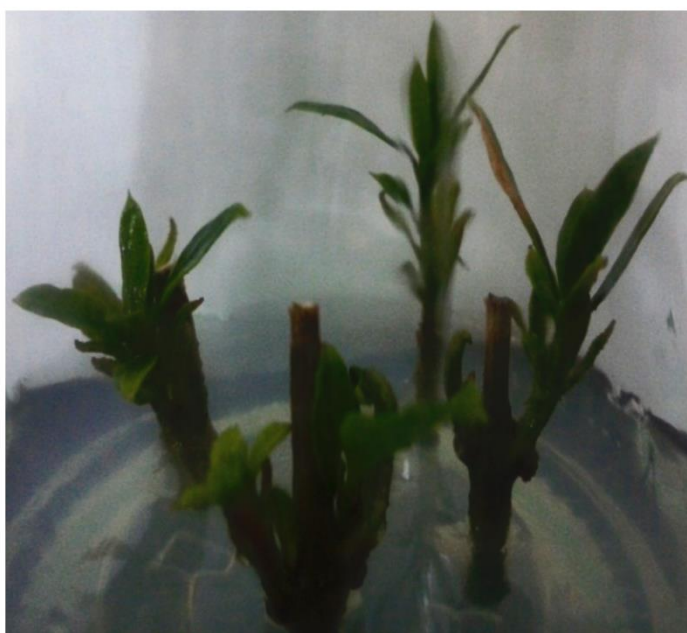
Fig. 1. (A) Initial olive explants (micro-cuttings) and (B) shoots ready for transfer to the rooting medium.

Culture Medium

Explants were cultured in a half-strength MS culture medium containing 1.5 mg of BAP hormone and 1 mg of GA3 hormone at pH 5.7. Then, they were sub-cultured in a fresh medium after four weeks. In the rooting culture medium, 1/2 MS medium was prepared with ZnO nanoparticles in three concentrations (2.43, 4.86, and 7.29 mg L^{-1}), zinc sulfate in two concentrations (43.342 and 4.86 mg L^{-1}), and without zinc (as a control sample). Each culture medium was supplemented with 3 mg L-1 of IBA hormone to enhance root growth. In each culture medium, three shoot explants of the previous stage, each 3 cm long, were cultured separately per cultivar, 'Caillette,' 'Blaidi,' and 'Dezfuli.' The experiments were conducted in the factorial arrangement using a completely randomized design with six replications (culture vessel). All culture media were measured in terms of callus formation percentage, callus fresh and dry weight, root length, root fresh and dry weights at the end of the experiment. The culture media were sterilized using an autoclave at 121°C and 15 psi pressure for 20 min. All cultures were incubated at 16 h of photoperiod and 25 °C temperature.

Statistical analysis

A statistical analysis of the data followed a normality test and involved the GraphPad Prism 8 software. The results appeared as mean values and standard deviations. Analysis of variance and Spline/LOWESS analysis had repeated measures (one/two ways) as statistical tests. IC50 values derived from generating a relevant curve. Differences were considered significant at $*p < 0.5$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

Results

Synthesis and characterization of ZnO NPs

A sol-gel method was used for synthesizing ZnO nanoparticles, characterized by various techniques to determine their physicochemical characteristics. DLS analysis facilitated the evaluation of the average hydrodynamic diameter and zeta potential. Based on Fig. 2A and B, the particles have an average hydrodynamic diameter of 243.8 nm and a zeta potential of 10.40 mV. In addition, the PDI for ZnO nanoparticles was approximately 0.233.

As observed in Fig. 3A, the FT-IR spectra revealed confirmations of ZnO NPs synthesis and indicated the number of functional groups at the NP surface. FT-IR measurements of ZnO NPs revealed different absorption peaks at 3475.32, 1587.11, 1454.21, and 720.52 cm-1. Additionally, UV-visible spectra showed that ZnO NPs, produced by the sol-gel method, had a stronger absorption band at 278 nm, indicating the fulfilment of nanoparticle synthesis (Fig. 3B).
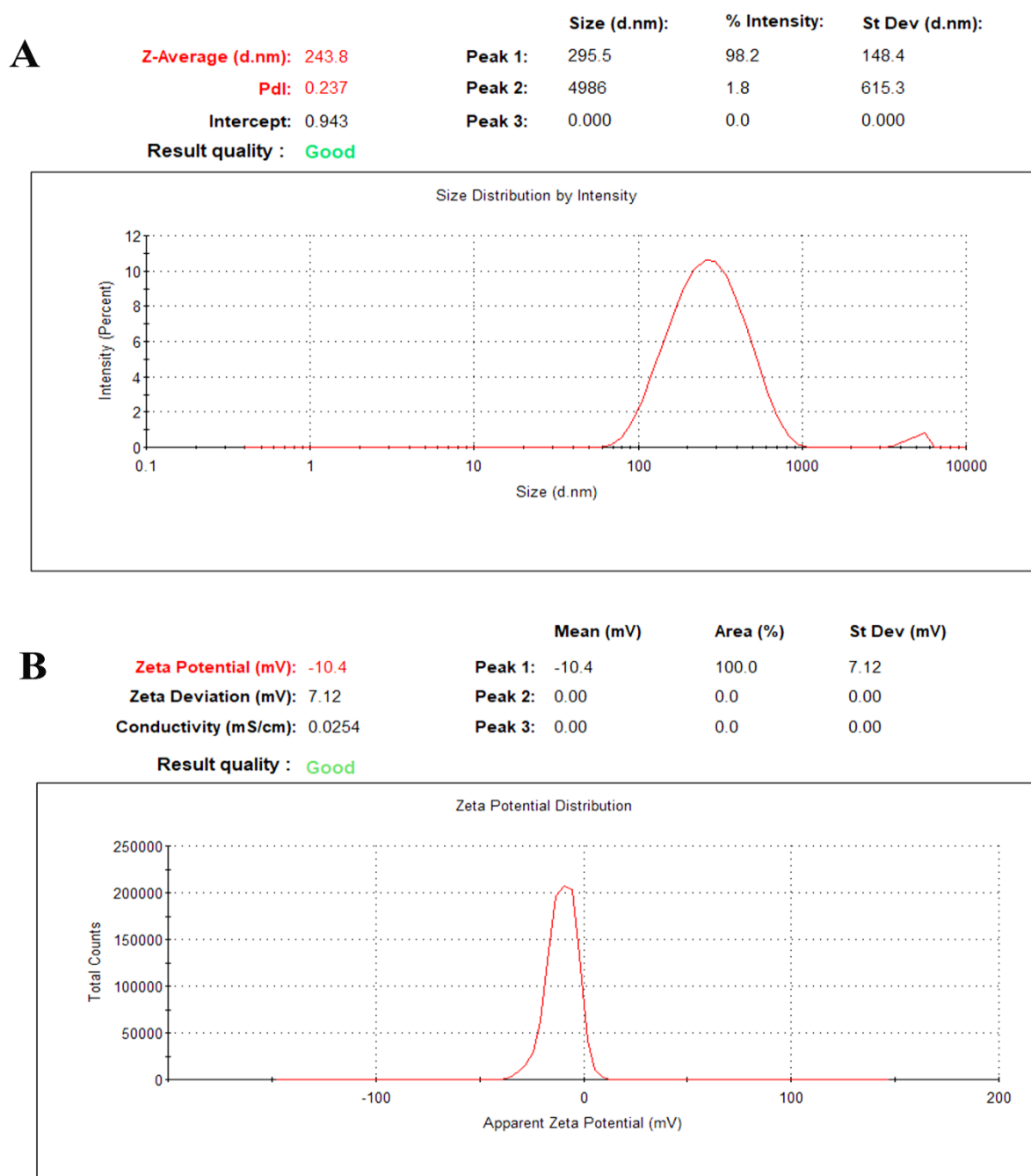
ZnO nanoparticles were synthesized by the sol-gel method and analyzed by FESEM to determine their morphology and distribution size. As observed in Fig. 4A, the particles appeared spherical, with uniform shapes and an even distribution. Similarly, the EDS profile revealed that a significant Zn signal was absorbed (78.32% Wt.) (Fig. 4B).

Callus production

The type of zinc source in the culture medium significantly affected callogenesis (P≤0.01) in the olive explants. The concentration of zinc oxide nanoparticles in the media was most effective on callus induction. All explants (100%) produced callus after exposure to the medium with different concentrations of zinc oxide nanoparticles (Fig. 5A). On the medium without zinc, the explants did not generate callus. On the medium containing zinc sulfate, the explants produced small amounts of callus (Fig. 5B).

Fresh weight & dry weight of callus

Depending on the type and concentration of zinc composition, the fresh weight and dry weight of the produced callus were significantly different. Explants cultured on media containing zinc oxide nanoparticles had the highest callus fresh weight and dry weight. With an increase in zinc oxide nanoparticle concentration, the callus fresh weight increased likewise. Lower values of fresh weight and dry weight were observed in explants grown on zinc sulfate-containing media, but callus did not form on zinc-deficient media. In addition, the average values observed from the cultivars revealed that the highest callus fresh weight and dry weight occurred in the 'Beldi' cultivar. The lowest callus fresh weight and dry weight occurred in the 'Dezfuli' cultivar (Fig. 6A and B).

A

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 243.8		Peak 1:	295.5	98.2	148.4
Pdl: 0.237		Peak 2:	4986	1.8	615.3
Intercept: 0.943		Peak 3:	0.000	0.0	0.000
Result quality : Good					

Size Distribution by Intensity

B

|			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -10.4		Peak 1:	-10.4	100.0	7.12
Zeta Deviation (mV): 7.12		Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0254		Peak 3:	0.00	0.0	0.00
Result quality : Good					

Zeta Potential Distribution

Fig. 2. Size and surface charge of SeNPs analysis by the DLS technique. (A) Hydrodynamic diameter average size (nm) of synthesized ZnO NPs, (B) Zeta-potential average (mV) of synthesized ZnO NPs.
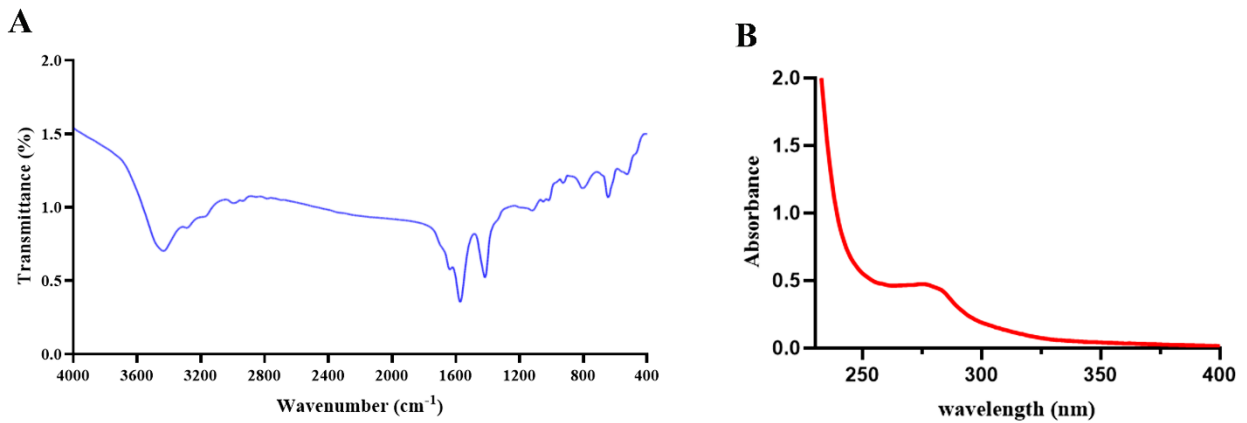
Fig. 3. Confirmation of synthesis ZnO NPs. (A) FT-IR spectrum of ZnO NPs, (B) UV-vis spectra of ZnO NPs.
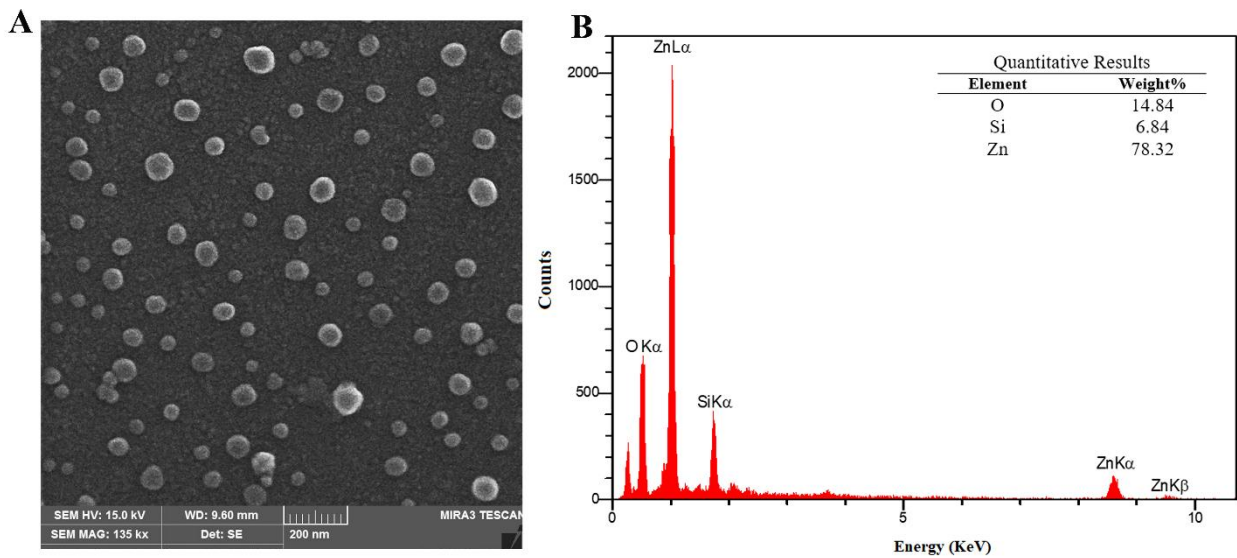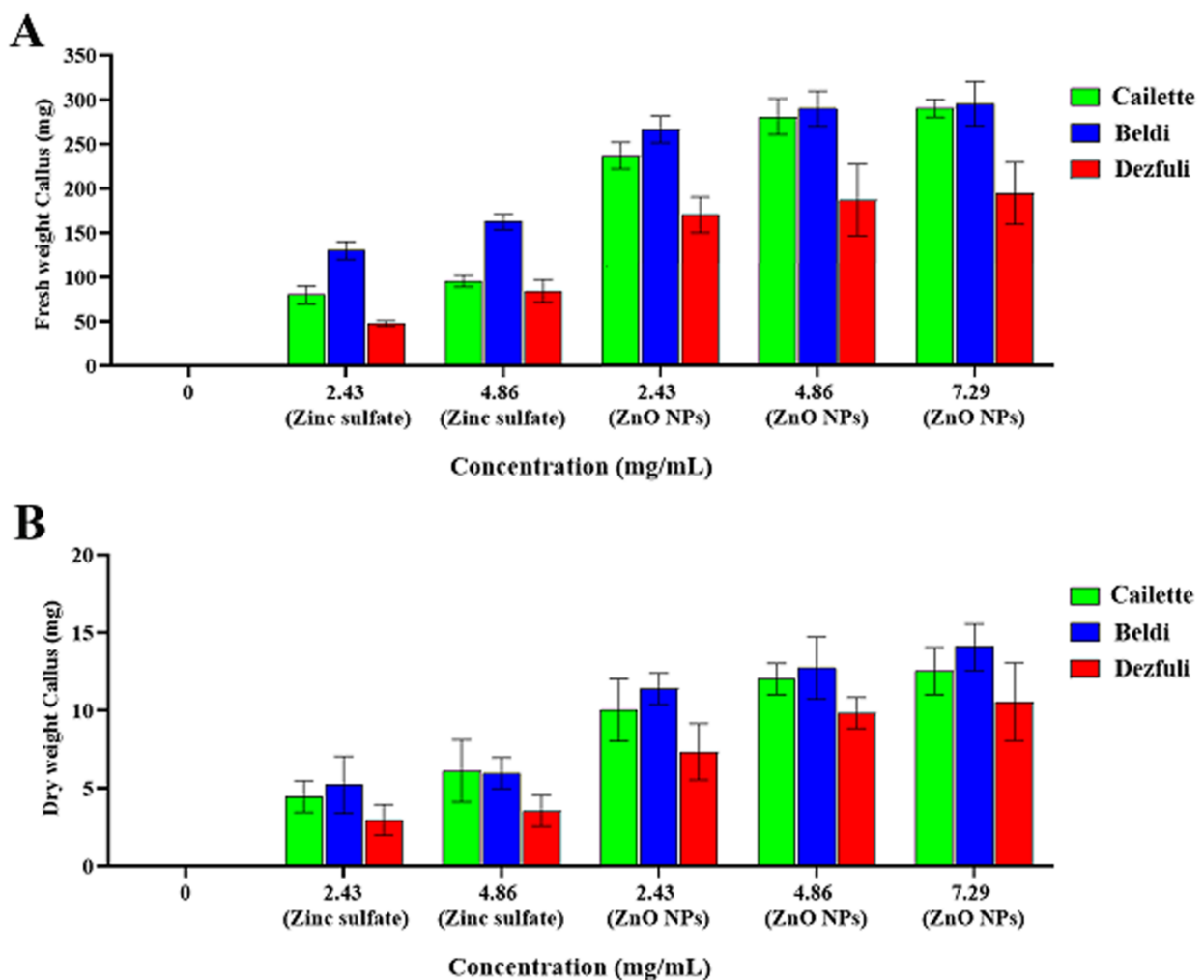

Fig. 4. Morphology and elemental distribution analysis. (A) FESEM analysis of synthesized ZnO NPs, (B) analysis of elemental distribution synthesized ZnO NPs by EDS.
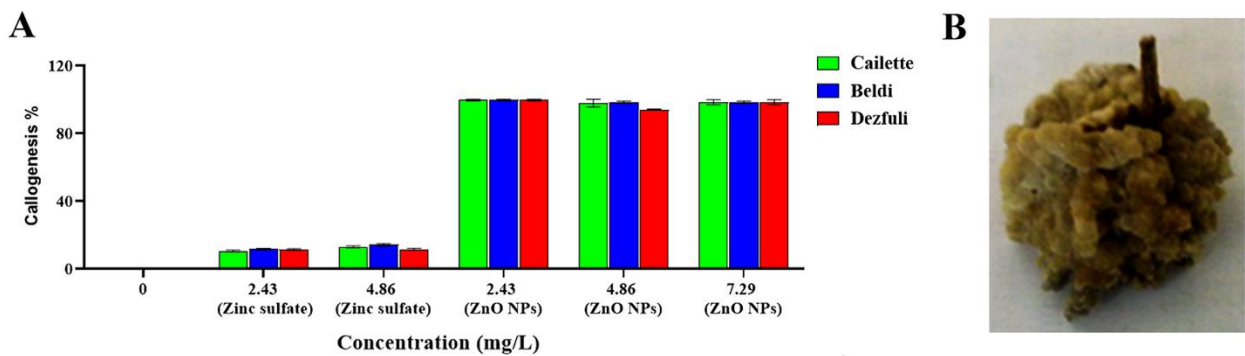
Fig. 5. (A) Callus induction in olive cultivar explants under *in vitro* conditions mediated by zinc sulfate and ZnO NPs, (B) Callus from olive micro-cuttings cultured in the ZnO NPs medium.
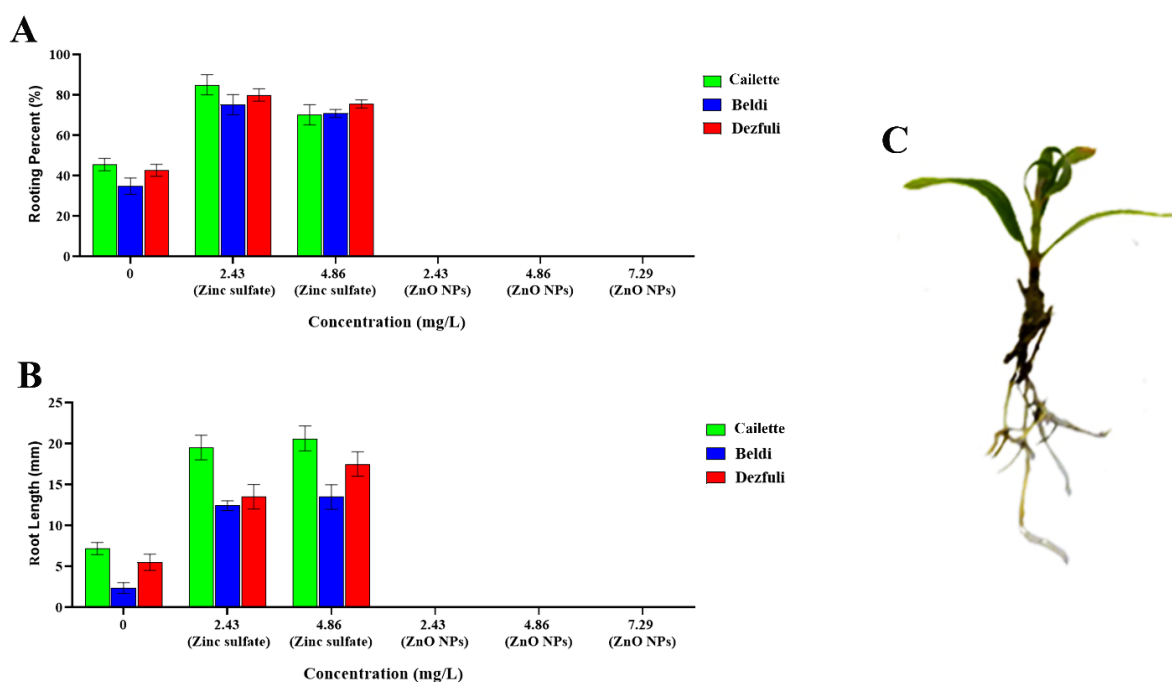
Fig. 6. *In vitro* study of the effect of ZnO NPs and zinc sulfate on (A) callus fresh and (B) dry weight of olive cultivar explants.

Rooting percentage

Based on the results, zinc oxide nanoparticles inhibited root induction in olive cultivar explants under *in vitro* conditions, unlike callus. Therefore, none of the explants grew roots when placed on various media containing zinc oxide nanoparticle concentrations. Zinc sulfate reportedly stimulated root initiation in olive explants in the meantime. Explants grown on media containing zinc sulfate (2.43 mg L-1) exhibited the highest average rooting percentage (80%), while explants grown on medium without zinc exhibited the lowest rooting percentage (41.2%). In making a comparison among the average rooting rates of the cultivars, the 'Caillette' had the highest rooting rate (68.8%), whereas 'Beldi' had the lowest rooting rate (60.2%) (Fig.7A).

Total root length

Regarding root length, the most extensive roots (17.2 mm) were obtained in the medium containing zinc sulfate at 4.86 mg L^{-1}, while the shortest roots (4.9 mm) appeared in zinc-free media. Furthermore, based on the cultivars, the highest average root length (15.8 mm) appeared in the 'Caillette' cultivar and the lowest (9.4 mm) in the 'Beldi' cultivar. However, zinc nanoparticles did not cause much root growth (Fig. 7B and C).

Fig. 7. *In vitro* study of the effect of ZnO NPs and zinc sulfate on (A) the rooting percentage and (B) root length in explants of olive cultivars. (C) Olive roots are produced in a culture medium containing zinc sulfate.

Fresh and dry roots weight

The root fresh and dry weights were similar. The highest root fresh weight (with an average of 23.3 mg) occurred in explants of the 'Caillette' cultivar, which grew on 2.43 or 4.86 mg L^{-1} sulfate. Explants of the 'Beldi' cultivar on medium without zinc exhibited the lowest fresh weight (11 mg on average), whereas the explants that grew on zinc medium had the highest fresh weight (21 mg on average). The highest root dry weight (2.3 mg) occurred in explants of the 'Caillette' cultivar, where zinc sulfate was either 2.43 or 4.86 mg L^{-1}. The lowest root dry weight (1.3 mg) occurred in explants of the 'Beldi' cultivar, which grew on zinc-free media (Fig. 8A and B).

Discussion

According to the results, ZnO nanoparticles induced good callus growth. The nanoparticles suppressed adventitious root growth at the basal end of olive explants, while zinc sulfate-induced callus growth and root growth. Since ZnO nanoparticles are highly penetrative and toxic, root production did not occur in explants treated with these particles. Several factors may affect

this, including chemical composition, size, and surface area of nanoparticles, besides the main effect of plant species (tissue sensitivity). Suspended ZnO nanoparticles reportedly inhibited ryegrass seed germination, and ZnO nanoparticles inhibited corn seed germination (Lin and Xing, 2007). However, these two types of nanoparticles did not affect radish, lettuce, turnip, and cucumber seeds. All six plant species showed a reduction in root growth after exposure to these two nanoparticles. According to other researchers, ZnO nanoparticles reduced the germination of many seed types and prevented the roots and stems from elongating. In addition to growth retardation (the main symptom of zinc toxicity), they also showed growth enhancement (El-Ghamery et al., 2003; Munzuroglu and Geckil, 2002). Several reports have shown that ZnO nanoparticles enhanced seed germination in some plants and reduced the effect of salinity stress (Adil et al., 2022; Zafar et al., 2022). The toxic effects of ZnO nanoparticles decreased by several approaches, including coating ZnO nanoparticles with two-dimensional materials and green synthesis using microemulsion (Al Jabri et al., 2022; Mukherjee et al., 2014).
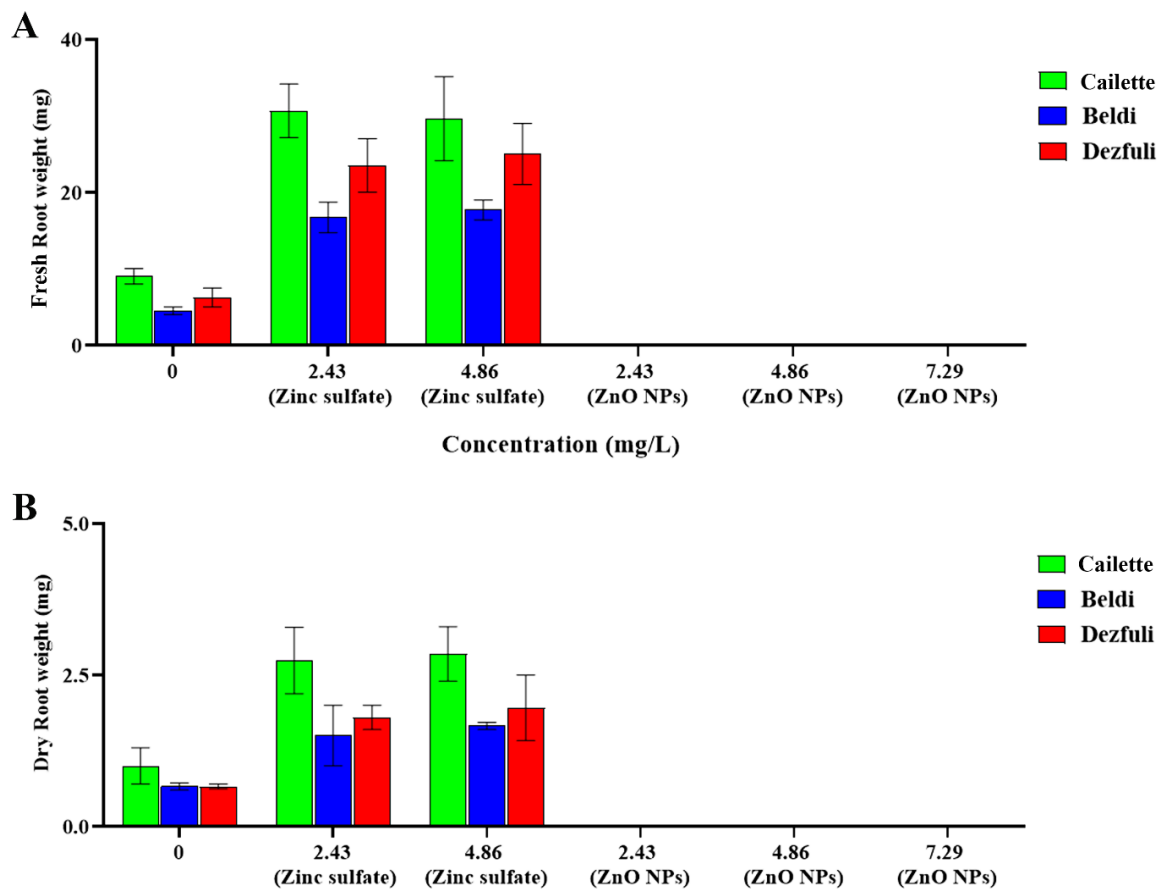
Fig. 8. *In vitro* study of ZnO NPs and zinc sulfate affecting (A) fresh root weight and (B) dry root weight of olive explants per cultivar.

ZnO nanoparticles act on plant cells and tissues in a variety of ways. The mechanism by which they do so is not well understood. The effect of zinc nanoparticles on plant cells and tissues derives from hormonal composition, biochemical balance, and molecular factors. Some factors play a more significant role in normal conditions, and others play a greater role in zinc toxicity.

Zinc is an essential element for plant growth and development, specifically its involvement in the biosynthesis of the hormone auxin, which stimulates the growth and rooting of plants. The zinc affects tryptophan through zinc finger transcription factors, capable of binding to tryptophan decarboxylase gene promoters and affecting tryptophan production (Pauw et al., 2004). First, the tryptophan is converted to indole-3-acetamide by tryptophan-2-monooxygenase (iaaM), followed by indole-3-acetamide conversion to indole by amidase (amiE). The auxin hormone induces adventitious roots in plants by increasing carbohydrates,

phenolic compounds, and rooting gene expressions while boosting the H_2O_2 content (Neves et al., 2012; Su et al., 2006; Bákány et al., 2021). Additionally, the enzyme super-oxidase dismutase (SOD) activity increases at the same time as free radicals increase in the plant cell in response to auxin simulation (Ilczuk and Jacygrad, 2016; Elmongy et al., 2020). The impact of zinc nanoparticles on plant growth and development is associated with their possible interference with auxin biosynthesis. The high level of auxin may lower the activity of the peroxidase enzyme and prevent the lignification process, which may explain another reason for the suppression of root formation in callus tissues formed on olive explants. Besides affecting the growth of roots and shoots, auxin hormones, including IAA, are also necessary for callus induction. Therefore, callus growth in all media that contained zinc oxide nanoparticles in this study demonstrated that the prevention of root induction on explants is not a result of

nanoparticles affecting the synthesis process of indole acetic acid.

Phenolic compounds act as cofactors by preventing IAA oxidase activity or removing free radicals responsible for the peroxidase reaction. Additionally, the phenolic compounds act as precursors to form lignin, thereby increasing adventitious root growth. According to Denaxa et al. (2021), the final effect of phenolic compounds on adventitious rooting depends on the type of bioactive compounds present in each plant. The hypothesis of rooting incapacity in olive explants due to zinc oxide nanoparticles negatively affecting the reduction of auxin hormone is not citable because olive plants contain a high polyphenolic content. Adventitious root production through auxin hormone signaling is affected by nitric acid. The increased expression of the NIA (nitrate reductase) gene supports this hypothesis (Scholl et al., 1974). Zinc acetate enhances the activity of the NIA enzyme, thereby increasing adventitious root formation, but zinc nanoparticles inhibit the activity of this enzyme and decrease root production (Abu-Abied et al., 2012).

Furthermore, previous research explored the effects of zinc nanoparticles on different types of proteins in plants, including enzymes and non-enzymatic proteins, as well as its impact on metal homeostasis and the regulation of gene expression in root cells. For some enzymes to remain stable and active, zinc plays an important role directly or indirectly. In plants, zinc exists in RNA polymerase, alcohol dehydrogenase, carbonic anhydrase, and superoxidase dismutase enzymes. Most carbonic anhydrases contain zinc ions in their active sites, which is why they work so well. This enzyme plays a role in maintaining an acid-base balance and transporting carbon dioxide (Occhipinti and Boron, 2019). The super oxidase dismutase enzyme contains zinc and copper metals (Zn/Cu SOD). In response to stress conditions such as drought and cold, this enzyme removes superoxide free radicals from plant cells (Azarin et al., 2022).

Moreover, the amount of MDA is the final product of lipid oxidation and is responsible for membrane damage. It decreases with the increase in superoxidase dismutase activity (Zhang et al., 2017). The zinc nanoparticles can adversely affect the activity of some proteins necessary for growth and development, such as metal-chelating compounds, YSL, ferric reductase defective 3 (FRD3), zinc-induced facilitator 1 (ZIF1), multidrug and toxin efflux (MATE), and LBD domain proteins. The molecular mechanism of metal homeostasis inside the cell involves the formation of metal complexes with ligands such as oligopeptides, organic acids, amino acids, or proteins, which are required to pass through cell membranes. Metal-chelating compounds such as metal-nicotinamide are thus essential when exposing plants to metals like zinc. YSL (yellow stripe-like) proteins allow the metal-nicotinamide complex to pass through the cell membrane (Balafrej et al., 2020). Nicotinamide is transferred from the cytosol to the vacuole by zinc-induced facilitator 1 (ZIF1) protein, forming a Zn-NA complex inside the vacuole. Accordingly, through this process, zinc maintains vacuolar homeostasis in plant cells (Haydon and Cobbett, 2007). The proteins of the LBD gene family, which have the LOB (LATERAL ORGAN BOUNDARIES) domain, play a crucial role in regulating the induction and growth of lateral and adventitious roots, as well as callus production, among other plant organs. Zhang et al. (2020) reported that these proteins influenced the auxin hormone response (Lee et al., 2009). Several proteins play a role in maintaining zinc balance in the cell, including MTPs (metal tolerance proteins), HMAs (heavy metal ATPases), NRAMP4 (Natural Resistance Associated Macrophage Protein 4), YSL (yellow stripe-like), and PCR2 (Plant Cadmium Resistance2) (Lan et al., 2013).

Zinc relocates mostly through the plasma membrane by ZIP family proteins. In plants, ZIP transporters play a vital role in zinc absorption and redistribution (Grotz et al., 1998). These proteins affect all organs and stages (Li et al., 2013). Some transcription factors regulate several ZIP family proteins. The transcription factors, known as bZIP transcription factors, regulate ZIP protein expression.

Two transcription factors, *bZIP19* and *bZIP23*, usually control gene expressions related to these proteins. After connecting in a dimer state, these two proteins can regulate the *ZIP* gene expression. The bZIP family proteins have a motif for binding to cis-regulatory elements called zinc deficiency response elements (ZDRE) and another motif, known as the leucine (Leu) zipper dimerization region, required to form dimers with other proteins in the family (Jakoby et al., 2002). Thus, *bZIP19* and *bZIP23* proteins first form a dimer, and upon activation, they induce the expression of ZIP family genes by binding to the ZDER regulatory sequence (located upstream of these genes). This dimer is known as a sensor for determining zinc status in plants. Zinc binds to a zinc-sensitive motif to connect divalent zinc ions (Lilay et al., 2021).

After the alignment of the olive genome, specifically the *bZIP19* and *bZIP23* amino acid sequences in this study, we found that the NCBI database contains two very similar sequences; they have a DNA binding site with NREAVRKYREKK and a lucine zipper domain with LEDEVIRRLTLNQQLMKRLQGQALLAEIARLCKCLL, which confirms previous findings by Bákány et al. (2021). Zinc concentration affects the expression of all ZIP genes except ZIP6 (Lira-Morales et al., 2019).

According to Fig. 9, 10, and Table 1, the protein network closely related to these two proteins has a very similar structure and function. ARFs (auxin regulating factors) are transcription factors that affect rooting by binding to the cis regions in genes related to auxin (Elmongy et al., 2020). In the procambial and surrounding parenchyma cells, auxin accumulation in the cut sites of *Arabidopsis* leaves triggers the expression of genes related to two home box transcription factors, including *WOX11* (WUSCHEL RELATED HOMEOBOX11) and *WOX12*. These genes increase the transformation of procambial/leaf parenchyma cells into root-forming cells (Ikeuchi et al., 2016). Evidence suggests that *WOX11* and *WOX12* genes assist in generating new meristems at the time of root production. The induction of *WOX11* gene expression relies on the presence of AuxREs (auxin response elements) in its promoter. This condition indicates that the ARF family of genes directly regulates the *WOX11* gene expression level in leaf samples (Liu et al., 2014). Also, some zinc finger proteins, such as bZIP11 or Zfp277, have reportedly bound to some ARF proteins and regulated their expression by facilitating their acetylation by histone acetylation machinery or by forming complexes with other proteins, respectively (Weiste Dröge-Laser, 2014; Negishi et al., 2010). Where large vacuoles are present, the excess zinc absorbed in callus cells is stored appropriately in these vacuoles. Thus, callus tissues can continue growing even with excess amounts of zinc. However, this excess zinc in hyper-accumulator plants such as olive is likely to accumulate more in the cell wall, leading to positive-feedback regulations of lignin synthesis gene expression in the cell wall, thereby increasing the physical resistance of tissues to root formation and suppressing the emergence of primary roots.

Conclusion

The results indicated that the type of zinc source in the culture medium significantly affected the olive explant response. Enrichment of the medium with zinc oxide nanoparticles induced callus on all olive explants (100%) at all concentrations but inhibited rooting. However, the zinc sulfate medium produced less callus but significantly enhanced the rooting. Therefore, the nanoparticles and zinc oxide stimulated dedifferentiation (callus formation) in olive explants but encouraged differentiation (organogenesis) *in vitro*. These results can explain the nature of these nanoparticles that derive from their unique synthesis.

Consent to participate
Consent was obtained from all individual participants involved in the study.

Consent to publish
The participants have consented to the submission of the case report to the journal.

Author contribution
The research conception and design were done by MM. Material preparation, data collection and analysis were performed by MT and MM. MM wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. SMHM offered technical advice. All authors read and approved the final manuscript.

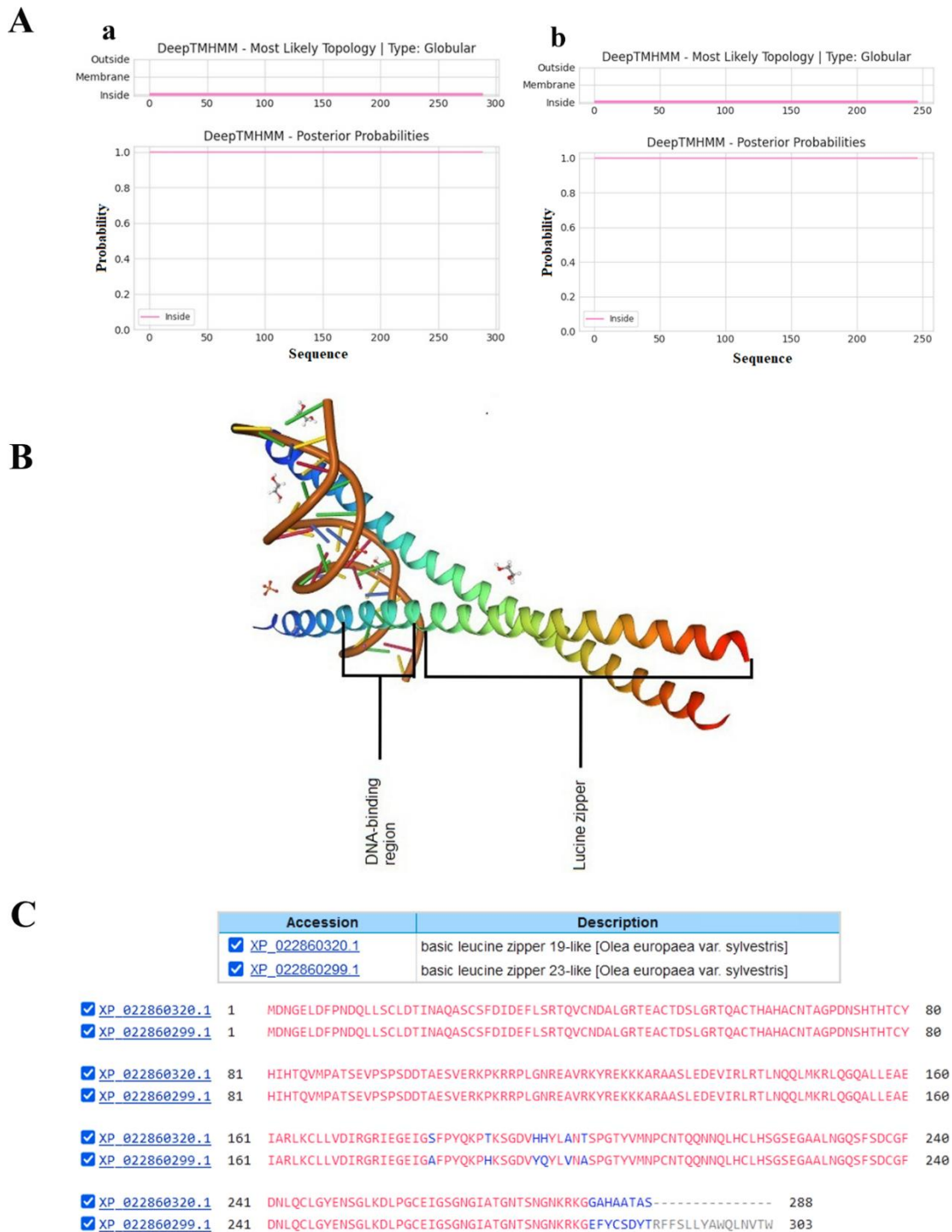## Conflict of Interest
The authors indicate no conflict of interest for this work.

Availability of data and materials
Data will be made available on request.

Fig. 9. (A) TMHMM output based on amino acid sequences of *bZIP19* (a) and *bZIP23* (b) proteins, indicating their membrane location. (B) Dimer proteins BZIP19/bZIP23, DNA binding sites, and Lucine zipper domains. Using the NCBI database and the Swiss database, the model is based on two genes (bZIP19, NCBI Reference Sequence: XM_023017314.1) from the olive genome (common olive taxid: 4146). (C) The identity of the amino acid sequences of *bZIP19* (NCBI Reference Sequence: XM_023004552.1, /protein id=XP_022860320.1) and *bZIP23* (NCBI Reference Sequence: XM_023004531.1, /protein_id="XP_022860299.1") related to the *olive genome*. The Olea europaea var. 'Sylvestris' (common olive taxid: 4146) using the COBALT tool (Constraint-based Multiple Alignment Tool) via the NCBI database.
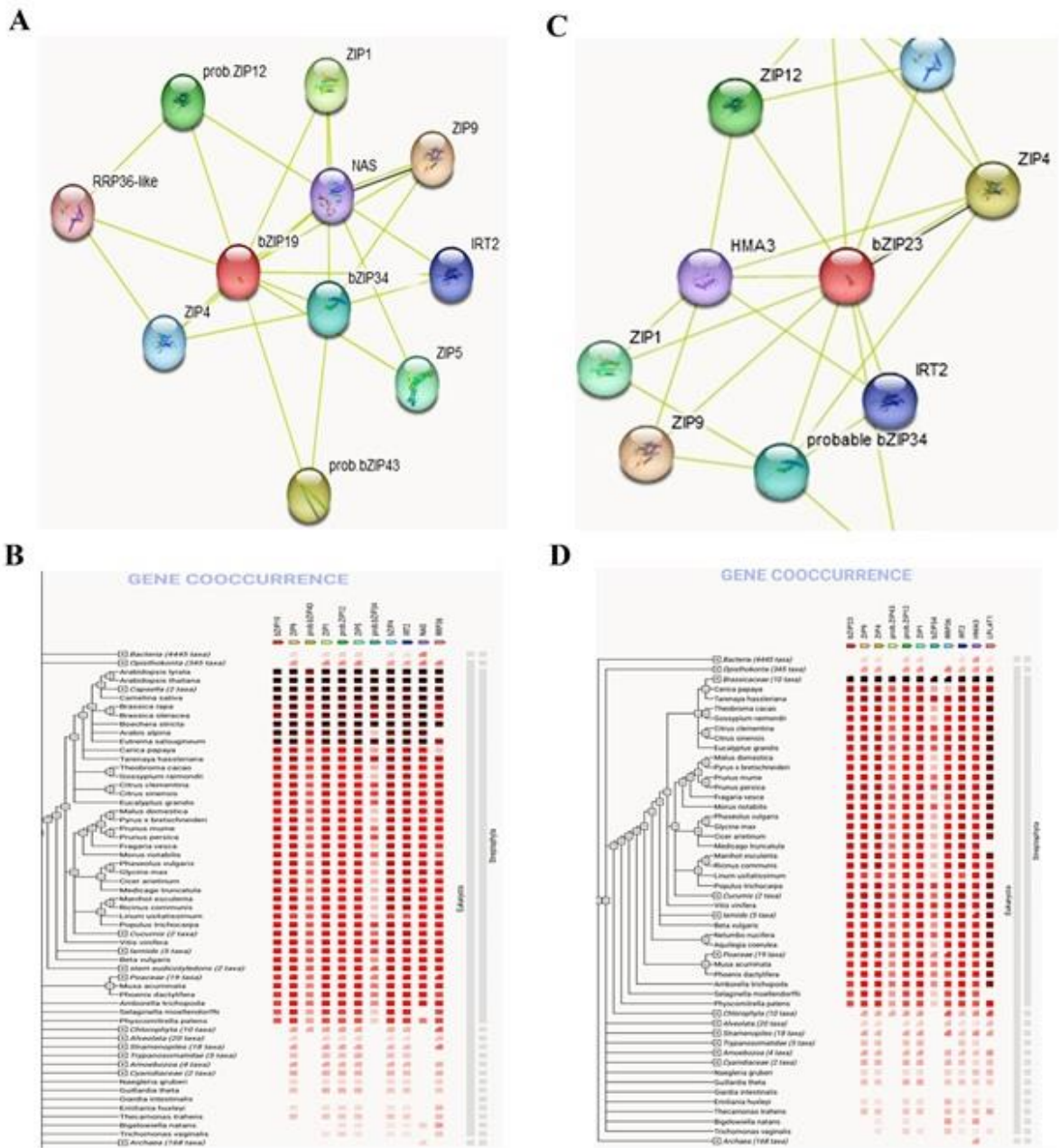
Fig. 10. Clusters of proteins associated with bZIP19 and bZIP23 transcription factors and their gene expression in other plants and non-plant environments.

Table 1. Dimer-related bZIP19/23 proteins and their functions.

Proteins direct interact with bZIP19 (*Olea europaea*)	Proteins direct interact with bZIP23 (*Olea europaea*)	Function (Based on STRING database https://string-db.org)
ZIP1 (zinc transporter 1-like)	ZIP1 (zinc transporter 1-like)	Zinc transporter 1 precursor. Transcript is induced in response to zinc deficiency in the root.
probable bZIP43 (basic leucine zipper 43-like)	probable bZIP43 (basic leucine zipper 43-like)	Sequence-specific DNA binding transcription factor activity; Involved in regulation of transcription, DNA-dependent; Located in chloroplast; Expressed in root and leaf.
ZIP4	ZIP4	Zinc transporter 4, chloroplastic. Transcript is induced in response to zinc deficiency in the root and shoot. Expression is regulated by copper, but response to copper deficiency is detected only after three weeks of deficiency.
probable,ZIP9	probable,ZIP9	Zinc transporter, involved in zinc uptake in roots. Targeted by BZIP19 transcription factor in response to zinc- deficient conditions.
probable, ZIP12	probable, ZIP12	
rRNA biogenesis RRP36-like protein	rRNA biogenesis RRP36-like protein	rRNA biogenesis RRP36-like protein.
probable, bZIP34	probable, bZIP34	Sequence-specific DNA binding transcription factor activity involved in the regulation of transcription; DNA-dependent.
IRT2 (fe(2^+) transport protein 1-like)	IRT2 (fe(2^+) transport protein 1-like)	Encodes a plasma membrane localized zinc/iron transporter, chloroplastic.
NAS (nicotianamine synthase)	-	Transcript levels rise in roots in response to zinc deficiency and rise in leaves in response to elevated zinc levels.
-		Cadmium/zinc-transporting ATPase HMA2 plays an important role in zinc transport and homeostasis; could be involved in cadmium detoxification (https://string-db.org).
	HMA3 (inactive cadmium zinc-transporting ATPase)	In hyper-accumulated species, *HMA3* was much more expressed in shoots than in roots. *HMA3* mRNA levels was highest in the mesophyll and bundle sheath of the vein (Mishra et al., 2017). It plays a vital role in the translocation or detoxification of Zn and Cd in plants. OsHMA3 transports Cd and plays a role in the sequestration of Cd into vacuoles in root cells (Takahashi et al., 2012).
-	LPLAT1 (lysophospholipid acyltransferase 1-like)	Catalyzes the reacylation step of the phospholipid remodeling pathway also known as the Lands cycle (Probable). The primary function of the Lands cycle is to provide a route for acyl remodeling to modify fatty acid (FA) composition of phospholipids derived from the Kennedy pathway (https://www.uniprot.org).

References

Abu-Abied M, Szwerdszarf D, Mordehaev I, Levy A, Belausov E, Yaniv Y. 2012. Microarray analysis revealed upregulation of nitrate reductase in juvenile cuttings of *Eucalyptus grandis*, which correlated with increased nitric oxide production and adventitious root formation. The Plant Journal 71(5), 787-99. https://doi.org/10.1111/j.1365-313X.2012.05032.x

Adil M, Bashir S, Bashir S, Aslam Z, Ahmad N, Younas T. 2022. Zinc oxide nanoparticles improved chlorophyll contents, physical parameters, and wheat yield under salt stress. Frontiers in Plant Science 13. https://doi.org/10.3389/fpls.2022.932861

Al-Habahbeh KA, Al-Nawaiseh MB, Al-Sayaydeh RS, Al-Hawadi JS, Albdaiwi RN, Al-Debei HS, Ayad JY. 2021. Long-term irrigation with treated municipal wastewater from the Wadi-Musa region: soil heavy metal accumulation, uptake and partitioning in olive trees. Horticulturae 7, 152. https://doi.org/10.3390/horticulturae7060152

Al Jabri H, Saleem MH, Rizwan M, Hussain I, Usman K, Alsafran M. 2022. Zinc oxide nanoparticles and their biosynthesis: Overview. Life 12, 59. https://doi.org/10.3390/life12040594

Li S, Zhou X, Huang Y. 2013. Identification and characterization of the zinc-regulated transporters, iron-regulated transporter-like protein (ZIP) gene family in maize. BMC Plant Biology 13, 114. https://doi.org/10.1186/1471-2229-13-114

Ayoub SJ, Qrunfleh MM. 2006. Seasonal variation in rooting 'Nabali' and 'Raseei' olive cuttings in relation to shoot content of endogenous plant hormones. Jordan Journal of Agricultural Sciences 2, 2. https://journals.ju.edu.jo/JJAS/article/download/1263/5959

Azarin K, Usatov A, Minkina T, Plotnikov A, Kasyanova A, Fedorenko A. 2022. Effects of ZnO nanoparticles and its bulk form on growth, antioxidant defense system and expression of oxidative stress related genes in *Hordeum vulgare* L. Chemosphere 287, 132167. https://doi.org/10.1016/j.chemosphere.2021.132167

Balafrej H, Bogusz D, Triqui ZEA, Guedira A, Bendaou N, Smouni A. 2020. Zinc hyperaccumulation in plants: A review. Plants 9(5), 562. https://doi.org/10.3390/plants9050562

Bákány B, Yin WB, Dienes B, Nagy T, Leiter É, Emri T. 2021. Study on the bZIP-Type transcription factors NapA and RsmA in the regulation of intracellular reactive species levels and sterigmatocystin production of *Aspergillus nidulans*. International Journal of Molecular Science 22(21), 11577. https://doi.org/10.3390/ijms222111577

Bayraktar M, Hayta-Smedley S, Unal S, Varol N, Gurel A. 2020. Micropropagation and prevention of hyperhydricity in olive (*Olea europaea* L.) cultivar 'Gemlik'. South African Journal of Botany 128, 264-273. https://doi.org/10.1016/j.sajb.2019.11.022

Binet MN, Lemoine MC, Martin C, Chambon C, Gianinazzi S. 2007. Micropropagation of olive (*Olea Europaea* L.) and application of mycorrhiza to improve plantlet establishment. In Vitro Cellular & Developmental Biology-Plant 43(5), 473-478. http://www.jstor.org/stable/29736201

Brito C, Dinis LT, Moutinho-Pereira J, Correia CM. 2019. Drought stress effects and olive tree acclimation under a changing climate. Plants (Basel) 8(7), 232. https://doi.org/10.3390/plants8070232

Castillo-González J, Ojeda-Barrios D, Hernández-Rodríguez A, González-Franco AC, Robles-Hernández L, López-Ochoa GR. 2018. Zinc metalloenzymes in plants. Interciencia 43(4), 242-248.

Chen LM, Cheng JT, Chen EL, Yiu TJ, Liu ZH. 2002. Naphthaleneacetic acid suppresses peroxidase activity during the induction of adventitious roots in soybean hypocotyls. Journal of Plant Physiology 159(12), 1349-1354. https://doi.org/10.1078/S0176-1617(04)70364-8

Chevallet M, Veronesi G, Fuchs A, Mintz E, Michaud-Soret I, Deniaud A. 2017. Impact of labile metal nanoparticles on cellular homeostasis. Current developments in imaging, synthesis and applications. Biochimica et Biophysica Acta (BBA)-General Subjects 1861(6), 1566-77. https://doi.org/10.1016/j.bbagen.2016.12.012

Denaxa NK, Vemmos SN, Roussos PA. 2021. Shoot girdling improves rooting performance of Kalamata olive cuttings by upregulating carbohydrates, polyamines and phenolic compounds. Agriculture 11(1), 71. https://doi.org/10.3390/agriculture11010071

El-Ghamery A, El-Kholy M, Abou El-Yousser M. 2003. Evaluation of cytological effects of Zn2+ in relation to germination and root growth of *Nigella sativa* L. and

Triticum aestivum L. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 537(1), 29-41. https://doi.org/10.1016/s1383-5718(03)00052-4

Elmongy MS, Wang X, Zhou H, Xia Y. 2020. Humic acid and auxins induced metabolic changes and differential gene expression during adventitious root development in Azalea microshoots. HortScience 55(6), 926-935. https://doi.org/10.21273/HORTSCI14885-20

Felenji H, Johari B, Moradi M, Gharbavi M, Danafar H. 2022. Folic acid-conjugated iron oxide magnetic nanoparticles based on Bovine Serum Albumin (BSA) for targeted delivery of curcumin to suppress liver cancer cells. Chemistry Africa 5(5), 1627-39. https://doi.org/10.1007/s42250-022-00425-1

Gharbavi M, Sharafi A, Motamed Fath P, Oruji S, Pakzad H, Manjili HK. 2021. Formulation and biocompatibility of microemulsion-based PMBN as an efficient system for paclitaxel delivery. Journal of Applied Biotechnology Reports 8(1). https://doi.org/10.30491/jabr.2020.114985

Gharbavi M, Mousavi M, Pour-Karim M, Tavakolizadeh M, Sharafi A. 2022. Biogenic and facile synthesis of selenium nanoparticles using *Vaccinium arctostaphylos* L. fruit extract and anticancer activity against in vitro model of breast cancer. Cell Biology International 46(10), 1612-24. https://doi.org/10.1002/cbin.11852

Gharbavi M, Johari B, Ghorbani R, Madanchi H, Sharafi A. 2023. Green synthesis of Zn nanoparticles and in situ hybridized with BSA nanoparticles for *Baicalein targeted* delivery mediated with glutamate receptors to U87-MG cancer cell lines. Applied Organometallic Chemistry 37(1), e6926. https://doi.org/10.1002/aoc.6926

Goh T, Toyokura K, Yamaguchi N, Okamoto Y, Uehara T, Kaneko S, Takebayashi Y, Kasahara H, Ikeyama Y, Okushima Y. 2019. Lateral root initiation requires the sequential induction of transcription factors LBD16 and PUCHI in *Arabidopsis thaliana*. New Phytology 224, 749-760. https://doi.org/10.1111/nph.16065

Grotz N, Fox T, Connolly E, Park W, Guerinot ML, Eide D. 1998. Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency. Proceedings of the National Academy of Sciences USA 9:95(12), 7220-4. https://doi.org/10.1073/pnas.95.12.7220

Haissig BE. 1972. Meristematic activity during adventitious root primordium development: influence of endogenous auxin and applied gibberellic acid. Plant Physiology 49, 886-892. https://doi.org/10.1104/pp.49.6.886

Han G, Lu C, Guo J, Qiao Z, Sui N, Qiu N. 2020. C2H2 zinc finger proteins: master regulators of abiotic stress responses in plants. Frontiers in Plant Science 11, 115. https://doi.org/10.3389/fpls.2020.00115

Harish V, Tewari D, Gaur M, Yadav AB, Swaroop S, Bechelany M, Barhoum A. 2022. Review on

nanoparticles and nanostructured materials: Bioimaging, biosensing, drug delivery, tissue engineering, antimicrobial, and agro-food applications. Nanomaterials 12(3), 457. https://doi.org/10.3390/nano12030457

Hasnidawani J, Azlina H, Norita H, Bonnia N, Ratim S, Ali E. 2016. Synthesis of ZnO nanostructures using sol-gel method. Procedia Chemistry 19, 211-6. https://doi.org/10.1016/j.proche.2016.03.095

Haydon MJ, Cobbett CS. 2007. Transporters of ligands for essential metal ions in plants. New Phytologist 174(3), 499-506. https://doi.org/10.1111/j.1469-8137.2007.02051.x

Hsieh SI, Castruita M, Malasarn D, Urzica E, Erde JM, Page D, Yamasaki H, Casero D, Pellegrini M, Merchant SS, Loo JA. 2013. The proteome of copper, iron, zinc, and manganese micronutrient deficiency in *Chlamydomonas reinhardtii*. Molecular & Cellular Proteomics 12(1), 65-86. https://doi.org/10.1074/mcp.M112.021840

Ikeuchi M, Ogawa Y, Iwase A, Sugimoto K. 2016. Plant regeneration: cellular origins and molecular mechanisms. Development 143(9), 1442-51. https://doi.org/10.1242/dev.134668

Ilczuk A, Jacygrad E. 2016. In vitro propagation and assessment of genetic stability of acclimated plantlets of *Cornus alba* L. using RAPD and ISSR markers. *In Vitro* Cellular & Developmental Biology-Plant 52, 379-90. https://doi.org/10.1007/s11627-016-9781-6

Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T. 2002. bZIP transcription factors in Arabidopsis. Trends in Plant Science 7(3), 106-11. https://doi.org/10.1016/s1360-1385(01)02223-3

Lan HX, Wang ZF, Wang QH, Wang MM, Bao YM., Huang J, Zhang HS. 2013. Characterization of a vacuolar zinc transporter OZT1 in rice (Oryza sativa L.). Molecular Biology Reports 40, 1201-1210. https://doi.org/10.1007/s11033-012-2162-2

Lee HW, Kim NY, Lee DJ, Kim J. 2009. LBD18/ASL20 regulates lateral root formation in combination with LBD16/ASL18 downstream of ARF7 and ARF19 in *Arabidopsis*. Plant Physiology 151, 1377-1389. https://doi.org/10.1104/pp.109.143685

Li X, Xing Y, Jiang Y, Ding Y, Li W. 2009. Antimicrobial activities of ZnO powder-coated PVC film to inactivate food pathogens. International Journal of Food Science & Technology 44(11), 2161-8. https://doi.org/10.1111/j.1365-2621.2009.02055.x

Li S, Zhou X, Huang Y. 2013. Identification and characterization of the zinc-regulated transporters, iron-regulated transporter-like protein (ZIP) gene family in maize. BMC Plant Biology 13(1), 114. https://doi.org/10.1186/1471-2229-13-114

Lilay GH, Persson DP, Castro PH, Liao F, Alexander RD, Aarts MG. 2021. *Arabidopsis bZIP19* and *bZIP23* act as zinc sensors to control plant zinc status. Nature Plants 7(2), 137-43. https://doi.org/10.1038/s41477-021-00856-7

Lin D, Xing B. 2007. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environmental Pollution 150(2), 243-50. https://doi.org/10.1016/j.envpol.2007.01.016

Lin YF, Aarts MG. 2012. The molecular mechanism of zinc and cadmium stress response in plants. Cellular and Molecular Life Sciences 69, 3187-206. https://doi.org/10.1007/s00018-012-1089-z

Lira-Morales JD, Varela-Bojórquez N, Montoya-Rojo MB, Sañudo-Barajas JA. 2019. The role of ZIP proteins in zinc assimilation and distribution in plants: current challenges. Czech Journal of Genetics and Plant Breeding 55(2), 45-54. https://doi.org/10.17221/54/2018-CJGPB

Liu H, Wang S, Yu X, Yu J, He X, Zhang S, Shou H, Wu P. 2005. ARL1, a LOB-domain protein required for adventitious root formation in rice. The Plant Journal 43(1), 47-56. https://doi.org/10.1111/j.1365-313X.2005.02434.x

Liu J, Sheng L, Xu Y, Li J, Yang Z, Huang H, Xu L. 2014. WOX11 and 12 are involved in the first-step cell fate transition during de novo root organogenesis in *Arabidopsis*. Plant Cell 6, 1081-1093. https://doi.org/10.1105/tpc.114.122887

Liu X, Dinh TT, Li D, Shi B, Li Y, Cao X. 2014. AUXIN RESPONSE FACTOR 3 integrates the functions of *AGAMOUS* and *APETALA* 2 in floral meristem determinacy. The Plant Journal 80(4), 629-41. https://doi.org/10.1111/tpj.12658

Mishra S, Mishra A, Küpper H. 2017. Protein biochemistry and expression regulation of cadmium/zinc pumping ATPases in the hyperaccumulator plants *Arabidopsis halleri* and *Noccaea caerulescens*. Frontiers in Plant Science 8, 835. https://doi.org/10.3389/fpls.2017.00835

Mukherjee A, Pokhrel S, Bandyopadhyay S, Mädler L, Peralta-Videa JR, Gardea-Torresdey JL. 2014. A soil mediated phyto-toxicological study of iron doped zinc oxide nanoparticles (Fe@ ZnO) in green peas (*Pisum sativum* L.). Chemical Engineering Journal 258, 394-401. https://doi.org/10.1016/j.cej.2014.06.112

Munzuroglu O, Geckil H. 2002. Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in *Triticum aestivum* and *Cucumis sativus*. Archives of Environmental Contamination and Toxicology 43, 203-13. https://doi.org/10.1007/s00244-002-1116-4

Negishi M, Saraya A, Mochizuki S, Helin K, Koseki H, Iwama A. 2010. A novel zinc finger protein Zfp277 mediates transcriptional repression of the Ink4a/arf locus through polycomb repressive complex 1. PLoS One 5, e12373. https://doi.org/10.1371/journal.pone.0012373

Neves N, Barros R, Antunes E, Calado J, Fortunato E, Martins R. 2012. Aluminum doped zinc oxide sputtering targets obtained from nanostructured powders: processing and application. Journal of the

European Ceramic Society 32(16), 4381-91. https://doi.org/10.1016/j.jeurceramsoc.2012.08.007

Occhipinti R, Boron WF. 2019. Role of carbonic anhydrases and inhibitors in acid-base physiology: insights from mathematical modeling. International Journal of Molecular Science 20(15), 3841. https://doi.org/10.3390/ijms20153841

Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M. 2007. ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in *Arabidopsis*. The Plant Cell 19(1), 118-30. https://doi.org/10.1105/tpc.106.047761

Pauw B, Hilliou FA, Martin VS, Chatel G, de Wolf CJ, Champion A. 2004. Zinc finger proteins act as transcriptional repressors of alkaloid biosynthesis genes in *Catharanthus roseus*. Journal of Biological Chemistry 279(51), 52940-8. https://doi.org/10.1074/jbc.M404391200

Porfírio S, Da Silva MDR, Cabrita MJ, Azadi P, Peixe A. 2016. Reviewing current knowledge on olive (*Olea europaea* L.) adventitious root formation. Scientia Horticulturae 198, 207-226. https://doi.org/10.1016/j.scienta.2015.11.034

Rigal A, Yordanov YS, Perrone I, Karlberg A, Tisserant E, Bellini C. 2012. The AINTEGUMENTA LIKE1 homeotic transcription factor PtAIL1 controls the formation of adventitious root primordia in poplar. Plant Physiology 160(4), 1996-2006. https://doi.org/10.1104/pp.112.204453

Rugini E. 1984. In vitro propagation of some olive (*Olea europaea sativa* L.) cultivars with different root-ability, and medium development using analytical data from developing shoots and embryos. Scientia Horticulturae 24(2), 123-134. https://doi.org/10.1016/0304-4238(84)90143-2

Scholl RL, Harper JE, Hageman RH. 1974. Improvements of the nitrite color development in assays of nitrate reductase by phenazine methosulfate and zinc acetate. Plant Physiology 53(6), 825-8. https://doi.org/10.1104/pp.53.6.825

Shin R, Burch AY, Huppert KA, Tiwari SB, Murphy AS, Guilfoyle TJ, Schachtman DP. 2007. The *Arabidopsis* transcription factor MYB77 modulates auxin signal transduction. Plant Cell 19, 2440-2453. https://doi.org/10.1105/tpc.107.050963

Su GX, Zhang WH, Liu YL. 2006. Involvement of hydrogen peroxide generated by polyamine oxidative degradation in the development of lateral roots in soybean. Journal of Integrative Plant Biology 48(4), 426-432. https://doi.org/10.1111/j.1744-7909.2006.00236.x

Sun T, Shi X, Friso G, van Wijk K, Bentolila S. 2015. A zinc finger motif-containing protein is essential for chloroplast RNA editing. PLoS Genetics 11(3), e1005028. https://doi.org/10.1371/journal.pgen.1005028

Takahashi R, Bashir K, Ishimaru Y, Nishizawa NK, Nakanishi H. 2012. The role of heavy-metal ATPases, HMAs, in zinc and cadmium transport in rice. Plant Signal Behavior 7(12) 1605-7. https://doi.org/10.4161/psb.22454

Tondey M, Kalia A, Singh A. 2022. A comparative evaluation of the effects of seed invigoration treatments with precursor zinc salt and nano-sized zinc oxide (ZnO) particles on vegetative growth, grain yield, and quality characteristics of *Zea mays*. Journal of Annals in Scientifc Technology 13, 40. https://doi.org/10.1186/s40543-022-00346-1

Umer Hassan M, Aamer M, Umer Chattha M, Haiying T, Shahzad B, Barbanti L, Nawaz M, Rasheed A, Afzal A, Liu Y, Guoqin H. 2020. The critical role of zinc in plants facing the drought stress. Agriculture 10(9), 396. https://doi.org/10.3390/agriculture10090396

Weiste C, Dröge-Laser W. 2014. The *Arabidopsis* transcription factor bZIP11 activates auxin-mediated transcription by recruiting the histone acetylation machinery. Nature Communications 5(1), 3883. https://doi.org/10.1038/ncomms4883

Yang B, Lee C, Xiang W, Xie J, He, JH, Kotlanka RK. 2009. Electromagnetic energy harvesting from vibrations of multiple frequencies. Journal of Micromechanics and Microengineering 19(3), 035001. https://doi.org/10.1088/0960-1317/19/3/035001.

Yang Q, Ma X, Luo S, Gao J, Yang X, Feng Y. 2018. SaZIP4, an uptake transporter of Zn/Cd hyperaccumulator *Sedum alfredii* Hance. Environmental and Experimental Botany 55, 107-17. https://doi.org/10.1016/j.envexpbot.2018.06.021

Yu T, Su W, Li W, Hong Z, Hua R, Li M. 2006. Synthesis, crystal structure and electroluminescent properties of a Schiff base zinc complex. Inorganica Chimica Acta 359(7), 2246-51. https://doi.org/10.1016/j.ica.2006.01.019

Zafar MW, Saleem MM, Destek MA., Caglar AE. 2022. The dynamic linkage between remittances, export diversification, education, renewable energy consumption, economic growth, and CO_2 emissions in top remittance-receiving countries. Sustainable Development 30(1), 165-75. https://doi.org/10.1002/sd.2236

Zhang L, Sun L, Zhang L, Qiu H, Liu C, Wang A, Deng F, Zhu AJ. 2017. Cu/Zn superoxide dismutase gene from *Saussurea involucrata* Kar. & Kir., SiCSD, enhances drought, cold, and oxidative stress in transgenic tobacco. Canadian Journal of Plant Science 97(5), 816-826. https://doi.org/10.1139/cjps-2016-0180

Zhang Y, Li Z, Ma B, Hou Q, Wan X. 2020. Phylogeny and functions of LOB domain proteins in plants. International Journal of Molecular Sciences 21(7), 2278. https://doi.org/10.3390/ijms21072278

Zhang M, Zhang S. 2022. Mitogen-activated protein kinase cascades in plant signaling. Journal of Integral Plant Biology 64, 301-34. https://doi.org/10.1111/jipb.13215

Zhao Y, Chen Y, Jiang C, Lu MZ, Zhang J. 2022. Exogenous hormones supplementation improve adventitious root

formation in woody plants. Frontiers in Bioengineering and Biotechnology 10, 1009531. https://doi.org/10.3389/fbioe.2022.1009531

Zhou Y, Zhao W, Lai Y, Zhang B and Zhang D. 2020.

Edible plant oil: global status, health Issues, and perspectives. Frontiers in Plant Science 11, 1315. https://doi.org/10.3389/fpls.2020.01315