



Nanoparticles as modulators of stress tolerance: Physiological and molecular insights into TiO₂ and ZnO effects in *Cucumis melo* L. subjected to salt shock

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ABSTRACT

Nanoparticles (NPs) have emerged as innovative tools in agriculture to enhance plant productivity and stress tolerance under challenging conditions. This study aimed to evaluate the short-term effects of TiO₂ and ZnO-NPs on melon plants (*Cucumis melo* L.) under control and salt stress shock conditions. Plants were treated with NPs via root application in hydroponically system, and physiological, biochemical, and molecular responses were analysed. TiO₂-NPs increased biomass, enhanced water potential, and the maintained photosynthetic efficiency during salt shock. In contrast, ZnO-NPs did not promote growth but triggered protective responses, including reduced lipid peroxidation and improved membrane stability. Transmission electron microscopy confirmed NPs localization in root and leaves, primarily near membranes and within vacuoles, suggesting their involvement in transport and redistribution mechanisms. NPs up-regulated root aquaporins, particularly *CmNIP5;1*, correlating with improved water transport and potential. TiO₂-NPs enhanced Fe redistribution in leaves, while ZnO-NPs reduced Cu levels and triggered an increase in Ca under salinity. Additionally, TiO₂-NPs promoted phenolic compound accumulation, enhancing antioxidant defences, whereas ZnO-NPs reduced these metabolites. In conclusion, TiO₂ and ZnO-NPs modulate key physiological and biochemical responses, improving stress tolerance and nutrient dynamics. These findings highlight their potential as innovative tools for sustainable agriculture and warrant further investigation into their mechanisms of action.

1. Introduction

In recent years, nanotechnology has reached all areas of life, leading to advances in the development of nanomaterials and the identification of potential applications. Progress has also been made in the use of nanoparticles (NPs) in agriculture, where the main objective is to increase crop yields and reduce the environmental footprint of traditional agrochemicals, as NPs may have reduced toxicity and increased chemical efficacy (Usman et al., 2020). However, the adverse effects and mode of action of these particles are

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poorly understood, particularly in agriculture, where research lags behind other fields such as biomedicine and before these nano-materials can be applied on an industrial scale, further research is needed to assess their impacts on plants, humans, and the environment to confirm their safety and non-toxicity (Sebesta et al., 2021).

In the field of agriculture, the most extensively studied NPs are titanium oxide (TiO₂-NPs) and zinc oxide (ZnO-NPs) (Ali et al., 2025). Toxicity tests have been conducted with these NPs, and the results demonstrate that only high doses (above 2000 mg L⁻¹) have the potential to cause adverse effects (Prasad et al., 2012). Metallic NPs have found to come in contact with cells and cause damage to cell membrane, DNA, and proteins (Khan et al., 2021). Nevertheless, the study and testing of metallic NPs is widespread, given the evidence that the positive effects can significantly outweigh the negative ones. This is particularly relevant in light of the hormesis of this type of materials, characterised by stimulation at low doses and inhibition at high doses, which has been a focus of research in recent years (Trela-Makowej et al., 2024). There are several recent studies indicate that low concentrations of these NPs not only promote plant growth but also enhance tolerance to abiotic stresses, such as drought and salinity (Zhao et al., 2020). The elucidation of the mechanisms by which these types of nanomaterials confer stress resilience to abiotic and biotic stresses remains an open and emerging area of research.

In response to stress and the development of tolerances, plants trigger various mechanisms, including osmotic and water regulation, the maintenance of ionic and nutrient homeostasis, the stability of cell membranes, or the synthesis of secondary metabolites in response to adverse environmental conditions (Yang and Guo, 2018). For example, different studies have investigated the role of NPs in these processes. ZnO nanoparticles alleviate the effects of drought by specifically enhancing wheat yield (*Triticum aestivum* L.) and nutrient accumulation in the grain (Dimkpa et al., 2020). Additionally, they mitigate salinity stress by promoting osmolyte synthesis and modulating the uptake of essential nutrients such as Ca and K (Lalarukh et al., 2022). The application of TiO₂-NPs to wheat through root, showed a beneficial effect on physicochemical parameters under drought stress (Mustafa et al., 2021); TiO₂-NPs induced the productions of secondary metabolite and antioxidant enzyme activities (lowered H₂O₂ content and decreased oxidative damage), reducing the salinity effects in Moldavian balm (Gohari et al., 2020). Foliar application of TiO₂-NPs to pakchoi (*Brassica chinensis* L.) improved photosynthetic efficiency and increased expression of genes involved in energy production for growth, as well as genes involved in water homeostasis, such as aquaporins (AQPs) (Wei et al., 2024). It has been demonstrated that various types of metal NPs can modulate the expression of AQPs and transcription factors associated with these proteins (Bárcana et al., 2022). For instance, the application of ZnO-NPs has been observed to enhance the expression of AQPs in barley plants, which plays an important role in determining stress tolerance (Akdemir, 2021), or SiO₂ NPs reduced the osmotic effect of salinity through the uptake of different AQPs (Mahmoud et al., 2022). AQPs are integral membrane proteins that facilitate the transport of water and other solutes across biological membranes. Among their subfamilies, the Nodulin 26-like intrinsic proteins (NIPs), specifically NIP2;2 and NIP5;1, have been identified as capable of transporting water and a diverse range of solutes (Johanson et al., 2001; Lopez-Zaplaná et al., 2020). They play a key role in the response of plants, particularly under stress conditions. These proteins are involved in the translocation and extrusion of various substances, thereby regulating homeostasis in the plant (Pommerrenig et al., 2015; Wang et al., 2017). This functionality is critical for understanding how plants manage abiotic stress, a challenge exacerbated by climate change.

In this context, the development of effective solutions to enhance crop resilience to abiotic stresses has become a crucial priority. The increasing prevalence of these adverse conditions has a detrimental impact on a multitude of crop species, including melon (*Cucumis melo* L.), a member of the Cucurbitaceae family with a significant economic influence, ranking as the fourth most prevalent fruit crop globally (FAO 2022, <http://faostat.fao.org>). The utilisation of nanotechnology, particularly the application of NPs to crops, presents a promising avenue for improving crop yield and enhancing crop resilience to environmental stresses (Thabet and Alqudah, 2024).

Based on previous research, it is hypothesized that TiO₂ and ZnO nanoparticles modulate various physiological and molecular mechanisms in plants under abiotic stress, including water transport regulation, membrane stability, and secondary metabolism. Through these mechanisms, NPs may enhance salinity tolerance. This study aims to provide a comprehensive understanding of how these nanoparticles influence plant responses to stress, with a novel focus on the mechanisms involved, since despite the actual findings, the exact role of NPs in modulating different stress-related pathways remains unclear. Specifically, we aimed to assess their effects on plant growth, secondary metabolism, and cell membrane integrity. Furthermore, we investigate whether these NPs act as protective agents against abiotic stress by analysing their short-term effects at the onset of salt shock, focusing on the initial response mechanisms. For these experiments, melon plants were treated with TiO₂-NPs or ZnO-NPs via root application in a hydroponic system, and their effects on photosynthetic efficiency, growth, water and osmotic potentials, membrane integrity, NIP aquaporin expression, and phenolic compound content were determined.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of a commercial melon variety (*Cucumis melo* L. var Mexel F1) were purchased from Sakata (Murcia, Spain). The seeds were soaked overnight in a beaker with aerated distilled water. Afterward, they were placed to germinate on moistened vermiculite. The experiment was conducted in a controlled environment chamber with a 16 h light and 8 h dark cycle, maintaining day/night air temperatures of 25 and 20°C, respectively. The relative humidity (RH) ranged from 60 to 80%, and photosynthetically active radiation (PAR) of 400 μmol m⁻² s⁻¹ was provided by a Pacific LED system (WT 470 C, LED80S/840 PSD WB L1600, Philips). The seedlings were cultivated in 8 L containers, with six plants per container. Each container was filled with Hoagland solution (pH 5.5–6.0), which was

continuously aerated. The composition of the Hoagland solution was: 6 KNO₃, 4 Ca(NO₃)₂, 1 KH₂PO₄, 1 MgSO₄ and 1 Fe-EDTA (M), and 25 H₃BO₃, 2 MnSO₄, 2 ZnSO₄, 0.5 CuSO₄, 0.5 (NH₄)₆MO₇O₂₄ (mM).

2.2. Applications of nanoparticles and salt treatments

Melon seedlings were grown for one week. Subsequently, the plants were subjected to NPs treatments, with TiO₂-NPs (50 mg L⁻¹) (Titanium (IV) oxide, < 150 nm particle size; Sigma-Aldrich, St. Louis, MO, USA) and ZnO-NPs (5 mg L⁻¹) (Zinc oxide nanoparticle, 8–16 nm particle size; Sigma-Aldrich, St. Louis, MO, USA) applied separately to the Hoagland solutions for one week. A short saline shock (100 mM NaCl) was then applied for 15 hours to half of the plants and measurements were carried out and samples were taken for further analysis of control and salt-treated plants after this time of treatment. This time frame was chosen based on studies indicating that early responses to salt shock are critical for understanding the mechanisms of stress perception and the activation of stress-related pathways (Shavrukov, 2013). Each treatment consisted of six biological replicates and plants were randomized within the growth system to minimize potential positional effects and environmental bias. Randomization was performed prior to treatment allocation and maintained throughout the experiment, which was repeated twice (Figure S1). Samples were lyophilised, frozen in liquid nitrogen and stored at -80°C, or oven-dried at 70°C. The fresh weights (FW) of roots and shoots were determined and plant material was placed in oven at 70°C for 7 days to obtain a constant dry weight (DW).

2.3. Relative Water Content

To determine the relative water content in leaf melon samples, leaf discs of approximately 1 cm diameter were cut from each treatment. The FW of the leaf was measured, followed by incubation for 24 hours in a water-filled Petri dish at 4°C. After measurement of the turgid weight of the leaves, they were then placed in an oven for 3 days at 70°C, and the DW was measured (Weatherley, 1950). The relative water content was determined by the formula given below:

$$RWC = (\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight}) \times 100$$

2.4. Chlorophyll Fluorescence Measurements

Chlorophyll fluorescence transients were evaluated on the youngest fully expanded leaf using a portable pulse modulated fluorometer (OS30p+ Chlorophyll Fluorometer, OptiSciences, UK). Before the measurements, leaves were dark adapted for 30 min. Measurement were carried out at different times: 4 h post application (PA), 3 days PA, 5 days PA, and 6 days PA of NPs treatment, point time that correspond to 15 h after shock saline treatment. Chlorophyll Fluorescence that detects efficiency (Fv/Fm) in photosystem II (PSII) and performance index (PI) parameters were selected to follow the photosystem II status of plants during the experiment.

2.5. Leaf water potential (Ψ_w) and osmotic potential (Ψ_s)

The leaf water potential (Ψ_w) was measured in the fourth fully-expanded leaf of four plants of each treatment using pressure chamber technique (Turner, 1988). The osmotic potential (Ψ_s) of three leaves was measured using a freezing-point depression osmometer (Digital Osmometer, Roebeling, Berlin) at 25 ± 1 °C (Navarro et al., 2003). For this, fresh leaves were sampled, placed at -20°C for at least 24 h and, after that, the cell sap was collected from the frozen plant material upon centrifuging. The sap obtained was diluted 1:10 and immediately measured, and the recorded values were converted to MPa.

2.6. Electrolyte Leakage

The conductivity of the distilled water was measured (Cw). Twenty leaf discs of 0.5 cm diameter and sections of 1 cm of roots (200 mg; 4 replicates per treatment) were placed in a tube containing 10 mL of distilled water. The tubes were left during 2 h at room temperature and shaken every 15 min. After that, the conductivity of the solution was measured and referred as C0. Then, the tubes were placed at 80 °C during 1 h and afterwards left until reaching room temperature. The solution conductivity to this time was referred as CT. The percentage of electrolyte leakage (EL) was calculated as follow:

$$EL (\%) = [(C0 - Cw) / (CT - Cw)] \times 100$$

2.7. Mineral content

Dry shoots and roots were ground into a fine powder and were analysed to determine the mineral content at the Ionomics Laboratory (CEBAS-CSIC, Murcia, Spain) as described by Nicolas-Espinosa et al., (2024) and following the ISO 11.885 (1996) standards

using inductively coupled plasma–optical emission spectrometry (ICP–OES) on a Thermo ICAP 6500 Duo Instrument (Thermo Fisher Scientific, Waltham, MA, USA).

2.8. Lipid peroxidation

Lipid peroxidation of the lyophilized material was determined, with the thiobarbituric acid reactive substances (TBARS) assay (Cakmak and Horst, 1991). 100 mg of lyophilized powder was mixed with 1 mL of 0.1 % trichloroacetic acid (TCA) and centrifuged at $12,000 \times g$ for 15 min. The supernatant (0.5 mL) was added to 1.5 mL of 2-thiobarbituric acid (TBA) in a 20 % TCA solution., incubated at 90°C for 20 min, cooled on ice and centrifuged at $12,000 \times g$ for 5 min. The absorbance of the supernatant was measured at 532 nm. The value for nonspecific absorption at 600 nm was subtracted to absorbance at 532 nm, and the amount of malondialdehyde (MDA)-TBA complex was calculated: $C = A/\epsilon$ ($\epsilon = 155 \text{ mM}^{-1} \text{ cm}^{-1}$).

2.9. Total Phenolic Content

Total phenolic compounds were extracted from 100 mg of lyophilized powder of the aerial part and roots of control and treated melon plants with 1.5 mL of methanol, H_2O , and formic acid (25:24:1). Extracts were prepared in a sonicator bath for 1 hour, vortexed every 15 min, and stored at 4°C overnight. After centrifugation ($10,000 \times g$ for 15 min), the supernatant was filtered through $0.22 \mu\text{m}$ Millex syringe filters (PVDF) by Merck Millipore (Billerica, MA, USA), into vials for HPLC-MS analysis. Analysis was conducted using High-Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS) at the Metabolomic and Proteomic Laboratory (ACTI - Murcia University, Spain) following the procedure described by (Albaladejo-Marico et al., 2024).

2.10. Aquaporins gene expression

Aerial part and root samples from four plant of each treatment were stored at -80°C . Subsequently, they were then ground using liquid nitrogen. Total RNA was extracted from 100 mg of each sample using the NZY Total RNA Isolation kit (Nzytech, Lisbon, Portugal) following the kit's instructions. The quantities and purities of RNA were determined using a Nanodrop 1000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA). The RT Master Mix for qPCR II Kit (HY-K0510A-MedChemExpress, Sollentuna, Sweden) was used to synthesise cDNA from 200 ng of total RNA following the manufacturer's instructions.

RT-qPCR was carried out in an Applied Biosystems 7500 Real-Time PCR system with $2 \mu\text{L}$ of cDNA samples (1:10 diluted) and $8 \mu\text{L}$ of reaction mixture (600 nM gene-group primers (Table 1) and $5 \mu\text{L}$ of SYBR Green Master Mix 2X (Applied Biosystems)). The primes used are those corresponding to the amplification of the gene *CmRAN* (GTP-binding nuclear protein RAN) used as house-keeping, and to the amplification of aquaporins *CmNIP2;2* y *CmNIP5;1* (Lopez-Zaplana et al., 2020). The amplification protocol comprised a two-step process: initial denaturation at 95°C for 10 min, followed by 40 cycles of 15 s of denaturation at 95°C and 1 min of annealing and extension at 60°C . Subsequently, a dissociation stage was performed. These conditions were used for both target and reference genes. Three technical replicates and three biological samples were tested for each treatment. Transcript levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method (Livak and Schmittgen, 2001) for both target and reference genes.

2.11. Transmission electron microscopy

Transmission electron microscopy (TEM) was performed to visualize root and leaf samples of melon plants in order to determine whether NPs are present in plant cells. Thin samples of both tissues were cut into small pieces and treated as described Martínez-Ballesta et al., (2016). Finally, sections of different samples were examined using a JEOL-1011 transmission electron microscope (JEOL, Tokyo, Japan) with a digital camera GATAN-ORIUSSC200 (Gatan, Pleasanton, CA, USA). For the TEM analysis, four biological replicates were used for each treatment group, including both leaf and root samples. From each biological replicate, one block was prepared, and three grids were obtained from each block. A total of five grids were randomly observed for each treatment and organ to ensure representativeness and minimize bias.

2.12. Statistical analysis

Statistical analysis and data presentation were conducted using R Studio software (R Core Team 2018). Prior to these tests, normality and a Grubbs' test were conducted to identify potential outliers. The acquired data were subjected to a *t*-test or one-way ANOVA followed by *post-hoc* Tukey test. Significance levels were indicated as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for

Table 1
Primer set for gene expression analyses.

Gene name	Forward (5'-3')	Reverse (5'-3')
<i>CmRAN</i> *	TGCACCCCTTTGGACTTCTTC	GATGTAGTAGCCATCCCGTAAAC
<i>CmNIP2;2</i> *	GAGAAGAATGAATCTGAAATAGG	GAAAAGAAGAACCAATTTTATGG
<i>CmNIP5;1</i> *	AGAATAAAGTTGAGAAGAAAAGG	GGCAAGTAGAACAATATAGCA

* (Lopez-Zaplana et al., 2020)

$p < 0.001$, and n.s. for not significant differences. All presented values represent means $\pm$ SE. R packages FactoMineR (Lê et al., 2008) and factoextra (Kassambara and Mundt, 2020) were used to generate a Principal Component Analysis (PCA).

3. Results

3.1. Photosynthetic efficiency and performance index in *Cucumis melo* after TiO_2 and ZnO nanoparticles application

The impact of TiO_2 and ZnO nanoparticles (NPs) on the maximum quantum efficiency of PSII (Fv/Fm) and the performance index (PI) in *C. melo* plants was assessed at different time points post-application (PA) (Fig. 1). At 4 hours PA, a significant decrease in Fv/Fm was observed in plants treated with ZnO NPs compared to the control. However, this decrease was not detected in plants treated with TiO_2 -NPs. By 3 days PA, TiO_2 -NPs treatment resulted in a reduction of Fv/Fm compared to the control. Additionally, by 6 days PA and after 15 hours of salt stress, plants treated with TiO_2 -NPs showed a significant increase in Fv/Fm compared to the salt control (Fig. 1A). Regarding the performance index (PI), this parameter was significantly higher at 4 hours PA in plants treated with TiO_2 -NPs when compared to the control. Nevertheless, PI decreased at 3 days post-application. In contrast, ZnO-NPs led to a significant decrease in PI compared to the control initially and at 6 days, with an increase observed at 3 and 5 days after NPs application (Fig. 1B).

3.2. Alterations in Biomass and Water Relations in *Cucumis melo* Plants Induced by TiO and ZnO NPs

The application of TiO_2 and ZnO NPs had a significant impact on biomass accumulation in *C. melo* under both control and salinity conditions. Plants treated with TiO_2 -NPs showed an increase (22 %) in FW compared to the control plants. In contrast, ZnO-NPs led to a

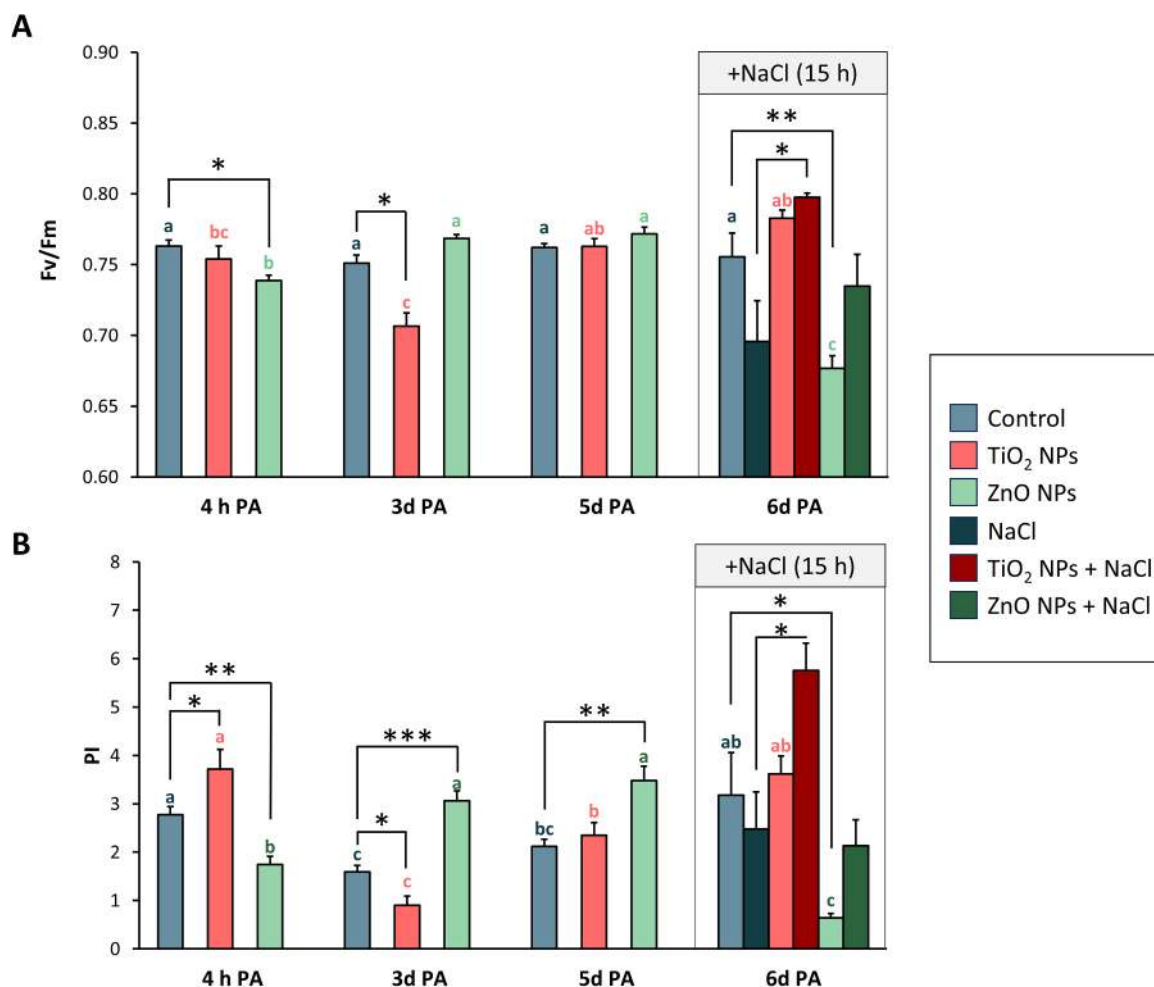


Fig. 1. Fv/Fm (A) and performance index (PI) (B) measured in melon plants at different times post application (PA) via roots of TiO_2 nanoparticles (NPs) and ZnO-NPs, and 100 mM NaCl. Each bar represents mean $\pm$ SE ($n = 4-6$). Asterisks indicate significant differences according to t -test of each group with their respective control without NPs (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Different letters indicate significant differences according to one way - ANOVA among different times of each group ($p < 0.05$) (each group is shown in a different font colour).

reduction in FW and DW, specifically under salinity stress (Fig. 2A-B). Additionally, the osmotic and water potentials were determined to assess the osmotic adjustment of the treated plants. Without salt stress shock, plants treated with TiO₂-NPs exhibited lower values of Ψ_s while both types of NPs induced values Ψ_w compared to untreated plants. In plants subjected to salt shock, nanoparticle treatments recorded the least negative potentials (Ψ_s and Ψ_w) compared to untreated plants (Fig. 2C-D).

3.3. Mineral analysis of *C. melo* treated with TiO₂ and ZnO NPs

A general mineral analysis of aerial part and roots of *C. melo* plants treated with TiO₂ and ZnO-NPs was carried out and it is shown in Fig. 3 and Table 2. The analysis of principal components carried out with the different variables corresponding to mineral elements (macronutrients, micronutrients and others) shows a clear separation of the groups, both in the aerial part (Fig. 3A) and in the roots (Fig. 3B). In particular, it can be seen how the groups corresponding to the plants treated with salinity are located in the upper left square, showing a strong correlation with the variable Na. In addition, we can observe how the group corresponding to ZnO-NPs shows a positive correlation with the variables Ca, P, Zn and Mo, both in the aerial part and in the roots. It is also remarkable how the TiO₂-NPs group correlates with variables corresponding to different metals.

Table 2 represents the results of the mineral analysis corresponding to macronutrients, micronutrients, Na, and Ti, as these were the most significant variables in this analysis. In the aerial part, TiO₂-NPs caused an increase in Ti levels, which appears to indicate a mobilisation of Ti from the roots, where an increase in Ti and a decrease in P and Mo levels have also been noted. With regard to ZnO-NPs, a decrease in Cu and an increase in Zn were observed in the aerial part of melon plants. Similarly, there was evidence of Zn mobilisation from the roots. In the root part, ZnO-NPs have been observed to induce an increase in the levels of Ca, Mo, Ti, and Zn. In plants that had undergone salt shock for 15 hours, it was observed that, in the aerial part, plants treated with ZnO-NPs exhibited a decrease in Cu, similar to that observed in the control conditions, and an increase in Zn compared to plants subjected to salinity but without NPs treatment. In the root part, both types of NPs were observed to cause an increase in Ca. Additionally, TiO₂-NPs elevated Ti levels and reduced Mo levels. Conversely, ZnO-NPs have been shown to solely elevate Zn levels. Among the alterations observed when comparing plants subjected to salinity with control plants, it was significant that under saline shock conditions, ZnO-NPs caused a decrease in B in the aerial part and in Mo and Mn in the root part. Conversely, TiO₂-NPs led to an increase in Fe in the aerial part, as well as an increase in Zn in the root part.

3.4. Membrane status in *C. melo* after TiO₂-NPs and ZnO-NPs treatments

To evaluate the state of the cell membranes of *C. melo* plants, two measurements were carried out. Firstly, electrolyte leakage (EL) was analysed, and secondly, lipid peroxidation was assessed. Both of these measurements were conducted on samples obtained from

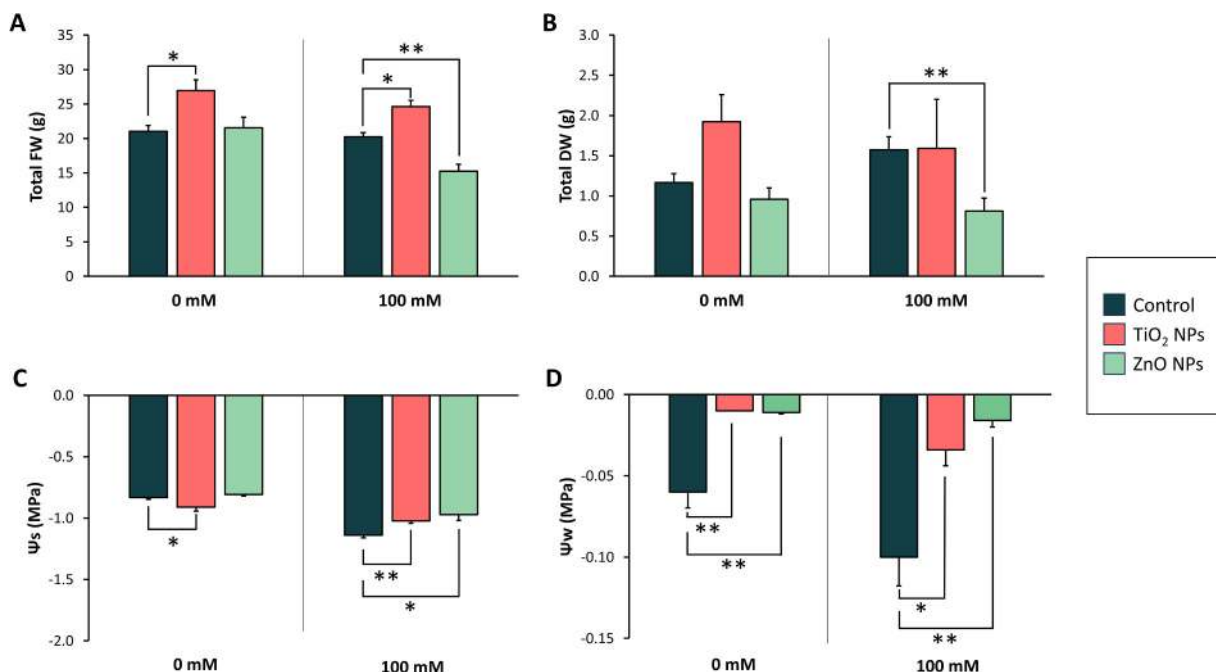


Fig. 2. Total fresh weight (FW) (A), total dry weight (DW) (B), leaf osmotic potential (Ψ_s) (C), and leaf water potential (Ψ_w) (D) of *Cucumis melo* plant treated with TiO₂ nanoparticles (NPs) and ZnO-NPs under control (0 mM NaCl) and salt stress (100 mM NaCl) conditions. Each bar represents mean $\pm$ SE ($n = 8-15$). Asterisks indicate significant differences according to t -test of each group with their respective control without NPs (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

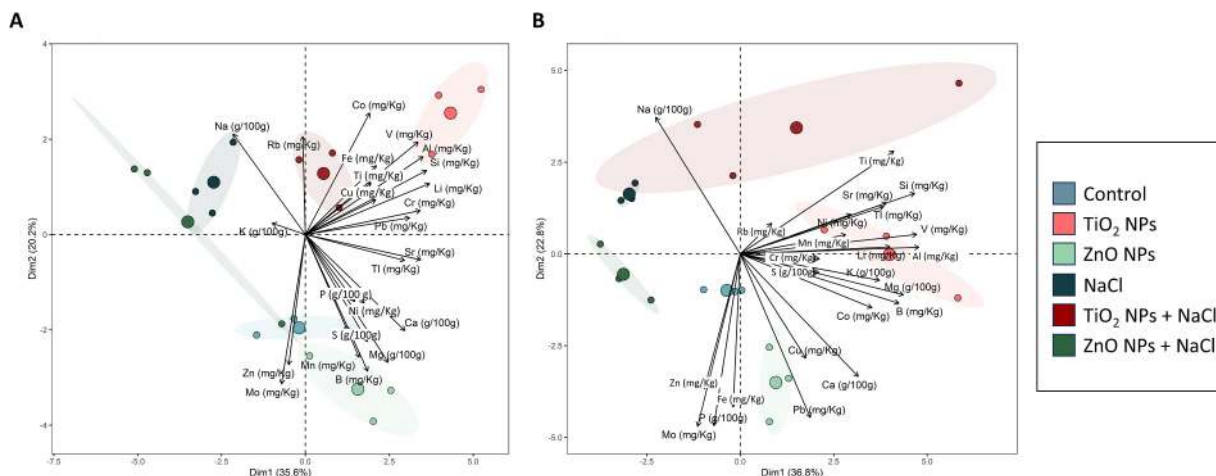


Fig. 3. Principal component analysis (PCA) of nutrients in the aerial part (A) and root (B) of *C. melo* plants treated with TiO_2 and ZnO-NPs. Arrows indicating loadings of each nutrient; 95 % confidence ellipses were plotted for each treatment, each small circle represents the data from an individual sample, while the large circle represents the centroid of mean values.

Table 2

Mineral analysis of *Cucumis melo* plant treated with TiO_2 nanoparticles (NPs) and ZnO-NPs under control (0 mM NaCl) and salt stress (100 mM NaCl) conditions. Data are mean $\pm$ SE (n = 3). Asterisks (*) denote significant differences within each group compared to the control without salt stress, based on a *t*-test ($p < 0.05$). Different letters indicate significant differences among treatments (control, TiO_2 -NPs, and ZnO-NPs) under either control or salt stress conditions, as determined by one-way ANOVA ($p < 0.05$).

Aerial Part	Control	TiO_2 -NPs	ZnO-NPs	NaCl	TiO_2 -NPs + NaCl	ZnO-NPs + NaCl
Ca (g/100 g)	6.40 $\pm$ 0.14 a	6.00 $\pm$ 0.28 a	6.38 $\pm$ 0.16 a	4.89 $\pm$ 0.15 a	5.46 $\pm$ 0.12 a	4.18 $\pm$ 0.27 a
K (g/100 g)	3.77 $\pm$ 0.07 a	3.38 $\pm$ 0.34 a	3.25 $\pm$ 0.11 a	3.73 $\pm$ 0.17 ab	3.59 $\pm$ 0.2 a	3.42 $\pm$ 0.11 b
Mg (g/100 g)	0.71 $\pm$ 0.03 a	0.67 $\pm$ 0.02 a	0.77 $\pm$ 0.02 a	0.64 $\pm$ 0.00 a	0.64 $\pm$ 0.01 a	0.56 $\pm$ 0.07 a
Na (g/100 g)	0.05 $\pm$ 0.01 a	0.01 $\pm$ 0.00 a	0.02 $\pm$ 0.00 a	0.86 $\pm$ 0.08 a*	0.73 $\pm$ 0.07 a*	0.70 $\pm$ 0.04 a*
P (g/100 g)	1.58 $\pm$ 0.05 a	1.37 $\pm$ 0.08 a	1.37 $\pm$ 0.05 a	1.31 $\pm$ 0.06 a*	1.41 $\pm$ 0.13 a	1.27 $\pm$ 0.08 a
S (g/100 g)	0.78 $\pm$ 0.01 a	0.59 $\pm$ 0.08 a	0.70 $\pm$ 0.06 a	0.60 $\pm$ 0.01 a*	0.67 $\pm$ 0.07 a	0.58 $\pm$ 0.07 a
B (mg/Kg)	50.58 $\pm$ 2.73 a	47.27 $\pm$ 2.66 a	55.62 $\pm$ 0.52 a	45.26 $\pm$ 0.61 a	47.00 $\pm$ 1.99 a	44.86 $\pm$ 2.57 a*
Cu (mg/Kg)	16.01 $\pm$ 0.4 a	15.12 $\pm$ 0.56 a	11.04 $\pm$ 0.74 b	13.95 $\pm$ 0.34 a*	14.96 $\pm$ 0.73 a	9.52 $\pm$ 1.19 b
Fe (mg/Kg)	142.52 $\pm$ 14.98 b	263.92 $\pm$ 22.41 a	166.67 $\pm$ 15.28 b	110.09 $\pm$ 1.36 a	178.63 $\pm$ 22.41 a*	216.49 $\pm$ 31.44 a
Mn (mg/Kg)	132.77 $\pm$ 3.73 a	124.04 $\pm$ 8.08 a	142.66 $\pm$ 4.33 a	112.00 $\pm$ 6.85 a	122.08 $\pm$ 7.95 a	123.71 $\pm$ 22.16 a
Mo (mg/Kg)	3.24 $\pm$ 0.09 a	1.96 $\pm$ 0.17 b	3.06 $\pm$ 0.26 a	2.70 $\pm$ 0.05 a*	2.17 $\pm$ 0.37 a	2.99 $\pm$ 0.55 a
Ni (mg/Kg)	0.49 $\pm$ 0.13 a	1.11 $\pm$ 0.09 a	2.91 $\pm$ 0.75 a	0.81 $\pm$ 0.39 a	0.75 $\pm$ 0.14 a	0.61 $\pm$ 0.19 a
Ti (mg/Kg)	1.60 $\pm$ 0.45 b	6.58 $\pm$ 0.73 a	2.48 $\pm$ 0.29 b	0.94 $\pm$ 0.11 a	3.85 $\pm$ 1.32 a	1.48 $\pm$ 0.55 a
Zn (mg/Kg)	138.03 $\pm$ 3.39 b	98.97 $\pm$ 9.96 b	480.31 $\pm$ 20.66 a	123.58 $\pm$ 1.72 b*	106.26 $\pm$ 11.77 b	365.21 $\pm$ 25.53 a*
Root	Control	TiO_2 -NPs	ZnO-NPs	NaCl	TiO_2 -NPs + NaCl	ZnO-NPs + NaCl
Ca (g/100 g)	0.51 $\pm$ 0.02 b	0.56 $\pm$ 0.03 b	0.71 $\pm$ 0.01 a	0.36 $\pm$ 0.00 b*	0.50 $\pm$ 0.04 a	0.48 $\pm$ 0.02 a*
K (g/100 g)	5.76 $\pm$ 0.01 a	5.64 $\pm$ 0.21 a	5.03 $\pm$ 0.23 a	4.67 $\pm$ 0.25 a*	4.88 $\pm$ 0.46 a	3.86 $\pm$ 0.34 a
Mg (g/100 g)	0.32 $\pm$ 0.01 a	0.34 $\pm$ 0.01 a	0.36 $\pm$ 0.00 a	0.26 $\pm$ 0.01 a*	0.34 $\pm$ 0.03 a	0.27 $\pm$ 0.01 a*
Na (g/100 g)	0.34 $\pm$ 0.14 a	0.39 $\pm$ 0.06 a	0.25 $\pm$ 0.06 a	1.97 $\pm$ 0.04 a*	2.1 $\pm$ 0.11 a*	1.86 $\pm$ 0.21 a*
P (g/100 g)	1.60 $\pm$ 0.06 a	1.39 $\pm$ 0.02 b	1.77 $\pm$ 0.04 a	1.41 $\pm$ 0.04 a	1.3 $\pm$ 0.06 a	1.49 $\pm$ 0.05 a*
S (g/100 g)	0.53 $\pm$ 0.01 a	0.52 $\pm$ 0.02 a	0.51 $\pm$ 0.03 a	0.51 $\pm$ 0.03 a	0.52 $\pm$ 0.02 a	0.48 $\pm$ 0.05 a
B (mg/Kg)	24.09 $\pm$ 0.31 a	26.37 $\pm$ 1.73 a	23.74 $\pm$ 0.69 a	21.34 $\pm$ 0.3 a*	22.31 $\pm$ 1.01 a	20.02 $\pm$ 0.14 a*
Cu (mg/Kg)	73.29 $\pm$ 1.34 a	77.86 $\pm$ 6.85 a	65.81 $\pm$ 3.98 a	51.19 $\pm$ 1.61 a*	53.37 $\pm$ 4.97 a*	61.27 $\pm$ 9.54 a
Fe (mg/Kg)	653.56 $\pm$ 30.92 a	701.51 $\pm$ 79.87 a	830.04 $\pm$ 47.67 a	514.17 $\pm$ 29.62 ab *	412.14 $\pm$ 56.79 b	976.44 $\pm$ 126.72 a*
Mn (mg/Kg)	140.17 $\pm$ 2.97 a	154.16 $\pm$ 15.18 a	115.77 $\pm$ 3.94 a	130.99 $\pm$ 18.55 a	130.22 $\pm$ 14.88 a	99.45 $\pm$ 1.57 a*
Mo (mg/Kg)	5.64 $\pm$ 0.13 b	3.71 $\pm$ 0.18c	7.38 $\pm$ 0.56 a	4.74 $\pm$ 0.13 a*	2.47 $\pm$ 0.07 b*	5.04 $\pm$ 0.28 a*
Ni (mg/Kg)	0.81 $\pm$ 0.05 a	1.06 $\pm$ 0.12 a	1.24 $\pm$ 0.15 a	0.99 $\pm$ 0.23 a	1.05 $\pm$ 0.18 a	0.75 $\pm$ 0.13 a
Ti (mg/Kg)	12.55 $\pm$ 1.22 b	182.8 $\pm$ 37.82 a	14.87 $\pm$ 2.12 b	4.22 $\pm$ 0.42 b*	239.08 $\pm$ 98.00 a	16.35 $\pm$ 3.34 b
Zn (mg/Kg)	282.61 $\pm$ 7.93 b	177.70 $\pm$ 18.10 b	3602.73 $\pm$ 66.88 a	332.08 $\pm$ 34.33 b	244.08 $\pm$ 9.40 b*	2517.64 $\pm$ 229.52 a*

the aerial part and from the roots (Fig. 4). The results demonstrate a reduction in the percentage of EL in the aerial parts of plants under control conditions when treated with the two types of NPs (-17 % with TiO_2 and -44 % with ZnO-NPs) (Fig. 4A). Additionally, the data show a notable reduction in lipid peroxidation (MDA-TBA complex) in the aerial parts of plants treated with ZnO-NPs, both under control conditions (-31 %) and when subjected to saline shock (-50 %) (Fig. 4C). No significant differences were observed in either of the two parameters measured in the roots (Figs. 4B and 4D). With regard to the impact of salt shock on plants, significant variations were observed in leaf MDA-TBA, with a reduction under salt shock in TiO_2 -NPs treated plants (Fig. 4C). Also, in EL within the root, with an enhancement in EL observed in the controls and in those treated with TiO_2 -NPs (Fig. 4B).

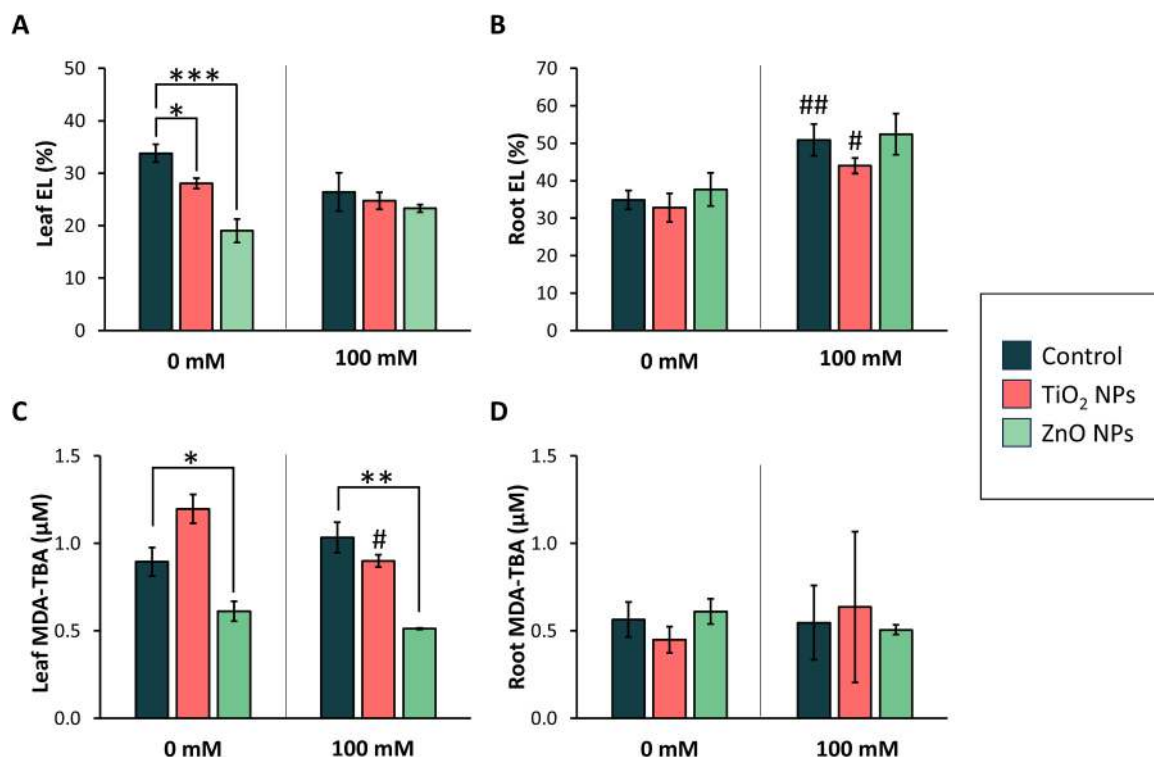


Fig. 4. Leaf Electrolyte leakage (EL) (A), root EL (B), Leaf (C) and root (D) lipid peroxidation (MDA-TBA complexes in μM) of *Cucumis melo* plant treated with TiO₂ nanoparticles (NPs) and ZnO-NPs under control (0 mM NaCl) and salt stress shock (100 mM NaCl) conditions. Each bar represents mean $\pm$ SE (n = 4–6). Asterisks indicate significant differences according to *t*-test of each group with their respective control without NPs (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and # symbol indicate significant differences according to the *t*-test (# $p < 0.05$, ## $p < 0.01$) to determine the independent effects of salt stress conditions.

3.5. Phenolic compounds

Phenolic compounds were analysed in both leaves and roots of melon plants under different conditions. Levels of isovanillic acid, caffeic acid, sinapic acid, and luteolin were detected in leaves (Fig. 5A–D), while only isovanillic and caffeic acids were detected in roots (Fig. 5E–F). The application of TiO₂-NPs resulted in a modification of the plant concentration of diverse metabolites. In the leaves, an increase in caffeic (57 %) and sinapic acid (57 %) was observed in plants that had undergone salt shock and had been treated with TiO₂-NPs (Fig. 5B–C). In the roots, a decrease in isovanillic acid was observed under control conditions (-59 %) (Fig. 5E). Conversely, the application of ZnO-NPs resulted in an increase (26 %) in luteolin under normal conditions in the leaves (Fig. 5D), while a generalised decrease in caffeic acid was observed in both the aerial part and root with the ZnO-NPs treatment (Fig. 5B and F).

3.6. NIP aquaporins expression analysis

The expression of two AQP was analysed, specifically two NIPs that have been identified as transporting a wide range of solutes in addition to water (Fig. 6 and Figure S2). In the aerial part, a decrease in the expression of *CmNIP2;2* was observed in plants treated with Zn-NPs under control conditions. In plants treated with NPs and subjected to saline shock, an increase in *CmNIP2;2* expression was observed when plants had been previously treated with TiO₂-NPs, while a repression of gene expression was seen with ZnO-NPs treatment (Fig. 6A). Regarding *CmNIP5;1* expression in both conditions, a decrease was observed in plants treated with ZnO-NPs (Fig. 6B). In the root part, *CmNIP2;2* expression increased in plants treated with TiO₂-NPs and decreased in those treated with ZnO-NPs. However, the opposite was observed when plants were subjected to salt shock: *CmNIP2;2* decreased in plants treated with TiO₂-NPs and increased in those treated with ZnO-NPs (Fig. 6C). Similarly, under control conditions, *CmNIP5;1* expression in the root increased with TiO₂-NPs treatment and decreased with ZnO-NPs treatment, whereas an increase in *CmNIP5;1* expression was observed in both treatments under saline shock (Fig. 6D).

3.7. TEM micrographs

Ultimately, TEM was employed to examine the ultrastructure of the roots (Fig. 7A–F) and leaves (Fig. 7G–L) of the treated plants, with the objective of detecting the presence of the NPs within the cells. Both types of NPs, applied via roots, in root sections, were

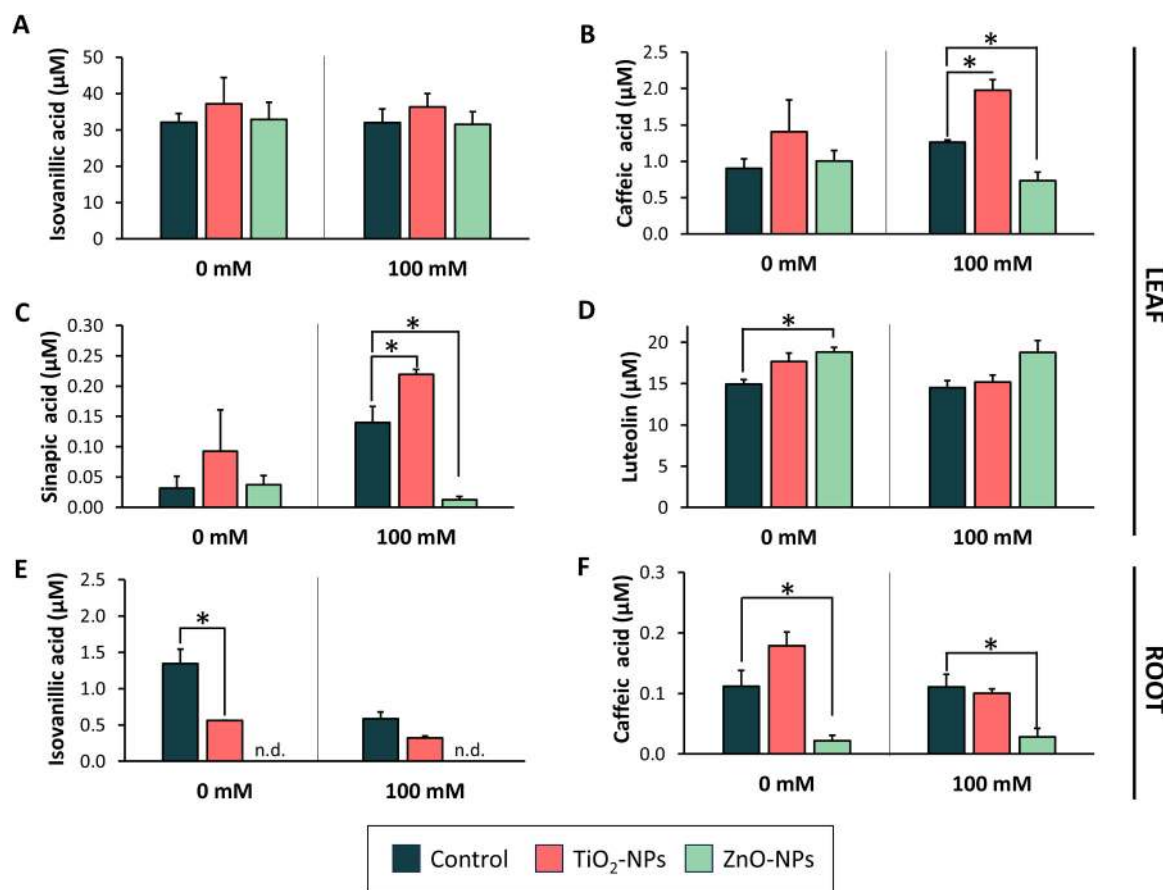


Fig. 5. Phenolic compounds (isovanillic acid, caffeic acid, sinapic acid, and luteolin) analysis of leaf (A-D) and root (E-F) from *Cucumis melo* plant treated with TiO₂ nanoparticles (NPs) and ZnO-NPs under control (0 mM NaCl) and salt stress shock (100 mM NaCl) conditions. Each bar represents mean ± SE (n = 3). Asterisks indicate significant differences according to t-test of each group with their respective control without NPs (* p < 0.05). n.d.: non-detected.

observed to be present within vacuoles and predominantly within intercellular spaces, as well as attached to membranes. TiO₂-NPs (Figs. 7B and 7E) are observed as high-contrast black dots with a regular structure, both in a dispersed form and in the form of aggregates. In contrast, ZnO-NPs (Figs. 7C and 7F) appear as a nebula with high black contrast relative to the surrounding cellular structures. These two types of distribution were not detected in any of the control samples examined, and when viewed under the microscope, they exhibited a high contrast compared to the rest of the sample surface, indicating that they were capturing a high electron flux. Furthermore, both TiO₂-NPs (Figs. 7H and 7K) and ZnO-NPs (Figs. 7I and 7L) were observed in the leaf sections, although at lower quantities than those present in the root.

4. Discussion

The use of novel technologies, including NPs of diverse types, in agricultural practices aims to promote efficiency in terms of yield and productivity. While previous studies have demonstrated the potential of TiO₂-NPs and ZnO-NPs in enhancing plant growth or playing an essential role in stress amelioration (Aqeel et al., 2022; Kandhol et al., 2022; Panahirad et al., 2024; Raliya et al., 2015), the underlying molecular mechanisms remain poorly understood, particularly with regard to water transport. In this study, we sought to identify the interactions between NPs and plants in order to understand their effect, both under normal conditions and in response to salt stress, paying particular attention to their role as protective agents, as summarised in Fig. 8. More specifically, our focus was on studying the effects of NPs during the primary plant response to the emergence of abiotic stress. Additionally, we compared the modes of action of two different NPs, which was a key factor in deciphering the mechanisms by which these materials influence crop responses. This study aimed to elucidate the interactions between NPs and plants under control and salt stress conditions, with a focus on their role as protective agents, as summarized in Fig. 8. Specifically, we examined their effects during the early plant response to abiotic stress and provided a comparative analysis of the distinct modes of action of TiO₂-NPs and ZnO-NPs. These insights represent a significant step toward deciphering the mechanisms by which NPs influence plant stress responses.

Previous work has shown that the application of NPs alters the photosynthetic state (Wei et al., 2024), which we also observed in

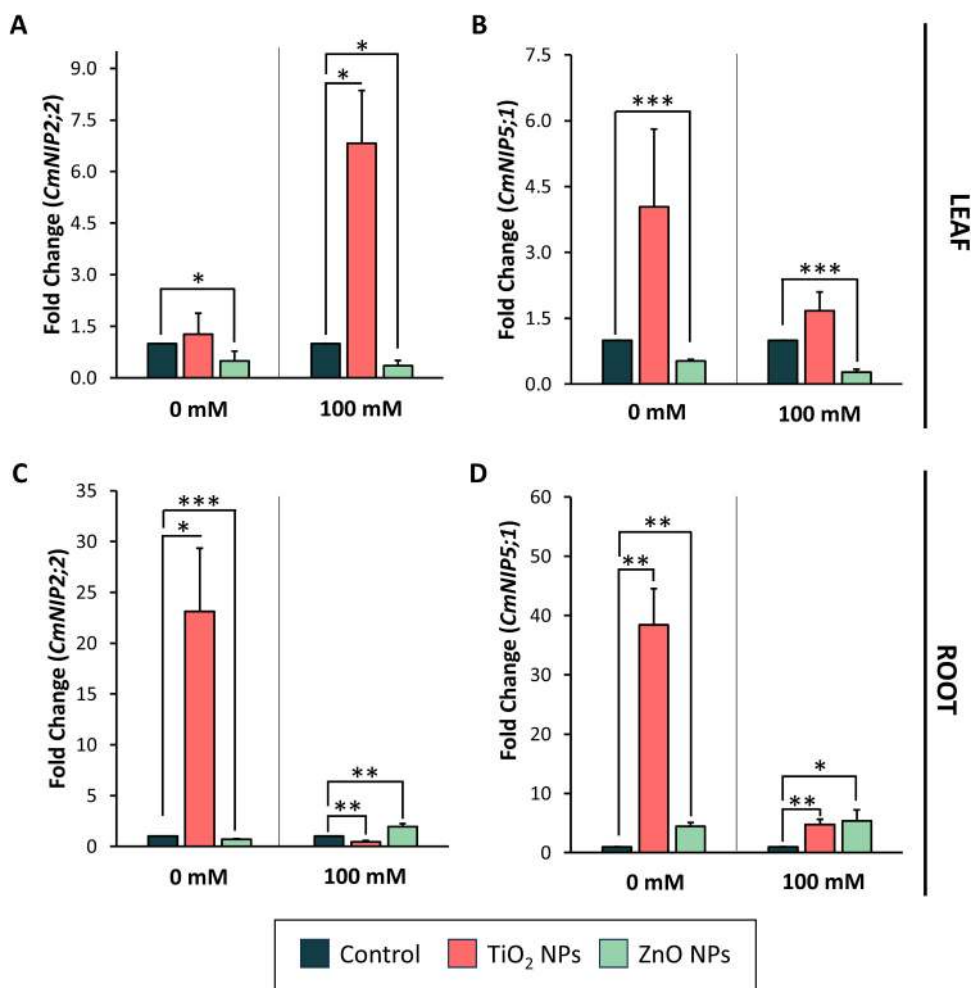


Fig. 6. Fold changes of *CmNIP2;2* and *CmNIP5;1* in aerial part (A-B) and root (C-D) of *Cucumis melo* plants treated with TiO₂ nanoparticles (NPs) and ZnO-NPs under control (0 mM NaCl) and salt stress shock (100 mM NaCl) conditions. Each bar represents mean $\pm$ SE ($n = 3-4$). Asterisks indicate significant differences according to *t*-test of each group with their respective control without NPs (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

our study. ZnO-NPs have an immediate effect in F_v/F_m , whereas TiO₂-NPs, showed no such reduction until 3 days later, indicating that melon plant modulates photosynthesis differently depending on the treatment. Furthermore, in the long term (6 days after application), ZnO-NPs had a negative effect, while TiO₂-NPs did not. In fact, this aligns with the results obtained for biomass, as plants treated with TiO₂-NPs showed a 22 % increase in total FW. Similar results have been reported in other crops, such as *Brassica chinensis* (Wei et al., 2024), *Oryza sativa* (Arshad et al., 2021), or *Triticum aestivum* (Ullah et al., 2020). In contrast, our study did not observe any photosynthetic benefit from the application of these NPs, although such effects have been reported in other studies, particularly with Ti NPs. These particles have been shown to capture light and have been shown to enhance photosynthetic activities (Wei et al., 2024). This discrepancy might be explained by the different application methods in our case, the NPs were applied through the roots rather than directly on the leaves, which could offset their light-capturing capacity. However, when plants treated with both types of NPs were exposed to salt shock, their photosynthetic efficiency remained stable compared to untreated plants, especially with TiO₂-NPs. This suggests that these treatments may act as protective agents against stress, maintaining photosynthetic efficiency at levels similar to control plants. As these NPs do not act directly on the leaf surface as light collectors, their protective role may involve influencing gene expression related to photosynthetic efficiency, such as *PetH* and *PetF*, which encode ferredoxin and participate in photosynthetic electron transport, as described previously by Wei et al., (2024).

In terms of osmotic adjustment, which is essential for maintaining homeostasis and plant growth, we found that both treatments increase leaf water potential under both non salinity and salinity conditions. This indicates that plants could experience restricted water availability (Munns, 2002). However, the fact that salt-treated plants also exhibited increased osmotic potential indicates their ability to maintain turgor, though water uptake and transport were likely restricted. Regarding how NPs regulate water potential, the higher osmotic and water potentials observed in treated plants suggest an improvement in water management within the plant rather than classical osmotic adjustment. This could involve better regulation of water transport, with aquaporins (AQPs) playing a central role. This hypothesis is supported by the differential expression of AQPs, particularly *CmNIP5;1*, which is involved in water transport

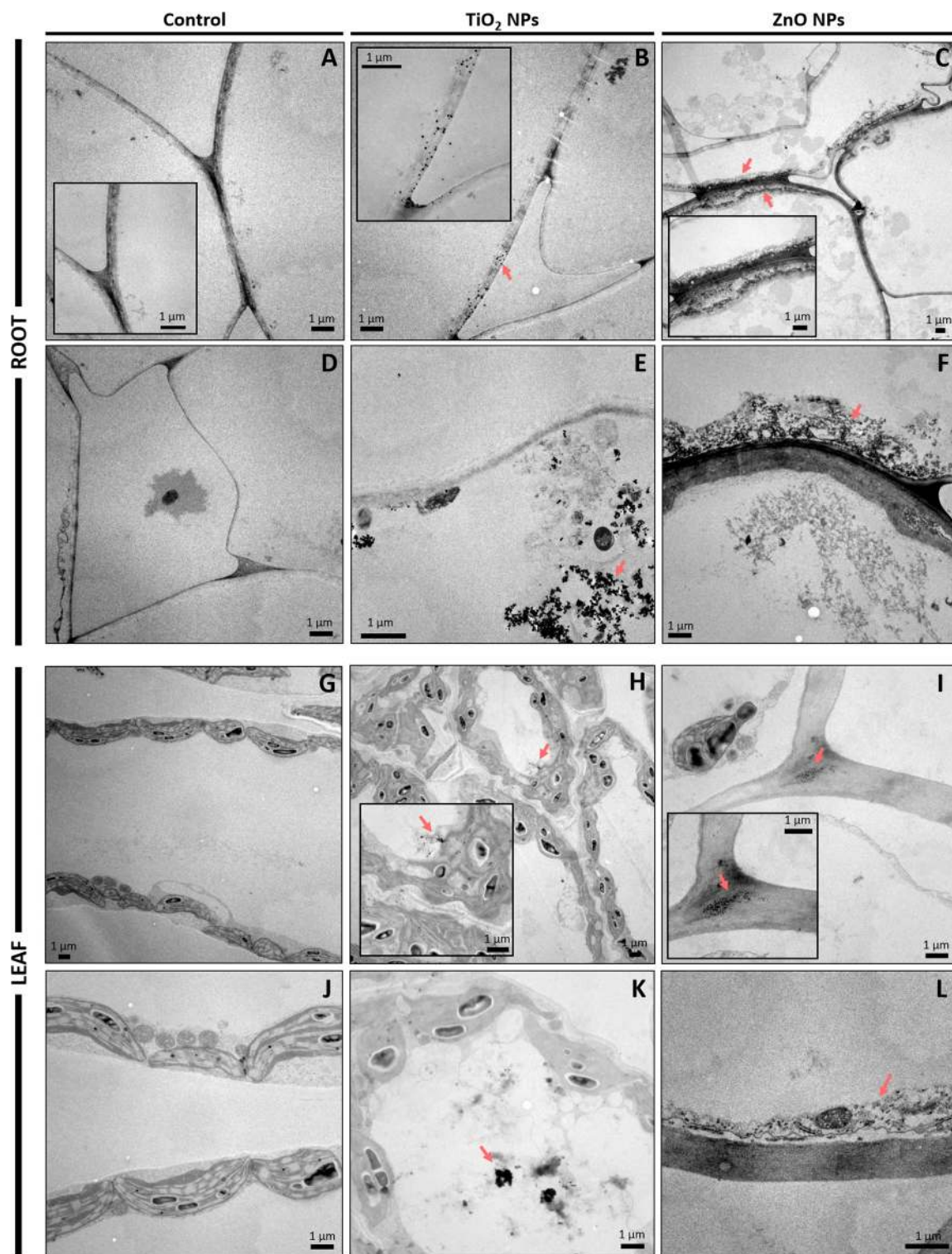


Fig. 7. Transmission electron microscopy (TEM) micrograph of different sections of root (A-F) and leaf (G-L) of *Cucumis melo* plants non-treated (control) (A, D, G, J) and treated with TiO₂ nanoparticles (NPs) (B, E, H, K) and ZnO-NPs (C, F, I, L). Condensed dark spots in case of TiO₂-NPs and dark nebulae in case of ZnO-NPs, shown with pink arrow, indicated presence of NPs. Scale bar = 1 μm.

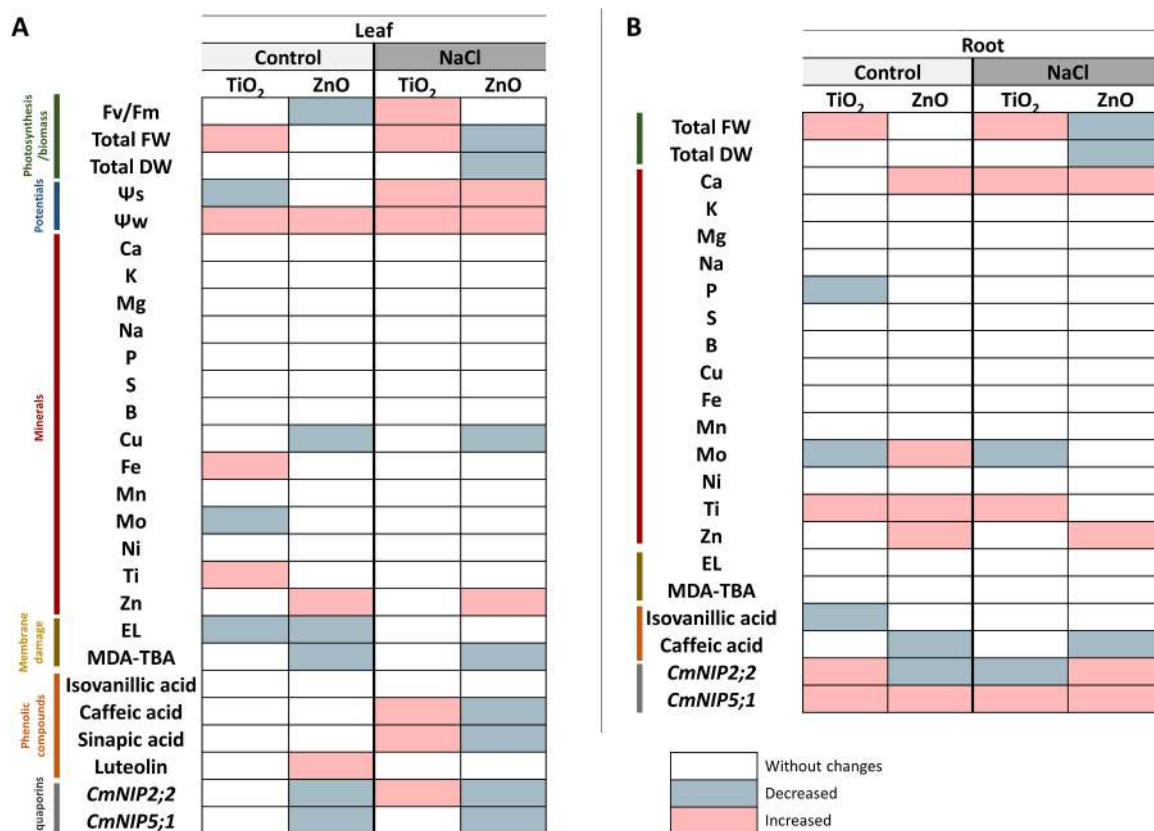


Fig. 8. Summary of the changes caused by TiO₂-NPs y ZnO-NPs treatments on leaves and roots of melon plants under control conditions and after salt shock.

(Lopez-Zaplana et al., 2022). In melon plants treated with both types of NPs under both control and stress conditions, *CmNIP5;1* was upregulated in roots, correlating with higher leaf water potential and more efficient water transport. This suggests that NP-treated plants may optimize water fluxes, maintaining cellular hydration and physiological processes under both normal and stress conditions. Several studies have shown that the expression of different aquaporins is modulated by the effect of metal nanoparticles (Akdemir, 2021; Mahmoud et al., 2022), which supports the idea that NPs could act as modulators of water transport mechanisms. Based on our findings, NPs appear to modulate AQP, with the upregulation of *CmNIP5;1* in NP-treated plants potentially linked to signaling pathways activated by different factors such as abscisic acid (ABA), a phytohormone known to increase in response to metal NPs (Tripathi et al., 2022) or calcium, which also rises in the roots of plants subjected to salinity and treated with NPs. These pathways are well-documented regulators of aquaporin gene expression and activity (Li et al., 2014), suggesting a mechanism through which NPs may enhance water transport. These mechanisms provide an adaptive advantage to NP-treated plants, mitigating the detrimental effects of salinity-induced osmotic stress and enhancing their ability to maintain turgor and physiological functions under adverse conditions.

In addition to the direct correlation between *NIP5;1* overexpression and enhanced aerial water status, other responses were observed. *NIP2;2* has been characterised by its ability to facilitate the silicon (Si) transport (Lopez-Zaplana et al., 2022), which has been demonstrated to elicit comparable effects with titanium (Ti), both tetravalent. An increase in *CmNIP2;2* expression was observed when TiO₂-NPs were applied, although only in the control conditions. This phenomenon was also documented in a previous study, wherein the expression of multiple NIP aquaporins was observed to increase in response to Ti (Pérez-Zavala et al., 2022). In another case, the expression of 19 AQP was modified as a consequence of foliar treatment with TiO₂-NPs as described by Wei et al. (2024). The overexpression of *CmNIP2;2* has been observed to correlate with increased Ti in the aerial part. However, under salinity conditions, *CmNIP2;2* was found to be down-regulated in roots and up-regulated in the aerial part. This indicates that the reduced expression in roots may function as an adaptive mechanism to restrict excessive Ti uptake under salt stress, while its up-regulation in the aerial part could facilitate the redistribution and accumulation of Ti in leaves, potentially for storage or detoxification purposes.

Higher water transport efficiency, coupled with the increased FW observed in plants treated with TiO₂-NPs, may also reflect an improvement in the cell membrane structure of aerial tissue of melon plants, as reflected by lower electrolyte leakage (EL). For ZnO-treated plants, the reduction in EL was accompanied by decreased lipid peroxidation, suggesting activation of the plant's detoxification system. Although ZnO-NPs did not enhance biomass, the plant appears to perceive these particles as potentially toxic, triggering protective responses that reduce membrane damage and improve water status. This activation of antioxidant enzymes by ZnO-NPs has

been documented in various studies on tomato, pepper, and melon plants (Faizan et al., 2021; García-López et al., 2019; Rehman et al., 2023). In the roots, no changes in membrane integrity were observed, as there were no alterations in EL or lipid peroxidation under any condition. This is significant because the roots serve as the entry point for the NPs. The absence of adverse effects on root physico-chemical structure is consistent with other studies (Lian et al., 2020). This suggests that NPs entry at the membrane level appears to be harmless, while still enabling the modulation and activation of signalling systems that impact the aerial parts. The presence of the NPs in membranes was confirmed via TEM. Both types of NPs preferentially localized near membranes and cell walls, where they can interact with cellular components, as previously described for TiO₂ and ZnO-NPs (Larue et al., 2012; Lin and Xing, 2008). Additionally, the presence of NPs within vacuoles suggests a potential storage mechanism for sequestering and mitigate toxicity. Furthermore, the translocation of NPs from roots to aerial parts tissues was evident, although their concentration in aerial parts was significantly lower than in the roots. Intracellular NPs mobility has been extensively described by Larue et al. (2012), highlighting cell-to-cell transport through plasmodesmata—a process we also observed in this study (Figure S3). Finally, the transport of water from roots to leaves, which is strongly influenced by transpiration, facilitates NPs movement through vascular vessels (Larue et al., 2012; Zhang et al., 2011).

Regarding to nutrient uptake and transport, it has been reported that the application of NPs can influence this process (Usman et al., 2020). In addition, in this process the solubility and ion release of metal NPs in hydroponic system are critical factors influencing their interactions with plants and the surrounding medium. In our study, different treatments altered nutrient levels in both aerial parts and roots, as shown by principal component analysis. Specifically, TiO₂ and ZnO-NPs significantly increased Ti and Zn concentrations in roots, and both elements were mobilised towards leaves. Although elevated levels of Ti and Zn could potentially be toxic (Lyu et al., 2017), no signs of toxicity were observed in the plants, as evidenced by unaltered biomass yield and the absence of cellular damage. Indeed, for Ti, its beneficial effects on plant growth may arise from enhanced nutrient uptake, such as Fe, or increased Fe activity in tissues, which regulates photosynthesis (Carvajal et al., 1995; Lyu et al., 2017). In this study, TiO₂-NPs markedly increased Ti levels throughout the plant, leading to a significant elevation in leaf Fe levels, possibly due to surface energy effects (Ma et al., 2010). In contrast, Zn reached moderately toxic levels in aerial tissues and highly toxic levels in roots. It can be hypothesized that the elevated solubility of NPs in aqueous solutions is the reason for their ability to release Zn²⁺ ions, which can directly influence plant nutrient uptake and physiological responses. This is in contrast to TiO₂-NPs, which are considered to be less soluble due to their ability to form stable colloidal suspensions with minimal ion release (Aruoja et al., 2009; Bhuvaneshwari et al., 2017). This corresponded with reductions in biomass and Fv/Fm in ZnO-NP-treated plants. However, these toxic Zn levels did not result in increased lipid peroxidation, as described in other studies (Anwaar et al., 2015). This could be due to activation of the ROS detoxification machinery, preventing membrane damage. Beyond Ti and Zn, NPs influenced other nutrients. Under salinity conditions, both treatments increased root Ca levels, countering the typical reduction in Ca uptake caused by salinity (Hadi and Karimi, 2012). As Ca has a protective effect under salinity stress (Davenport et al., 2005) this suggests a protective role for NPs. Conversely, ZnO-NPs reduced Cu levels in aerial tissues under both conditions, likely due to competition between Cu and Zn for root absorption sites (Imtiaz et al., 2003).

Our findings (Fig. 8) suggest that TiO₂-NPs and ZnO-NPs interact with plant tissues through distinct mechanisms, influencing the plant's response to salinity. The profile of phenolic compounds provides evidence of this phenomenon, showing opposite trends under saline conditions. TiO₂-NPs promoted the accumulation of caffeic and sinapic acids, while ZnO-NPs decreased these compounds. Phenolic compounds, including caffeic acid, have been demonstrated to play a pivotal role in non-enzymatic antioxidant defence mechanisms by neutralising ROS and mitigating oxidative damage (Bose et al., 2014). The observed increase in these compounds in the presence of TiO₂-NPs suggests that the plants employed a phenolic-mediated pathway to cope with oxidative stress induced by salinity. Conversely, the reduction of caffeic acid under ZnO-NPs treatment might indicate a divergent mechanism of ROS detoxification. However, it is crucial to acknowledge that, despite the decline in caffeic acid levels, plants treated with ZnO-NPs exhibited a reduction in lipid peroxidation in their leaves, as evidenced by a decrease in MDA content under both control and saline conditions (Fig. 4C). This finding suggests that ZnO-NPs may activate alternative antioxidant pathways, such as enzymatic defences, to mitigate oxidative stress. Consequently, while TiO₂-NPs appear to enhance phenolic-based antioxidant responses, ZnO-NPs likely engage distinct mechanisms to maintain oxidative balance. These observations underscore the intricate nature of plant-nanoparticle interactions and suggest that both NPs modulate stress responses, albeit through different pathways.

Despite the fact that the present study primarily investigates the short-term effects of NPs on melon plants following salt shock stress, the potential long-term impacts of these NPs on plant health and the environment deserve further consideration. Prolonged exposure to NPs has the potential to result in their accumulation in plant tissues or the surrounding environment, which may lead to phytotoxicity or alterations in soil homeostasis (Dev et al., 2017). From an environmental perspective, the persistence and mobility of these NPs in the ecosystem are critical factors that should be the focus of future research. ZnO-NPs, for example, have been observed to dissolve and release Zn²⁺ ions, which have been shown to be toxic at high concentrations (Xu et al., 2016). Similarly, although more stable, TiO₂ NPs have been found to accumulate in soil and aquatic systems, potentially affecting non-target organisms. Therefore, it is crucial to assess different concentrations to strike a balance between benefits and avoidance of toxicity. Future studies should therefore address these gaps by assessing the long-term effects of NPs under field conditions, evaluating their accumulation in plant tissues and monitoring their impact on soil health.

5. Conclusion

The present study demonstrates that the application of TiO₂-NPs and ZnO-NPs in melon plants modulates several physiological responses, both under control and saline conditions, providing protective effects through distinct mechanisms. TEM analysis confirmed the presence of both NPs in root and aerial tissues, localising near membranes, cell walls, and vacuoles, suggesting intracellular

activity. Both NPs modified osmotic adjustment, reducing water uptake and transport while increasing leaf water potential. These effects correlated with the overexpression of *CmNIP5;1* in the roots, promoting water movement. ZnO-NPs reduced lipid peroxidation and electrolyte leakage, activating detoxification mechanism, while TiO₂-NPs increased FW, improved cell stability, and reduced membrane damage. Under saline stress, TiO₂-NPs induced the accumulation of caffeic and sinapic acids, compounds known for their role in scavenging ROS and protecting cells from oxidative damage. This suggests that TiO₂-NPs might activate phenylpropanoid biosynthesis pathways as part of their protective mechanism. Interestingly, ZnO-NPs did not induce this phenolic accumulation, indicating divergent modes of action between the two nanomaterials. These findings suggest that TiO₂-NPs and ZnO-NPs elicit distinct molecular and physiological responses in plants. TiO₂-NPs appear to provide more consistent benefits under both control and saline conditions, making them a promising candidate for enhancing plant resilience. Further studies should investigate the precise molecular pathways involved in nanoparticle-plant interactions, including their potential effects on ROS homeostasis, hormonal signalling, and aquaporin regulation, to better understand their long-term implications for crop improvement under stress scenarios.

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CRediT authorship contribution statement

Albaladejo-Marico Lorena: Writing – review & editing, Methodology, Investigation, Formal analysis. **Thameur Afwa:** Writing – review & editing, Methodology, Investigation. **Garcia-Martinez Antonio:** Investigation. **Carvajal Micaela:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition. **Yepes-Molina Lucia:** Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eti.2025.104101](https://doi.org/10.1016/j.eti.2025.104101).

Data availability

Data will be made available on request.

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GLOSSARY

AQPs: aquaporins
 DW: dry weight
 EL: electrolyte leakage
 Fv/Fm: chlorophyll fluorescence
 FW: fresh weight
 MDA-TBA: malondialdehyde - 2-thiobarbituric acid
 NIP: nodulin 26-like intrinsic proteins
 NPs: nanoparticles
 PCA: principal component analysis
 PI: performance index
 TBARS: thiobarbituric acid reactive substances
 TCA: trichloroacetic acid
 TEM: transmission electron microscopy
 TiO₂: titanium dioxide
 ZnO: zinc oxide
 Ψ_w: water potential
 Ψ_s: osmotic potential.