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Green-Synthesized Zinc Oxide Nanoparticles Mitigate Salt Stress in Soybean

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Abstract

Soybean [*Glycine max* (L.) Merrill] is a high value leguminous crop characterized by its excellent protein and oil qualities. The legume is also excellent as human food, animal feed, industrial raw material and for its ability to improve soil quality through nitrogen fixation. However, while this crop has all these attractive attributes and benefits, its agricultural production is seriously affected by salt stress. Thus, any research effort aimed at understanding the physiological response of this crop to salt stress is worthwhile. In this study, we report the green synthesis of zinc oxide nanoparticles (ZnO-NPs) and use of such NPs as a priming agent, to mitigate salt stress in a PAN 1644R soybean variety that is commonly grown in the tropics. Findings showed that while salt stress (100 mM NaCl) significantly affected key plant attributes such as seed germination (e.g., delay), early seedling development (e.g., stunted radicles), morphological features (e.g., pale leaves), physiological traits (e.g., low chlorophyll content) and physiological processes (e.g., reduced photosynthesis), the ZnO-NPs (at 200 mg·L^{-1} dosage) used counteracted all these effects, allowing plants to grow optimally with enhanced statuses. We then concluded that the ZnO-NPs mitigate salt stress in soybean, underscoring the importance of this study to food security and sustainable agricultural practices.

Keywords: soybean; salt stress; ZnO-NPs; germination; early seedling development; morphological features; physiological traits; physiological processes

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1. Introduction

Soybean [*Glycine max* (L.) Merrill] is an important leguminous crop that provides excellent and quality nutrients to humans and livestock [1,2]. The crop is also important to industries as a source of raw materials and agriculture for its ability to improve soil quality through nitrogen fixation [3,4]. However, despite all of these attractive attributes and benefits, soybean production is severely affected by salt stress, because the crop is a glycophyte that lacks effective mechanisms for tolerating high soil salinity [5].

Typically, salt stress affects plants by disturbing the ionic and water equilibrium, which results in both ionic stress and osmotic stress [6,7]. In the early stages of plant development, salt stress inhibits water absorption, root development, cell elongation, and leaf formation, while in the later stages, salt accumulation hinders enzyme activity and inhibits photosynthesis, resulting in premature senescence [8]. Additionally, salt stress

triggers the production of reactive oxygen species (ROS), which are responsible for oxidative damage to lipids, proteins, and nucleic acids, disrupting normal metabolic processes [9]. Overall, all these effects of salt stress result in poor development, reduced growth, low productivity and ultimately death of the affected plant [10].

Naturally, even though different plants have developed varying mechanisms of tolerating salt stress, this stress factor still poses a huge challenge, particularly in crop production because it affects nearly every developmental stage of plant growth, including germination, vegetative growth, and reproduction [11]. Growth suppression occurs due to a decrease in osmotic potential that prevents roots from absorbing water and nutrients. Major negative impacts would then be noticed at the level of the entire plant through death and/or a decline in outputs [12].

Considering that nowadays there is a strong growing demand for sustainable agricultural practices using eco-friendly approaches to improve crop tolerance to various stress factors, the probable use of priming agents such as nanoparticles (NPs) could be a plausible solution to the production of soybean under the persistent challenge of salt stress worldwide [13]. Technically, priming agents are defined as substances, usually containing naturally occurring compounds, that when applied to plants, can help in mineral nutrition, abiotic stress tolerance, crops yields, and quality characteristics [14–17]. In this study, we report the banana-based synthesis of zinc-oxide nanoparticles (ZnO-NPs) and their use as a priming agent on soybean seeds subjected to salt stress to demonstrate the capacity of these nanocomposites to mitigate such stress.

2. Materials and Methods

The experimental procedures carried out in this study are summarized in the workflow below (Figure 1) and fully described in proceeding sections.

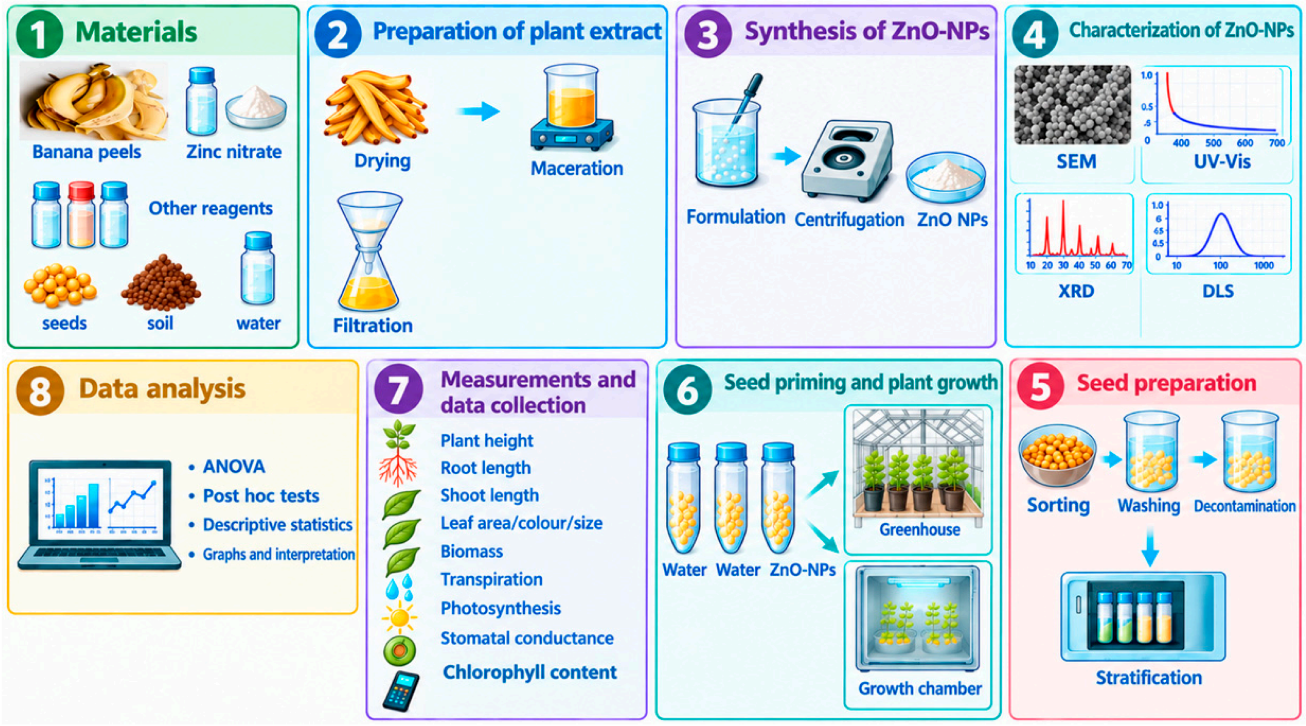


Figure 1. Layout of experimental procedures.

2.1. Source of Materials

The ZnO-NPs used in this study were synthesized in the Nanotechnology Research Laboratory, Chemistry Department, North-West University (NWU), Republic of South

Africa (RSA). Bananas were purchased from a local market (Megacity, Mmabatho, North West, South Africa) for their peel extract to serve as a source of the reducing and stabilizing agent during NP formulation. Zinc nitrate (precursor), sodium tripolyphosphate, sodium chloride, sodium hydroxide, ethanol and Triton X-100 of high-grade level were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Commercial bleach was purchased from a local market (Mmabatho). All aqueous solutions were prepared using deionized water. Water, filter papers and potting soil used in this study were sterilized by autoclaving at 121 °C for 15 min. The soybean cultivar PAN 1644R seeds used in this study were purchased from PANNAR (Pvt) Ltd. (Durban, Kwa-Zulu Natal, South Africa), while the potting soil mix used in this study was bought from Culterra (Muldershift, Gauteng, South Africa). All plant-related studies were then carried out in the Plant Biotechnology Research Laboratory, Botany Department, NWU, South Africa.

2.2. Preparation of the Plant Extract

Banana peels were removed from the fruit and washed thoroughly with tap water. The peels were then cut into 1 cm^3 pieces for easy drying at 60 °C overnight. An aqueous extract of the banana peels was then prepared by heating 4 g of the dried chopped peels in 100 mL of deionized water at 60 °C for 2 h, stirring at 500 rpm. The solution was then filtered in an Erlenmeyer flask using a 124 mm Whatman filter paper (No 1). Next, 50 mL of water was then added to 50 mL of the filtrate, to generate an extract.

2.3. Preparation of the ZnO-NPs

The desired ZnO-NPs were synthesized as described by Zungu et al. [18] using the banana peel extract generated above (Section 2.2) as a source of bio-reducing and stabilizing agents. Briefly, to 100 mL of the banana peel extract, 50 mL of 3.3 mmol·L^{-1} $Zn(NO_3)_2.6H_2O$ solution was added drop-wise while stirring gently at 100 rpm for 10 min. The pH of the resultant mixture was adjusted to 10 using a 1 mol·L^{-1} NaOH solution. The mixture was then incubated at 50 °C for 24 h to allow further reaction. After 24 h, the reacted mixture was centrifuged at 1500× g for 10 min. The pellet was kept while the supernatant was discarded. The pellet was rinsed once with 5 mL of ethanol and twice more with 10 mL of deionized water. The rinsed pellet was then dried in an oven at 60 °C for 48 h, producing the intended ZnO-NPs.

2.4. Characterization of the NPs

The synthesized ZnO-NPs were characterized according to AbdElhady [19], using different physicochemical methods to ascertain their fitness for use in the study.

2.4.1. Scanning Electron Microscopy (SEM)

This method was used to identify the morphology, size and agglomeration state of the NPs. Briefly, 2 mg of the generated ZnO-NPs was dispersed in 1 mL of 100% (v/v) ethanol followed by sonication for 15 min at room temperature, to generate a colloidal suspension. Next, 2 µL of the generated colloidal suspension was placed on a 300-mesh carbon-coated copper grid, and the setting left to dry at room temperature for 2 h. The set grid was then loaded onto a JEOL JEM 2100 microscope (JEOL Incorporated, Peabody, MA, USA) for imaging and the generated images analyzed using the ImageJ (1.54t) software (National Institutes of Health, Bethesda, MD, USA).

2.4.2. Ultraviolet Visible (UV-Vis) Spectrophotometry

This method, which depends on peaks of the spectrum at different wavelength ranges, was used to determine the formation of the zinc metal oxide NPs. That is, 1 mg of the NPs suspended in 1 mL of H_2O was added to a 1 mL disposable transmittance cuvette.

Absorbance spectrum was then measured between 400 and 700 nm wavelengths, using a 365 UV/V is spectrophotometer (Separation Scientific, Cape Town, Western Cape, South Africa).

2.4.3. X-Ray Diffraction (XRD) Analysis

This method was used to identify the composition and crystal quality of the synthesized NPs. An AXS X-ray D8 Advance diffractometer (Bruker, Berlin, Germany) equipped with a Cu-K_α radiation source and LynxEye positive sensitive detectors was used. Briefly, 0.5 mg of the dried NPs suspended in 500 µL of H_2O was mounted on a glass-slide sample holder and adjusted to the correct height. Measurements were then run and taken at a wavelength of 0.154 nm, voltage of 40 kV and current of 30 mA within a range of fixed 2θ angles and a typical step size of 1.00° per angle. In other words, the analysis was operated in a continuous θ-θ scan ranging from 2θ = 5 to 80 degree angles, locked in coupled mode with Cu-K_α radiation. Data was then collected using Bruker (TopSpin 4.x) software (Bruker, Johannesburg, Gauteng, South Africa) followed by analysis with Origin 2025 (10.2 series) software to demonstrate characteristic diffraction peaks.

2.4.4. Dynamic Light Scattering (DLS)

This method was used to measure and determine the sizes, charges (zeta potential), stability and polydispersity indices of the synthesized NPs. In short, 1 mg of the dried NP was suspended in 5 mL dH_2O and filtered using a 0.45 µm filter to obtain a clear solution. Next, 1 mL of the filtered NP was added to a disposable transmittance cuvette to measure its size, zeta potential and polydispersity indices using a Malvern Zetasizer (Separation Scientific, Cape Town, Western Cape, South Africa).

2.4.5. Fourier Transform Infrared (FTIR) Spectroscopy

This method was used to identify phytochemicals of the banana peel extract and their associated functional groups. Briefly, 2 mg of the dried NPs suspended in 1 mL of H_2O was placed onto a glass-slide sample holder of an AXS X-ray D8 Advance diffractometer (Bruker, Berlin, Germany) followed by subjection of the mounted NPs to infrared light over a wavenumber range of 4000 to 400 cm^{-1} and resolution of 4 cm^{-1}. Functional groups associated with the synthesized NPs were then clustered according to their spectral wavelengths, and ultimately analyzed using the OriginPro 2019b software.

2.5. Preparation of Solutions

Sterile water and 200 $mg{\cdot}L^{-1}$ ZnO-NPs solution were prepared for use as priming solutions (Figure 2), while 100 mM NaCl solution was prepared and used to induce salt stress (Figure 3).

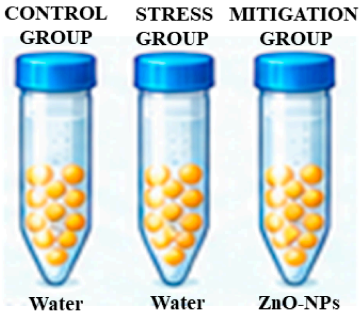


Figure 2. Priming of the soybean seeds. Seeds in the control and stress groups were primed with water, while those in the mitigation group were primed with 200 $mg{\cdot}L^{-1}$ ZnO-NPs.

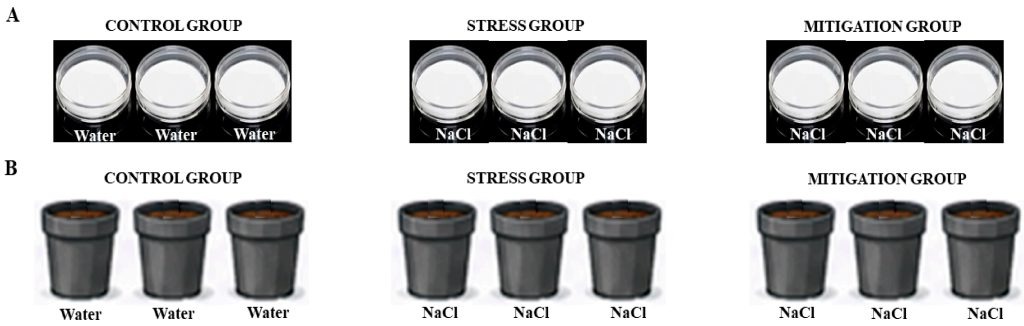


Figure 3. Preparation of Petri dishes and pots for the study. Each Petri dish was set to contain a filter paper, while each pot was set to contain soil. (**A**) Each Petri dish in the control group was treated with 2 mL of sterile water, while each Petri dish in the stress and mitigation groups was treated with 2 mL of 100 mM NaCl solution before placing the primed seeds on the filter papers to germinate and maintain seedlings in a growth chamber. (**B**) Each pot in the control group was treated with 200 mL of sterile water, while each pot in the stress and mitigation groups was treated with 200 mL of 100 mM NaCl solution before sowing the primed seeds in the soil to germinate and maintain plants in a greenhouse.

2.6. Seed Sorting, Washing, Decontamination and Stratification

The PAN 1644R soybean seeds were sorted through suspension in sterile water and selecting only those that settled to the bottom of the container for further use and discarding those that floated. The selected seeds were then washed twice with 20 mL of sterile water through suspension for 1 min and vortexing for 1 min before discarding the used water. The washed seeds were then surface-sterilized for 2 min in 20 mL of 30% (v/v) hypochlorite (commercial bleach) and 0.02% (v/v) Triton X-100 solution through suspension for 1 min and vortexing for 1 min before discarding the used solution. The decontaminated seeds were then washed thoroughly (three times) with 60 mL of sterile distilled water. After decontamination, the seeds were stratified (to break dormancy and induce germination) by submergence in sterile distilled water for 48 h at 4 °C.

2.7. Seed Priming

After stratification, a total of 54 seeds were primed so that 27 could be used in the Petri dish-based experiment while the other 27 were used in the pot-based experiment. Briefly, three falcon tubes were labelled as control group, stress group and mitigation group (Figure 2), and 18 seeds were placed in each tube. In the control-group tube, 20 mL of sterile water was added, in the stress-group tube, 20 mL of water was also added, while in the mitigation-group tube, 20 mL of a 200 mg·L^{-1} ZnO-NPs solution was added. After this, the three tubes were left to stand at room temperature for 4 h. After 4 h, seeds were carefully removed from their respective solutions and blotted on sterile tissue papers before being placed in similarly labelled fresh falcon tubes (Figure 2).

2.8. Preparation of Petri Dishes and Pots

A total of nine Petri dishes were collected and in each Petri dish, a 3 HW 90 mm Munktell filter paper was placed. The nine Petri dishes were then divided into three groups of three with the first group labelled as control group, the second group labelled as stress group and the third group labelled as mitigation group (Figure 3A). In the control group, each filter paper was flooded with 2 mL of sterile water before the Petri dishes were closed with their lids. In the stress and mitigation groups, each filter paper was flooded with 2 mL of 100 mM NaCl solution before the Petri dishes were closed with their lids (Figure 3A). Alongside this setting, a total of nine pots were collected and each pot was filled with soil mix composed of 3 parts peat-based soil and 2 parts vermiculite. The nine pots were then

divided into three groups of three with the first group labelled as control group, the second group labelled as stress group and the third group labelled as mitigation group (Figure 3B). In the control group, the soil mix in each pot was flooded with 200 mL of sterile water while in the stress and mitigation groups, the soil mix in each pot was flooded with 200 mL of 100 mM NaCl solution (Figure 3B).

2.9. Petri Dish-Based Experiment

This experiment was specifically designed to focus on the analysis of seed germination and early seedling development in soybean. Briefly, 9 seeds in the falcon tube of the control group (Figure 2) were transferred into the three Petri dishes of the control group (Figure 3A), ensuring that each Petri dish contained 3 seeds on top of the moistened filter paper. The same approach was followed for the 9 seeds in the falcon tube of the stress group (Figure 2) with the three Petri dishes of the stress group (Figure 3A) and also for the 9 seeds in the falcon tube of the mitigation group (Figure 2) with the three Petri dishes of the mitigation group (Figure 3A). Afterward, the nine Petri dishes with seeds were incubated in a TL-300 growth chamber (Jeio Tech, Seoul, Korea) under controlled conditions (23 °C; 18 μmol·m^{-2}·s^{-1} photon, 16 h light/8 h dark, and 70% humidity). The incubated seeds were watered every 48 h with 2 mL of sterile water per Petri dish until the experiment was completed. Data on germination was recorded every day for 10 days, while early seedling growth parameters were measured after 15 days. The experiment was repeated two more times.

2.9.1. Determination of the Germination Parameters

Various equations were used to determine the emergence of roots and shoots during seed germination. The equations are given below based on Kader [20], and Khalaki et al. [21].

(i) Germination index

$$\sum (n1 \times 10) + (n2 \times 9) + (n3 \times 8) + \cdots + (n10 \times 1), \quad (1)$$

where n1, n2 . . . n10 represent the number of germinated seeds on the first, second, and subsequent days until the last day, day 10; 1 to 10 are standard weightings given to the number of germinated seeds per day, the highest given to day one. A higher germination index value denotes a higher percentage and rate of germination.

(ii) Germination rate index

$$G1/_1 + G2/_2 + G3/_3 + \cdots + Gx/_x, \quad (2)$$

where G1 is the germination percentage at the first counting day after sowing, G2 is the germination percentage at the second counting day after sowing, and so on. A higher germination rate index denotes greater and faster germination.

(iii) Mean germination time

$$\frac{\sum ni \times di}{N}, \quad (3)$$

where ni is germinated seeds per day, di = counting day, and N is the total number of seeds sown. A lower mean germination time denotes a rapidly germinated seed population.

(iv) Germination percentage

$$\frac{\text{Germinated seeds}}{\text{total seeds sown}} \times 100\%, \quad (4)$$

Here, the final number of seeds germinated is multiplied by 100 to obtain the germination percentage. Thus, a high germination percentage value would denote a high ger-

mination level for the seed population while the opposite indicates a low germination percentage value.

2.9.2. Observation of the Morphological Traits of Seedlings

Seedlings were visually observed for differences in various physical traits such as number of leaves, number of roots, length of stems, length of roots, etc., per each treatment group (control, stress, and mitigation), and the observed differences recorded.

2.9.3. Measurement of the Morphological Traits of Seedlings

Tissue Number

The number of lateral roots (LRN) per plant was determined by individually counting the roots followed by calculation of their average. Using this approach, number of leaves (LFN), roots (RTN), and seedlings (SDN) were determined.

Tissue Length

The length of shoots (SHL) and radicles (RDL) were measured in millimeters (mm) using an ACCUD 150 mm digital Vernier caliper (Vermont Sales, Midrand, Gauteng, South Africa) and such measurements were taken whilst tissues were still attached to plants [18]. Using this approach, the following were determined: SHL, RDL, leaf length (LFL) and seedling length (SDL).

Tissue Weight

To obtain the fresh and dry weight measurements for each tissue [18], seedlings were carefully removed from the Petri dishes and placed onto a dry paper towel in order to eliminate any excess water. A surgical blade was then used to physically separate the roots, lateral roots, stems and leaves from the seedlings. Fresh weight was measured in grams (g) using a PS750/C/2 analytical balance (Radwag, Radom, Poland), while dry weight was also measured in g by first placing each tissue in a VO-02 drying oven (JP SELECTA, Barcelona, Spain) set at 65 °C for 72 h, followed by weighing of the tissue on the analytical balance. Using this approach, the following weights were determined: shoot fresh weight (SHFW), shoot dry weight (SHDW), root fresh weight (RTFW), root dry weight (RTDW), seedling fresh weight (SDFW) and seedling dry weight (SDDW).

2.10. Pot-Based Experiment

This experiment was specifically designed to focus on the analysis of whole plant morphology and physiology. Briefly, the remaining 9 seeds in the falcon tube of the control group (Figure 2) were transferred into the three pots of the control group (Figure 3B), ensuring that in each pot, 3 seeds were sown 3 cm deep in the soil. The same approach was also followed for the remaining 9 seeds in the falcon tube of the stress group (Figure 2) with the three pots of the stress group (Figure 3B) and also for the remaining 9 seeds in the falcon tube of the mitigation group (Figure 2) with the three pots of the mitigation group (Figure 3B). Afterward, the nine pots with the seeds were placed in a greenhouse under controlled growth conditions of long days (16 h light) and short nights (8 h darkness) at temperatures of 25 °C daytime and 22 °C night-time. The sown seeds were irrigated every second day with 200 mL of sterile water per pot until the experiment was completed. Data on morphological features and physiological traits and processes was recorded after 60 days. The experiment was repeated two more times.

2.10.1. Observation of the Morphological Features of Plants

After 60 days of growth, plants were visually inspected for variations in physical features such as the colour of leaves, density of leaves and density of roots per each set of

the experimental conditions (control, stress, and mitigation). The observed variations were noted and recorded.

2.10.2. Measurement of the Physiological Processes of Plants

The rates of photosynthesis, transpiration and stomatal conductance were measured simultaneously on live plants [22] using an LCpro-SD infrared gas analyzer (IRGA) (ADC Bio-Scientific, Hertfordshire, UK). In this procedure, one leaf (middle leaflet of the second trifoliate leaf) from a plant was selected and enclosed into the leaf chamber of the instrument, which was set to retain all environmental parameters at ambient state while recording measurements from the leaf. Measurements were then taken at 10 s intervals for 3 min during the day between 10 a.m. and 1 p.m. The recorded time series were then summarized as means before statistical testing. Respective graphs for the three physiological processes (photosynthesis, transpiration and stomatal conductance) per each set of the experimental conditions (control, stress, and mitigation) were then plotted against time and interpreted. Ideally, one plant was selected at random from each of the three experimental groups (control, stress, and mitigation), and one leaf also selected randomly from that selected plant for measurements. In other words, one leaf was randomly selected from 9 plants per treatment. The procedure was then repeated twice more but ensuring that no leaf was measured more than once.

2.10.3. Measurement of the Morphological Parameters of Plants

Length

The length of stems (STL) and roots (RTL) were measured and determined as outlined in the Tissue Length section [18].

Weight

To obtain the fresh and dry weight measurements for each tissue, pots were flooded with water followed by a careful removal of plants from the soil. Excess and adhering soil particles onto the roots were carefully removed by hand and plants were placed on a dry paper towel to blot them. After this, the method outlined in the Tissue Weight section above was then followed. Using this approach, the following weights were determined: stem fresh weight (STFW) and stem dry weight (STDW), leaf fresh weight (LFFW), leaf dry weight (LFDW), RTFW and RTDW.

2.10.4. Measurement of the Physiological Traits of Plants

Stomatal Number

The nail polish imprint method as described by Voleníková and Tichá [23] was used. Briefly, three leaf tissues were randomly selected, one from each of the three experimental groups (control, stress, and mitigation), as outlined in Section 2.10.2. Each leaf was plucked off the plant at the base of its stock and its blade cut with a sharp razor, and tweezers used to fit it on a microscope slide. A clear nail polish was then applied onto the lower side (abaxial) of the leaf to make an impression of the surface, and allowed to dry. Following this, adhesive tape was placed and carefully pressed onto the polished leaf surface. The tape with its developed imprint was then carefully removed from the leaf surface and placed on a microscope slide. A high-resolution Primo Star light microscope (Carl Zeiss, Cape Town, Western Cape, South Africa) with a field of view of 0.05 mm was then used to analyze leaf impressions that were imprinted on the tape. Micrograph pictures of the impressions were then taken using a digital camera fixed to the microscope. The number of stomata (SN) per leaf surface was then counted physically in the field of view (FoV) of the micrographs.

Chlorophyll Content

This was determined according to Senthilkumar et al. [24], whereby an MC100 chlorophyll concentration meter (Apogee Instruments, Logan, UT, USA) was used, having first been calibrated by holding down its measuring arm. Following this, a leaf was put into the measuring head of the meter ensuring that the leaf fully covered the measuring area. Out of this setting, the concentration of the leaf chlorophyll (a and b) was then shown on the screen of the device for recording chlorophyll content (CC). Leaves from each of the three experimental groups (control, stress, and mitigation) in triplicate forms were randomly selected, as outlined in Section 2.10.2.

Relative Water Content

This was determined according to a method by Gonzalez and Gonzalez-Vilar [25] wherein leaves from each of the three experimental groups (control, stress, and mitigation) were randomly selected (in triplicate forms), as outlined in Section 2.10.2. Using a sharp razor blade and tweezers, a leaf tissue was plucked off the plant at the base of its stock. Its fresh weight (FW) was then measured and determined as described in the Tissue Weight section. Following this, its turgid weight (TW) was also measured and determined. This was done by letting the leaf float in a closed volumetric flask partially filled with distilled water for 3 h at room temperature under normal light; after the 3 h, the leaf was removed, blotted, and its weight measured and recorded. Following this, its dry weight (DW) was then measured and determined as described in the Tissue Weight section. Relative water content (RWC) was then calculated using the equation below.

$$\text{RWC (\%)} = [(\text{FW} - \text{DW})/(\text{TW} - \text{DW})] \times 100 \quad (5)$$

where FW = fresh weight, TW = turgid weight, and DW = dry weight.

2.11. Statistical Analysis

Data was examined using one-way analysis of variance (ANOVA) ($n = 3$; $p \leq 0.05$) to establish the differences in plant responses to the various treatments used in the study (control, stress and possible mitigation). Whenever ANOVA revealed significant differences within a treatment set, means were then separated using the post hoc Student–Newman–Keuls (SNK) multiple range test.

3. Results

3.1. Characterization of the Synthesized NPs

Five physicochemical techniques (UV-Vis, SEM, XRD, DLS and FTIR) were used to characterize the ZnO-NPs synthesized in this study so as to ascertain their suitability for further use (Figure 4). As is seen in the figure, the generated UV-Vis spectrum (A) has a distinct peak at wavelength 314 nm, indicating successful formation of the synthesized NPs. Additionally, the generated SEM micrograph (B) and XRD diffractograms (C) suggest formation of spherical and crystalline NPs. The sharp DLS curves (D) suggest that the formed NPs were synthesized via a well-controlled and highly homogeneous growth process. The FTIR spectrum (E) indicates the presence of various functional groups in the synthesized NPs.

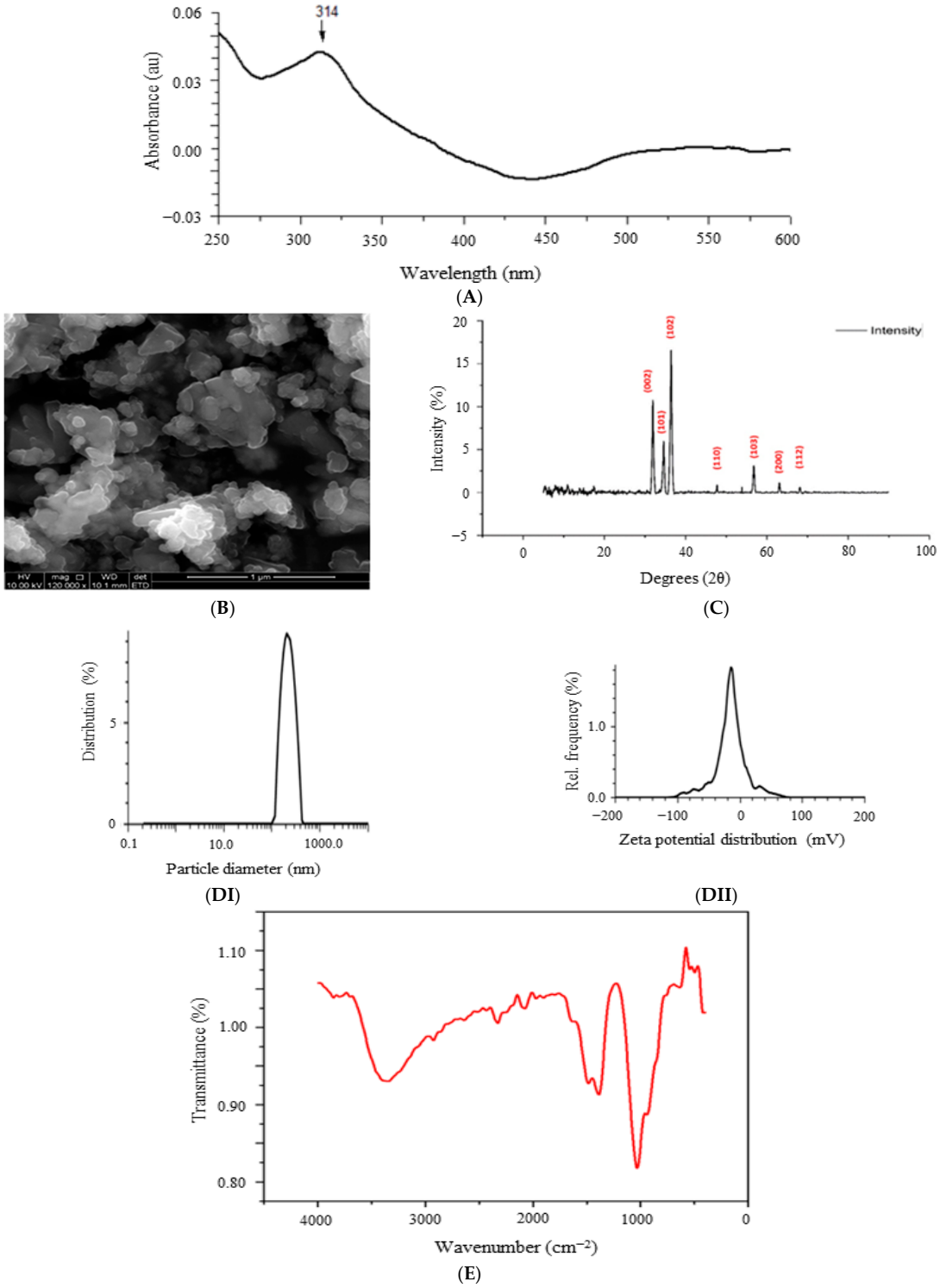


Figure 4. Characterization of the banana-based synthesized ZnO-NPs with different physicochemical techniques. (**A**) UV-Vis spectrum demonstrating formation of the metal oxide NPs; (**B**) SEM micrographs showing the morphology and agglomeration state of the formed NPs; (**C**) XRD diffratograms confirming the crystalline phase and structure of the synthesized NPs; (**DI**,**DII**) DLS curves showing the particle size (**DI**) and zeta potential (**DII**) of the formed NPs; and (**E**) FTIR sketch indicating presence of functional groups (absorption peaks in the range 4000–400 cm^{-1} wavenumbers) in the synthesized NPs. In the red sketch of (**E**), the broad band at 3364 cm^{-1} represents an O-H group, the weak band at 2917 cm^{-1} represents a C-H stretch, the other weak band at 2328 cm^{-1} represents the C≡N vibrational starching, the band at 2082 cm^{-1} represents a C=C vibrational stretch, the band at 1482 cm^{-1} represents aromatic rings, while the strong band at 1035 cm^{-1} represents a C-H group.

3.2. Petri Dish-Based Experiment

3.2.1. Determination of the Germination Parameters

Germination parameters of the soybean seeds primed with water and grown under no stress (control set-up), soybean seeds primed with water and grown under salt stress (stress set-up), and soybean seeds primed with ZnO-NPs and grown under salt stress (mitigation set-up) were measured and recorded daily for 10 days from the onset of germination in a growth chamber. Measurements and recordings were based on emergence of the radicle from the seed and targeting germination index (GI), germination rate index (GRI), germination percentage (GP), and mean germination time (MGT) (Figure 5). As is seen in the figure, comparing seeds in the control set-up group to seeds in the stress set-up group, it is apparent that the GI (A), GRI (B) and GP (C) parameters were all significantly reduced by salt stress, while on the other hand the MGT (D) parameter was significantly increased. However, when comparing seeds in the stress set-up group to seeds in the mitigation set-up group, all germination parameters were notably and significantly improved in the latter group of seeds. Essentially, salt stress reduced GI with a fold change of 1.33 while the ZnO-NPs increased it with a similar fold change (Figure 5A). Salt stress also reduced GRI with a fold change of 1.65, but ZnO-NPs increased it by 2.65-fold (Figure 5B). In Figure 5C, salt stress reduced GP with a fold change of 1.62 while the ZnO-NPs increased it with the same fold change of 1.62. In Figure 5D, salt stress increased MGT with a fold change of 1.83 while the ZnO-NPs decreased it by 1.29-fold.

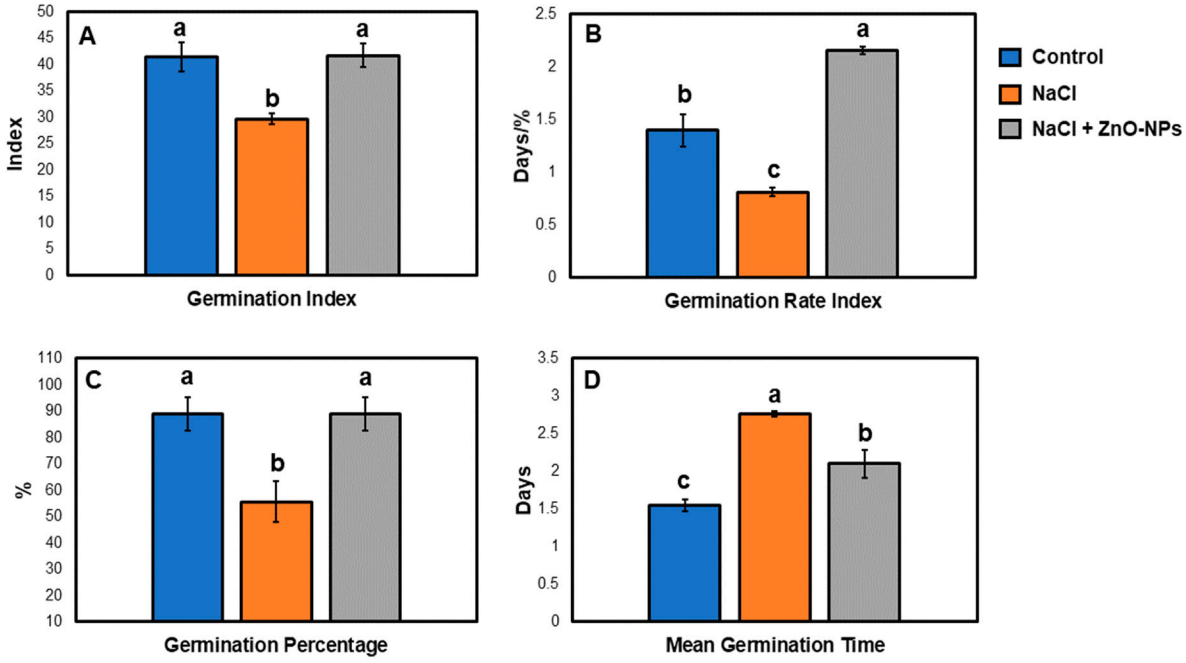


Figure 5. Germination parameters of soybean seeds primed with water and grown under no stress (blue bars), soybean seeds primed with water and grown under salt stress (orange bars), and soybean seeds primed with ZnO-NPs and grown under salt stress (grey bars). Seeds were incubated in a growth chamber for 10 days to allow gemination, and different parameters were then measured and recorded from day 1 till day 10. The measured and recorded parameters were GI (**A**), GRI (**B**), GP (**C**) and MGT (**D**). Data presented are mean values ($n = 3$, $p < 0.05$), error bars are standard errors, and lower-case letters refer to the statistical analysis performed using one-way ANOVA and post hoc SNK multiple range tests.

3.2.2. Observation of the Morphological Traits of Seedlings

In each set-up group (control, stress and mitigation), 15 days from the onset of germination in the growth chamber, seedlings were visually observed for differences in their

morphological traits (Figure 6). Comparing seedlings generated from seeds primed with water and grown under no stress (a) to those generated from seeds primed with water and grown under salt stress (b), it is evident that the number of roots, length of roots, and number of lateral roots were affected (reduced) by salt stress. However, when comparing seedlings generated from seeds primed with water and grown under salt stress (b) to those generated from seeds primed with ZnO-NPs and grown under salt stress (c), it is apparent that the ZnO-NPs counteracted all such observed effects of salt stress.



Figure 6. Morphological traits of soybean seedlings generated from seeds primed with water and grown under no stress (**a**), seeds primed with water and grown under salt stress (**b**), and seeds primed with ZnO-NPs and grown under salt stress (**c**).

3.2.3. Measurement of the Morphological Traits of Seedlings

At day 15 from the onset of seed germination, the parameters of various tissues of soybean seedlings generated from seeds primed with water and grown under no stress (control set-up), seeds primed with water and grown under salt stress (stress set-up), and seeds primed with ZnO-NPs and grown under salt stress (mitigation set-up) were measured and recorded. Measurements and recordings were separately taken for the aerial (Figure 7) and underground (Figure 8) tissues, targeting parameters such as shoot length (SHL), shoot fresh weight (SHFW), shoot dry weight (SHDW), radicle length (RDL), radicle fresh weight (RDFW), radicle dry weight (RDDW), lateral roots length (LTRL) and lateral roots number (LTRN). As is seen in both figures, comparing the parameters of seedlings generated from the control-group seeds to those of seedlings generated from the stress-group seeds, it is apparent that all measured parameters were significantly affected by salt stress; on the other hand, when comparing parameters of seedlings generated from the stress-group seeds to those of seedlings generated from the mitigation-group seeds, ZnO-NPs notably and significantly counteracted all the observed effects of salt stress. Essentially, as shown in Figure 7, salt stress reduced SHL with a fold change of 1.73 (A), SHFW by 1.5-fold (B) and SHDW by 1.43-fold (C); however, ZnO-NPs counteracted the effect of salt stress by increasing SHL with a fold change of 2.41, SHFW by 1.5-fold and SHDW by 1.43-fold (C). In Figure 8, salt stress reduced RDL with a fold change of 2.58 (A), increased LTRL by 1.5-fold (B), and reduced LTRN by 2.0-fold (C), RDFW by 1.92-fold (D), and RDDW by 1.72-fold (E). However, ZnO-NPs counteracted the effect of salt stress by increasing RDL with a fold change of 6.75 (A), reducing LTRL by 1.86-fold (B), and increasing LTRN by 3.0-fold (C), RDFW by 2.65-fold (D), and RDDW with a fold change of 2.45 (E).

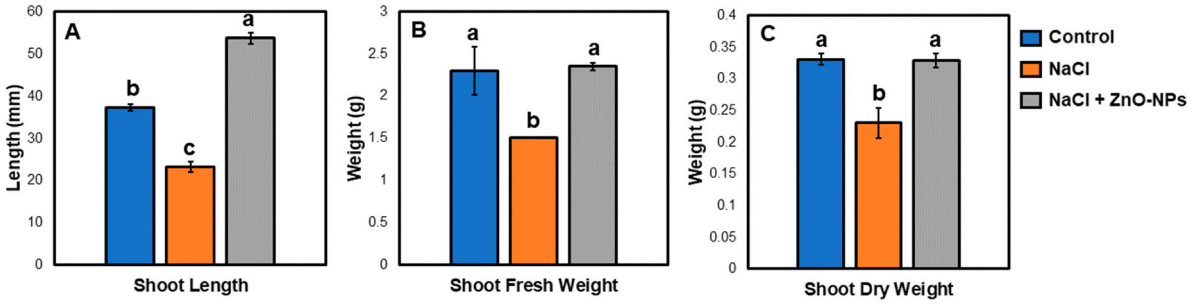


Figure 7. Growth parameters of the aerial tissues of soybean seedlings generated from seeds primed with water and grown under no stress (blue bars), seeds primed with water and grown under salt stress (orange bars), and seeds primed with ZnO-NPs and grown under salt stress (grey bars). Seeds were incubated in a growth chamber for 10 days to allow germination followed by a further 5-day incubation to allow seedling development. At day 15, growth parameters of the aerial tissues of the seedlings were then measured, compared and recorded. The targeted parameters were SHL (**A**), SHFW (**B**) and SHDW (**C**). Data presented are mean values ($n = 3$, $p < 0.05$), error bars are standard errors and lower-case letters refer to the statistical analysis performed using one-way ANOVA and post hoc SNK multiple range tests.

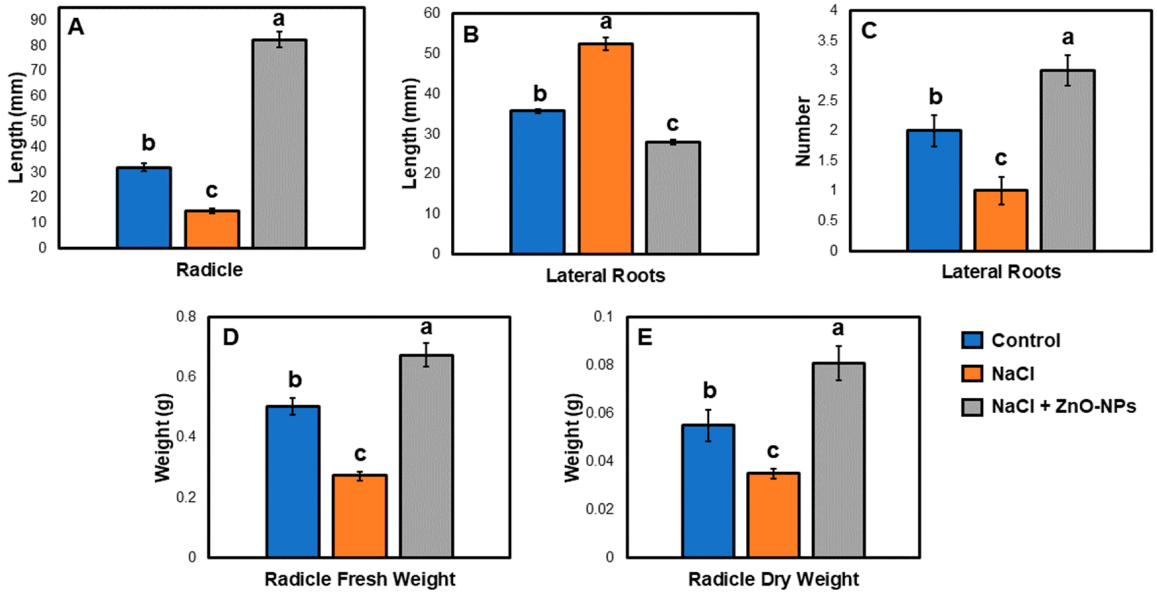


Figure 8. Growth parameters of the underground tissues of soybean seedlings generated from seeds primed with water and grown under no stress (blue bars), seeds primed with water and grown under salt stress (orange bars), and seeds primed with ZnO-NPs and grown under salt stress (grey bars). Seeds were incubated in a growth chamber for 10 days to allow germination followed by a further 5-day incubation to allow seedling development. At day 15, growth parameters of the different underground tissues of the seedlings were then measured, compared and recorded. The targeted parameters were RDL (**A**), LRL (**B**), LRN (**C**), RDFW (**D**) and RDDW (**E**). Data presented are mean values ($n = 3$, $p < 0.05$), error bars are standard errors and lower-case letters refer to the statistical analysis performed using one-way ANOVA and post hoc SNK multiple range tests.

3.3. Pot-Based Experiment

3.3.1. Observation of the Morphological Features of Plants

In each set-up group (control, stress, and mitigation), 60 days from the onset of germination in the greenhouse, plants were visually observed for differences in their morphological traits (Figure 9). Comparing plants generated from seeds primed with water and grown under no stress (control group) to those generated from seeds primed with water and grown under salt stress (stress group), it is clear that leaf number, leaf size, leaf

colour, stem length, root length and root density were affected (reduced) by salt stress. However, comparing plants generated from seeds primed with water and grown under salt stress (stress group) to those generated from seeds primed with ZnO-NPs and grown under salt stress (mitigation group), it is noticeable that the ZnO-NPs could counteract the noted effects of salt stress.



Figure 9. Morphological traits of soybean plants generated from seeds primed with water and grown under no stress (control group), seeds primed with water and grown under salt stress (stress group), and seeds primed with ZnO-NPs and grown under salt stress (mitigation group).

3.3.2. Measurement of the Morphological Parameters of Plants

At day 60 from the onset of seed germination in potting soil in the greenhouse, the parameters of various organs of soybean plants generated from seeds primed with water and grown under no stress (control set-up), seeds primed with water and grown under salt stress (stress set-up), and seeds primed with ZnO-NPs and grown under salt stress (mitigation set-up) were measured and recorded. Measurements and recordings were separately taken for the aerial (Figure 10) and underground (Figure 11) parts of the plants, targeting parameters such as stem length (STL), stem fresh weight (STFW), stem dry weight (STDW), root length (RTL), root fresh weight (RTFW), root dry weight (RTDW), leaf fresh weight (LFFW) and leaf dry weight (LFDW). As is seen in both Figures 10 and 11, comparing the parameters of plants generated from the control-group seeds to those of plants generated from the stress-group seeds, it is apparent that all the measured parameters were significantly affected by salt stress; on the other hand, when comparing the parameters of plants generated from the stress-group seeds to those of plants generated from the mitigation-group seeds, ZnO-NPs notably and significantly reversed all such observed effects of salt stress. As shown in Figure 10, salt stress reduced STL with a fold change of 2.49 (A), STFW by 1.16-fold (B), STDW by 1.2-fold (C), LFFW by 1.38-fold (D), and LFDW with a fold change of 1.381 (E); however, ZnO-NPs counteracted the effect of salt stress by increasing STL with a fold change of 2.75 (A), STFW by 1.37-fold (B), STDW by 1.2-fold (C), LFFW by 1.38-fold (D), and LFDW with a fold change of 1.381 (E). As indicated in Figure 11, salt stress reduced RTL with a fold change of 1.57 (A), RTFW by 1.61-fold (B) and RTDW by 1.24-fold (C); however, ZnO-NPs reversed the effect of salt stress by increasing RTL with a fold change of 2, RTFW by 1.61-fold (B) and RTDW by 1.24-fold (C).

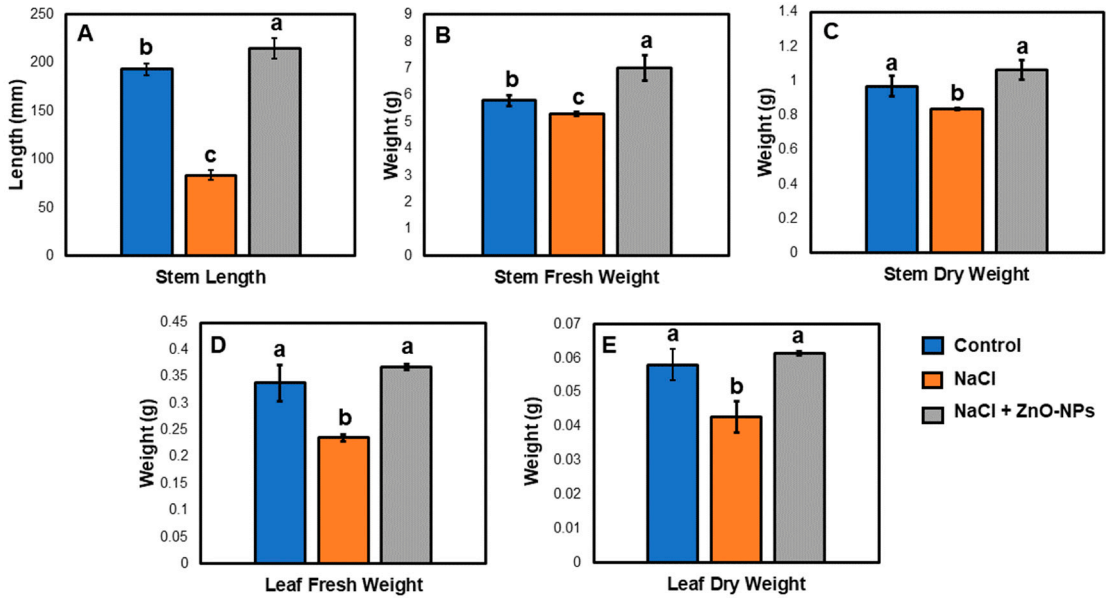


Figure 10. Growth parameters of the aerial parts of soybean plants generated from seeds primed with water and grown under no stress (blue bars), seeds primed with water and grown under salt stress (orange bars), and seeds primed with ZnO-NPs and grown under salt stress (grey bars). Seeds were grown in potting soil in a greenhouse and kept under constant irrigation for 60 days. At day 60, growth parameters of the different aerial tissues of plants generated from the seeds were then measured, compared and recorded. The targeted parameters were STL (**A**), STFW (**B**), STDW (**C**), LFFW (**D**) and LFDW (**E**). Data presented are mean values ($n = 3$, $p < 0.05$), error bars are standard errors and lower-case letters refer to the statistical analysis performed using one-way ANOVA and post hoc SNK multiple range tests.

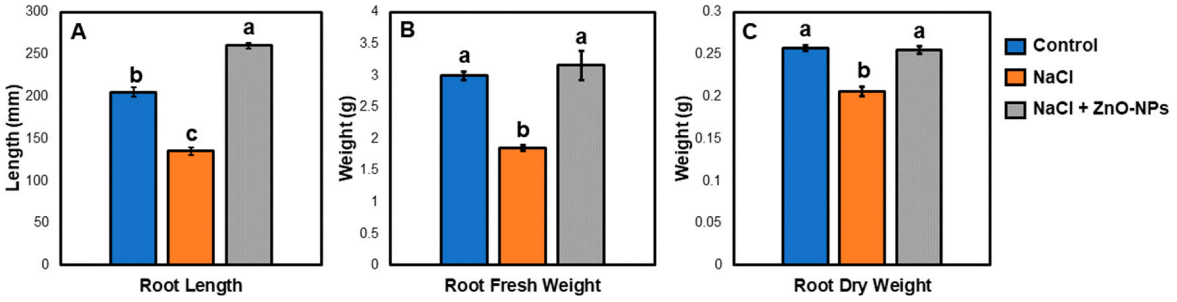


Figure 11. Growth parameters of roots of soybean plants generated from seeds primed with water and grown under no stress (blue bars), seeds primed with water and grown under salt stress (orange bars), and seeds primed with ZnO-NPs and grown under salt stress (grey bars). Seeds were grown in potting soil in a greenhouse under constant irrigation for 60 days. At day 60, growth parameters of the underground tissues of plants generated from the seeds were then measured, compared and recorded. The targeted parameters were RTL (**A**), RTFW (**B**) and RTDW (**C**). Data presented are mean values ($n = 3$, $p < 0.05$), error bars are standard errors and lower-case letters refer to the statistical analysis performed using one-way ANOVA and post hoc SNK multiple range tests.

3.3.3. Analysis of the Physiological Traits of Plants

Besides visually observing and measuring morphological traits of soybean plants generated from the differently primed seeds growing under different conditions (control, stress and mitigation), other key parameters (physiological traits) such as stomatal number (SN), chlorophyll content (CC), and relative water content (RWC) were also analyzed and recorded (Figure 12). As is seen in the figure, comparing analyses done on plants generated from seeds primed with water and grown under no stress to those done on plants generated from seeds primed with water and grown under salt stress, all the measurements indicated

significant salt-stress effects; on the other hand, when comparing analyses done on plants generated from seeds primed with water and grown under stress to those done on plants generated from seeds primed with ZnO-NPs and grown under stress, the ZnO-NPs notably and significantly counteracted all effects caused by salt stress. For SN, salt stress reduced it from an average of 4 (AI) to 3 (AII) while ZnO-NPs counteracted the effect of salt stress by increasing SN to an average of 7 (AIII). For CC, salt stress reduced it with a fold change of 1.66 while ZnO-NPs counteracted this effect of salt stress by increasing CC with a similar fold change of 1.66 (B). For RWC, salt stress reduced it with a fold change of 1.05 while ZnO-NPs counteracted the effect of salt stress by increasing RWC with a fold change of 1.03 (C).

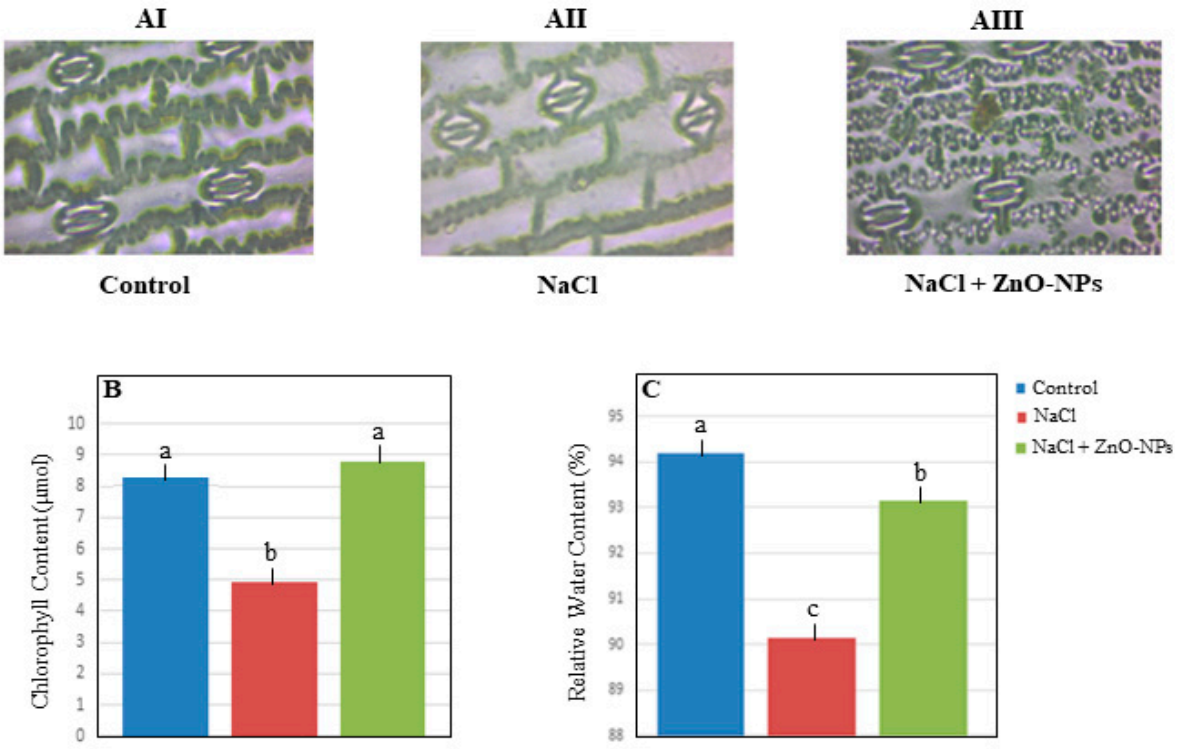


Figure 12. Physiological traits of soybean plants generated from seeds primed with water and grown under no stress ((**AI**), blue bars), seeds primed with water and grown under salt stress ((**AII**), red bars), and seeds primed with ZnO-NPs and grown under salt stress ((**AIII**), green bars). Seeds were grown in potting soil in a greenhouse under constant irrigation for 60 days. At day 60, biochemical traits of plants generated from the seeds were then measured, compared and recorded. The targeted parameters were SN (**AI**–**AIII**), CC (**B**) and RWC (**C**). Data presented in (**B**,**C**) are mean values ($n = 3$, $p < 0.05$), error bars are standard errors and lower-case letters refer to the statistical analysis performed using one-way ANOVA and post hoc SNK multiple range tests.

3.3.4. Measurement of the Physiological Processes of Plants

At day 60 from the onset of seed germination in potting soil in the greenhouse, different physiological processes of plants generated from the differently primed and grown seeds (control, stress and mitigation) were measured and recorded (Figure 13). Measurements and recordings were taken using an IRGA during the day at 10 s intervals for 3 min, targeting the following processes: photosynthesis (A), transpiration (B), and stomatal conductance (C). In the figure, comparing processes measured from plants generated from seeds primed with water and grown under no stress (control group) to those measured from plants generated from seeds primed with water and grown under salt stress (stress group), all processes were generally affected by salt stress; on the other hand, when comparing processes measured from plants generated from seeds primed with water and grown under

salt stress to those measured from plants generated from seeds primed with ZnO-NPs and grown under stress (mitigation group), it is evident that ZnO-NPs could counteract the observed effects of salt stress. For instance, for photosynthesis (A), between 100 and 180 s, plants from the stress group had the lowest rate of ~0.8 μmol $m^{-2}s^{-1}$, followed by plants from the control group with a rate of between ~0.8 and ~1.3 μmol $m^{-2}s^{-1}$, and lastly, with plants from the mitigation group with a rate of between ~1.3 and ~5.0 μmol $m^{-2}s^{-1}$. For transpiration (B), throughout the whole assaying period, plants from the stress group had the lowest rate of between ~0.06 and ~0.18 μmol $m^{-2}s^{-1}$, followed by plants from the control group with between ~0.19 and ~0.22 μmol $m^{-2}s^{-1}$, and lastly, with plants from the mitigation group with a rate of between ~0.33 and ~0.47 μmol·m^{-2}·s^{-1}. For stomatal conductance (C), between 40 and 180 s, once again, plants from the stress group largely had the lowest rate of close to 0 μmol·m^{-2}·s^{-1}, followed by plants from the control group with ~0.01 μmol·m^{-2}·s^{-1}, and lastly, with plants from the mitigation group with a rate of ~0.02 μmol·m^{-2}·s^{-1}.

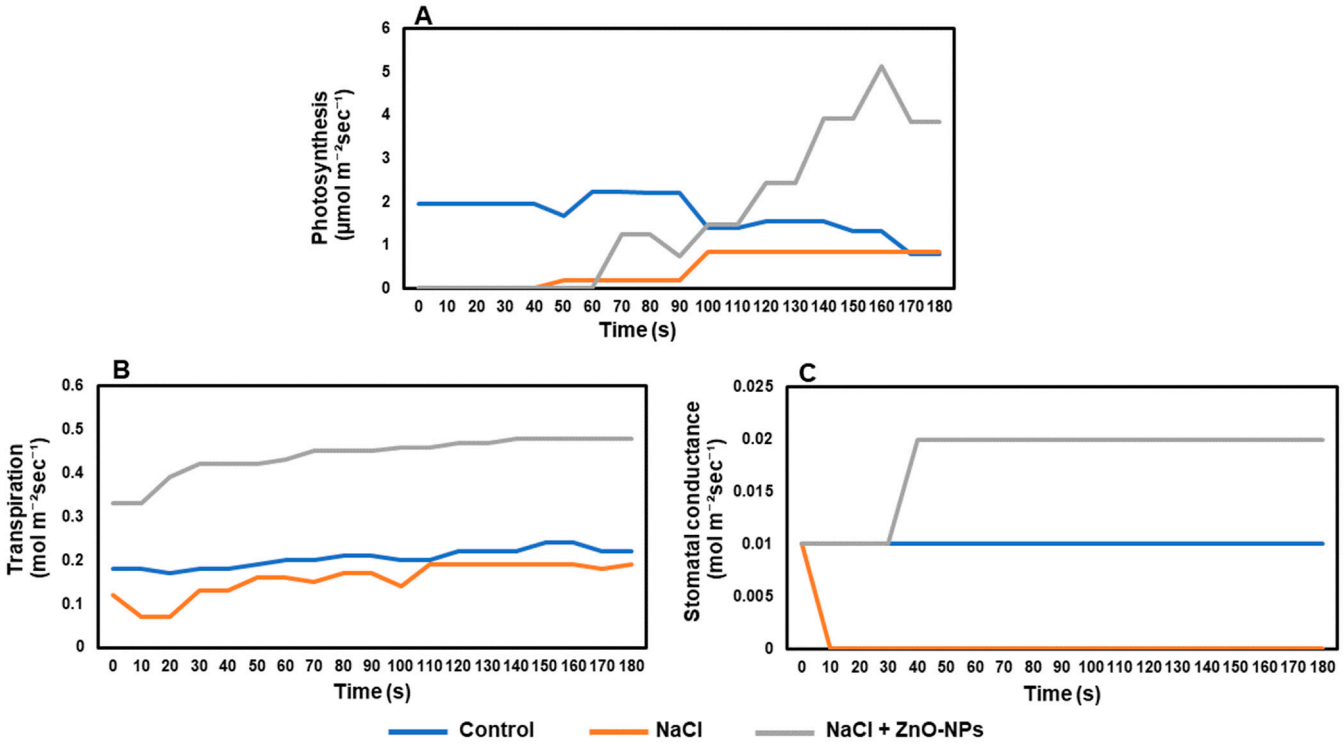


Figure 13. Physiological processes of soybean plants generated from seeds primed with water and grown under no stress (blue bars), seeds primed with water and grown under salt stress (orange bars), and seeds primed with ZnO-NPs and grown under salt stress (grey bars). Seeds were grown in potting soil in a greenhouse under constant irrigation for 60 days. At day 60, physiological processes of plants generated from the seeds were then measured, compared and recorded. The targeted processes were photosynthesis (**A**), transpiration (**B**), and stomatal conductance (**C**), and for each process, measurements were taken during the day at 10 s intervals for 3 min using an IRGA. Data presented are mean values (n = 3) and error bars are standard errors based on one-way ANOVA and post hoc SNK multiple range tests ($p < 0.05$).

4. Discussion

Soybean [*Glycine max* (L.) Merrill] is one of the most important oil crops in the world with tremendous importance as a food legume [26]. Oil from this legume finds its way into a variety of uses for domestic and agricultural purposes in the form of various food and feed preparations for humans and animals [27]. Besides its use in domestic and agricultural settings, soy oil also has multifarious uses in industries and commercial enterprises related

to production of plastics, paper, ink, paints, and varnishes [28]. Lately, use of soy oil as biodiesel has opened another avenue of renewable energy for industries and the transport sector [29]. As a leguminous crop, soybean is capable of utilizing atmospheric nitrogen through biological fixation and is therefore less dependent on synthetic nitrogenous fertilizers that have the risk of causing environmental pollution. The crop offers great potential as a protein supplement to fight malnutrition [30,31]. However, despite all these benefits associated with this legume, soybean cultivation or production is severely affected by salt stress or salinity in the soil [32].

Salt stress remains the major devastating environmental stress factor affecting salt-sensitive plants such as soybean [33], because it induces ionic imbalance, osmotic stress and oxidative stress in plants, which all have a profound impact on plant morphology, biomass, and biochemical processes, leading eventually to plant injuries and/or death [34,35]. In agricultural soils, generally, salt stress occurs as a result of the increased use of poor-quality water for irrigation, application of chemical fertilizers, and the designing and use of poor drainage systems [36]. Currently, it is estimated that more than 20% of all cultivated soils in the world is affected by salt, increasing at a rate of over 10% per year, so that by the year 2050, 50% of it will be affected [37]. As a result, crop cultivation and food production are severely affected, ultimately threatening food security. Current reports available on crop production and food security indicate that the global annual crop yield and economic losses due to salt stress are around 10% and $27.3 billion per year, respectively [38,39]. Thus, in order to maintain crop production under these continued challenging environmental conditions, some sustainable and affordable agricultural practices should be developed and practiced.

Nowadays, most farmers are slowly turning into ‘green technology’, which involves use of metal-based nanoparticles (NPs) such as the zinc oxide NPs (ZnO-NPs) [40]. The rationale being that smaller NPs with suitable surface qualities are more likely to be absorbed by plants for growth and development [40]. Most interestingly, several recent studies have reported that priming of plants with ZnO-NPs, either via foliar spraying (leaf priming) or seed treatment (seed priming), significantly enhances performance and production under salt stress [18,34,41–43]. This priming aspect, also known as stress priming or conditioning, is defined as a physiological state, which involves the induction and activation of plant defences against environmental challenges (current and future) [43]. In this primed state, the plant’s response and resistance to stressful conditions is very rapid and efficient [44,45]. Additionally, the plant also stores the information of the priming agent until later exposure to the initial triggering stimulus—an effect systematically referred to as ‘stress memory’ in plant defence [43]. In line with this concept, we therefore, assessed in this study the role of ZnO-NPs in alleviating salt stress in soybean.

To begin, ZnO-NPs were synthesized based on the green synthesis method [46], using banana peel extract as the source of reducing and stabilizing agents on the Zn element [13]. This specific synthesis method was preferred among other known existing methods because it is simple, safe, cost effective, environmentally friendly, relatively reproducible, and results in more stable particles [13,47]. After synthesis, the generated NPs were characterized using various physicochemical techniques, to ascertain the NPs’ suitability for use in the study. UV-Vis was used to determine formation of the NPs; SEM to determine the morphology and agglomeration state of the formed NPs; XRD to determine the composition and crystal quality of the formed NPs; DLS to determine the particle size distribution, zeta potential and polydispersity index of the formed NPs; and FTIR to detect presence of functional groups in the formed NPs. Figure 4 shows that well-dispersed, agglomerated NPs with spherical and crystalline structures possessing various characteristic functional groups, were formed, thereby ascertaining the suitability of the synthesized NPs for use in our

study. These kinds of ZnO-NPs have also been formulated [48–50] and used previously in other studies [18,19,51,52].

When these synthesized NPs were used, findings from our study showed that priming seeds with 200 mg·L^{-1} ZnO-NPs significantly improves germination and early seedling development in soybean under salt stress (Figures 5–8). In plants, seed germination and early seedling development are the initial stages of plant development and major limiting factors on the establishment and stand of plants [53,54]. When properly established, seed germination and early seedling development also enhance resistance to both biotic and abiotic stresses in plants [55]. So, any disturbance to these two critical phases will have massive and permanent impacts on plant growth and its ultimate productivity [56]. Seed priming is an important modern technique applied to plants to improve seed germination and early seedling development [57–59]. The approach has been reported to enhance nutrient uptake, water use efficiency and metabolic activities to ensure rapid and effective plant establishment under stressful field conditions [57]. These enhancements are thought to be due to the importance of Zn as an element in the biochemical processes that initiate germination. These changes are associated with dormancy loss, degradation of inhibitors, maximal reactive oxygen species (ROS) production, promotion of imbibition, and activation of germination-mediating enzymes [59]. Other studies related to ours include a recent one by Chakhmakhchyan et al. [59] in which seed germination and early seedling growth were enhanced in two soft wheat (*Triticum aestivum* L.) genotypes (V1 and V2) under salt stress after priming with 50 and 100 mg·L^{-1} ZnO-NPs. Additionally, another report by Sharma et al. [60] showed that nano-priming of seeds with 20 and 40 ppm of ZnO-NPs improved early germination and radicle emergence in rice. Srivastav et al. [61] also reported comparable increases in germination and seedling vigour in wheat and maize after the application of ZnO-NPs due to high amylase activity.

Findings from our study also showed that priming seeds with 200 mg·L^{-1} ZnO-NPs significantly improves morphological attributes of soybean plants growing under salt stress (Figures 9–11). In plants, in all tissues that promote development, salt stress is known to restrict growth by inhibiting cell division and elongation at meristematic sites [62] and decreasing water and ionic potentials in the soil, which then hinders the absorption of water and nutrients by plants [63–65]. Seed priming has been reported to enhance nutrient uptake, water use efficiency, and plant performance, which then help to increase crop productivity and yield under salt stress conditions [66]. This is also likely to be as a result of the primary role Zn has in activating cell division and elongation at meristematic sites [47]. Notably, the influence of ZnO-NPs reported in this study on the morphological attributes of soybean has also been demonstrated in other plants, where the nano-priming of *Oryza sativa* seeds with increasing concentrations (0, 5, 10, 15, and 25 ppm) of pristine ZnO-NPs encouraged an increase in plant height under salt stress [67]. Additionally, another study also reported an increase in height and shoot and root dry weights of salt-stressed wheat plants generated from ZnO-NPs-primed seeds [40]. Furthermore, Gupta et al. [44] reported that applying ZnO-NPs to salt-stressed bean plants led to significant improvements in fresh and dry weights.

Finally, findings from our study also showed that priming seeds with 200 mg·L^{-1} ZnO-NPs significantly improves the physiological attributes of soybean plants growing under salt stress (Figures 12 and 13). In plants, salt stress is known to decrease stomata number and chlorophyll content through oxidative damage induced by the presence of ROS [68]. Salt stress is also known to causes the infelicitous closure of stomata, which then slows down gaseous exchange (stomatal conductance), transpiration and photosynthesis [69]. Besides affecting photosynthesis through the slowing down of gaseous exchange, salt stress also affects this key physiological process by causing a decrease in the concentration of

chlorophylls and carotenoids, leading to distortions in the ultrastructure of chloroplasts and the photosystem II centre [70]. Priming of seeds with ZnO-NPs continues to be an important emerging technique for enhancing crop performance in agriculture. The strategy is believed to involve modulation of early biochemical and physiological processes in plants [71]. In a study by Kumar et al. [71] in tomato (*Solanum lycopersicum* L.), seedlings generated from seeds nano-primed with 50 ppm ZnO-NPs exhibited elevated levels of total chlorophyll, proline and total phenol alongside enhanced antioxidant enzyme (superoxide dismutase, catalase and peroxidase) activities. Also, a concurrent reduction in malondialdehyde content indicated lower lipid peroxidation and reduced oxidative damage [71]. These effects collectively suggested improved homeostasis and metabolic efficiency in the tomato plant [71], which could have been the same effect from the 200 mg·L^{-1} ZnO-NPs used in this study on soybean. Additionally, priming of seeds with increasing concentrations (0, 5, 10, 15, and 25 ppm) of pristine ZnO-NPs was also shown to promote increases in stomatal number and total chlorophyll content in *O. sativa* [68]. In maize, under saline conditions, plants generated from seeds primed with 0.04% ZnO-NPs were found to maintain higher photosynthetic and transpiration rates compared to plants generated from untreated seeds [72]. The rates of photosynthesis and transpiration were also shown to be improved by priming seeds with ZnO-NPs in wheat under salt stress [73]. Finally, a study by Gupta et al. [44] also evidenced that ZnO-NPs application on salt-stressed bean plants could increase the rate of photosynthesis.

Overall, considering that salt stress and other abiotic stress factors such as drought, non-optimal temperatures, and low soil fertility will continue to challenge crop and food production globally [74], findings of this study provide a promising system, especially if practised at a larger scale, to curb food insecurity and improve economic development. This is particularly important for the semi-arid and arid regions, where soils are mostly affected by salts. The study is also instrumental in that it integrates the concepts of nanotechnology with those of plant biotechnology to provide a sustainable and innovative approach that improves the crucial practices of food security and sustainable agriculture. However, before ZnO-NPs are used in agriculture, it is advisable that extra studies on the toxicity, persistence, residue, and environmental impact of these NPs are undertaken to establish their optimum dose and/or safe application window. In addition, further research on these ZnO-NPs is needed to elucidate their underlying molecular mechanisms of action and validate such findings under field conditions.

5. Conclusions

Findings of this study established that priming of soybean seeds with 200 mg·L^{-1} ZnO-NPs significantly improved performance of this legume under salt stress. Enhanced outcomes included, among others, accelerated germination, high germination percentage, reduced mean germination time, improved seedling and full-grown plant vigour, size and biomass, increased stomatal number and chlorophyll content, and elevated photosynthetic, transpiration and stomatal conductance rates. Applying ZnO-NPs at low concentrations enhances the cost-effectiveness of seed priming by minimizing material requirements. Moreover, the reduced dosage decreases environmental risks by limiting NP accumulation and toxicity to non-target organisms. Thus, these factors contribute to our method's plausible sustainability and potential for large-scale implementation.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	analysis of variance
CC	chlorophyll content
DLS	dynamic light scattering
DW	dry weight
FoV	field of view
FTIR	Fourier transform infrared
FW	fresh weight
GI	germination index
GP	germination percentage
GRI	germination rate index
IRGA	infrared gas analyzer
LFDW	leaf dry weight
LFFW	leaf fresh weight
LFL	leaf length
LFN	leaf number
LTRL	lateral roots length
LTRN	lateral roots number
MGT	mean germination time
NPs	nanoparticles
RDDW	radicle dry weight
RDFW	radicle fresh weight
RDL	radicle length
ROS	reactive oxygen species
RTDW	root dry weight
RTFW	root fresh weight
RTL	root length
RTN	root number
RWC	relative water content
SDDW	seedling dry weight
SDFW	seedling fresh weight
SDL	seedling length
SDN	seedling number
SEM	scanning electron microscopy
SHDW	shoot dry weight
SHFW	shoot fresh weight

SHL	shoot length
SN	stomatal number
SNK	Student–Newman–Keuls
STDW	stem dry weight
STFW	stem fresh weight
STL	stem length
TW	turgid weight
UV-Vis	ultraviolet visible
XRD	X-ray diffraction
ZnO-NPs	zinc oxide nanoparticles

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