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Zinc oxide nanoparticles mediated salinity stress mitigation in *Pisum sativum*: a physio-biochemical perspective

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Abstract

Salinity is the major abiotic stress among others that determines crop productivity. The primary goal is to examine the impact of Zinc Oxide Nanoparticles (ZnO NPs) on the growth, metabolism, and defense systems of pea plants in simulated stress conditions. The ZnO NPs were synthesized via a chemical process and characterized by UV, XRD, and SEM. The ZnO NPs application (50 and 100) ppm and salt (50 mM and 100 mM) concentrations were carried out individually and in combination. At 50 ppm ZnO NPs the results revealed both positive and negative effects, demonstrating an increase in the root length and other growth parameters, along with a decrease in Malondialdehyde (MDA) and hydrogen peroxide concentrations. However, different concentrations of salt (50 mM and 100 mM) had an overall negative impact on all assessed parameters. In exploring the combined effects of ZnO NPs and salt, various concentrations yielded different outcomes. Significantly, only 50 mM NaCl combined with 50 ppm ZnO NPs demonstrated positive effects on pea physiology, leading to a substantial increase in root length and improvement in other physiological parameters. Moreover, this treatment resulted in decreased levels of MAD, Glycine betaine, and hydrogen peroxide. Conversely, all other treatments exhibited negative effects on the assessed parameters, possibly due to the high concentrations of both stressors. The findings offered valuble reference data for research on the impact of salinity on growth parameters of future agriculture crop.

Keywords Nanofertilizer, Salinity stress, Physiological changes, Bio-fortification, Salt stress

Introduction

Salinization is a global problem as soil salinity plays a role, in determining crop yields which leads to decreased productivity in areas where the soil has high salt concentrations [1]. In Pakistan's farmland, 6.68 million hectares of soil are impacted by salt. Among these Punjab accounts for 2.67 million hectares with medium to elevated salt levels [2, 3]. Exposure to high salt stress can cause several problems in the plant, like slower growth, poor development, yellowing of leaves, messed-up hormones, and less effective antioxidants [4]. When plants experience osmotic stress, it can lead to a decrease in their growth and the amount they produce. This happens because osmotic stress changes some important

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processes in the plant's body [5]. For example, in peas, having too much sodium and chloride ions can make the plant's growth slow down [6, 7]. Abiotic stresses cause a reduction in the amount of chlorophyll [8], affecting how the plant exchanges gases with the air, and changing the plant's water and mineral uptake balance [9, 10]. Peas are a major cash crop globally, accounting for approximately 40% of all pulse trade. Field peas are rich in carbohydrates and provide highly digestible nutrients, with about 86 to 87% digestibility, making them an excellent choice for livestock feed. In Pakistan, peas are grown on a total area of 23.58 thousand ha and production is 149.02 thousand tons with a regular yield of 6.32 tones/ha [11].

Before sowing seed priming is a significant strategy to enhance the intime growth and yield of the valued crops [12]. Among sustainable approaches, it promptly increases the quality of trait and seed development resulting in enhanced plant growth [13, 14]. In agriculture seed priming is a traditional practice applied to stimulate seed germination and plant development [15]. Several studies have stated that seed priming acts as a defense for seed storage and enhances seed, seed emergence, germination rate, and growth by altering the physiological states such as nutrient uptake, water use efficiency, and refining tolerance to abiotic and biotic stresses [14, 15].

ZnO NPs are widely recognized as the most extensively manufactured NPs globally [16], second only to carbon nanotubes [17, 18], gold, silver and titanium dioxide NPs [19–21]. ZnO NPs are produced at a rate of between 550 and 5550 tons per year, which is 10–100 times higher than any other nonmaterial [22, 23]. The significance of ZnO NPs stems from their tiny dimensions, distinctive shapes, and captivating physical and chemical properties [24]. ZnO NPs are deemed one of the most vital nanomaterials, whose applications are expanding mostly due to their tiny sizes [25–27], distinctive shapes [28], and appealing physical and chemical properties [24, 29]. In Zn deficit areas specifically, it has been commonly used as a nano-fertilizer to enhance plant germination and growth [30, 31]. NPs may raise the nutrient availability to plants which ultimately improves crop production [32]. In recent studies due to extensive application ZnO NPs have shown a positive impact on plant growth and physiology in the agriculture sector [33]. The main objective of this study was to developed a new protocal for cash crops to cope against salinity stress particularly *P sativum* growth to enhanced morphological, physiological and biochemical perspective. For this purpse, our hypothesis was that ZnO NPs treatment may ameliorate detrimental effects of salinity by affecting ROS mediated ionic homeostasis in plant tissues conditions. The dual role of ZnO NPs as plant growth promoter and stress mitigator was evaluated by assessing the plant biomarker such as MAD, glycine betaine, and hydrogen peroxide. Furthermore, this study also illustrated impact of ZnO NPs on plant growth in stress conditions which leads towards dehydration of cell and also influenced on the salt imbalance. Moreover, this study give a complete mechanism of cell physiology in production of ROS in result of salinity stress and their defense system trigger by the ZnO NPs for normal function enzymes and their respective reactions.

Materials and methods

Synthesis of zinc oxide nanoparticles

The ZnO NPs was synthesized as a colloidal suspension using a sol- gel technique. Briefly, 0.5 mol/L (8.78 g) of zinc acetate dihydrate (99.5%) was dissolved in 80 mL of 2-propanol (99.9%) at 50 °C. The solution was diluted to 840 mL using 2-propanol. Then, 160 mL of a 0.5 mol/L (3.2 g/160 mL) sodium hydroxide (99.5%) solution (prepared using a 7:1 mixture of 2-propanol and di-H_2O) was added at 0 °C within 1 min under stirring. The mixture was immersed in a water bath preheated to 65 °C for 2 h. After seven days of further aging at room temperature, the solvent was removed by centrifugation at 7000 rpm for 15 min. The centrifugation process was repeated until the by-products will completely remove. The precipitate was dried in an oven at 120 °C for 8 h. Finally, the powder was calcined in a muffle furnace at 400 °C for 2 h. The calcinations of the sample were done at 500 °C for 2 h to attain ZnO NPs [34].

Characterization of ZnO NPs

UV-vis spectroscopy analysis

ZnO NPs were examined with the help of ultraviolet spectrometry. In this technique, ZnO NPs were monitored by periodic sampling of aliquots, and time spectra of the ZnO NPs solution were measured. These spectra were recorded after every 15 min, and the UV spectrum of all aliquots was monitored as a function of time retention on the UV spectrophotometer operated at a resolution of 1 nm.

X-ray diffraction analysis

For a comprehensive understanding of the structural characteristics of the ZnO NPs, X-ray diffraction (XRD) analysis was employed. The X-ray diffractometer used in this study was a Shimadzu Model: XRD 6000. This analysis was conducted on ZnO NPs in their powdered form. The XRD investigation covered a range of diffraction angles from 20 to 80 degrees at 2 theta, corresponding to a wavelength of 0.154 nm. The XRD, by examining the X-ray's interaction with the ZnO NPs structure, provided insights into their crystalline properties [35].

FTIR analysis

To identify the various functional groups present in the ZnO NPs, an infrared spectra analysis was conducted in

transmission mode. This was achieved using a Fourier-Transformed Infrared Spectrophotometer (FT/IR-610, JASCO). The wavelength range selected for this analysis spanned from 400 to 4000 cm^-1. The FTIR helped in identifying the specific chemical groups within the ZnO NPs by examining their unique infrared absorption patterns.

Scaaning electron microscopy

The morphology and texture of ZnO NPs were explored by Quanta Inspect scanning electron microscope, operating at 25 kV in vacuum. For this purpose, ZnO NPs were used in the powder form.

Seed priming

The seeds of peas were obtained from National Agriculture Research Center (NARC) Islamaabd. The supreme variety of peas was soaked in a prepared solution of ZnO NPs. To prepare a 50ppm solution 0.25 g of ZnO NPs was added to 500 ml distilled water. Similarly, 0.50 g of ZnO NPs was added to 500 ml distilled water to prepare a 100 solution. Now we soaked 50 seeds in three different Petri dishes at concentrations of 0 ppm, 50 ppm, and 100 ppm. The conditions for Pea seeds priming were fully immersed in priming agents for 3 h at room temperature in the dark [36, 37]. After these 5 seedlings were transplanted from each petri dish into an earthen pot.

Experimental design

Seeds were sown in clean earthen pots (22 cm in diameter) containing clay-sandy soil (2:1 *v/v*). Pots were filled with 5 kg of thoroughly mixed soil; five seedlings were transplanted from the Petri dishes to each earthen pot. Water was used according to the requirements of the crop. Salt treatments were imposed to 15-day-old seedlings by adding 250 mL NaCl solution to the soil. The salt irrigation persisted for 2 weeks (5 day interval). The harvest of the vegetative stage was performed after 15 days of the salt treatment; plant samples were collected for estimation of growth parameters. For the experimental layout, CRD (completely randomized design) with 3 replications was used. Temperature was recorded as 25–27 °C at day time and 15–18 °C at night time. Humidity was recorded from 56 to 62% at day time and 76–81% at night time.

When plants became mature and ready to be harvested, yield parameters were measured as mentioned in Table 1.

Table 1 Treatment details regarding the ZnO NPs application under control and salinity stress

Treatments	Concentrations
C	Control
N1	50 ppm ZnO NPs
N2	100 ppm ZnO NPs
S1	50 mM NaCl
S1*N1	50 mM NaCl + 50 ppm ZnO NPs
S1*N2	50 mM NaCl + 100 ppm ZnO NPs
S2	100 mM NaCl
S2*N1	100 mM NaCl + 50 ppm ZnO NPs
S28N2	100 mM NaCl + 100 ppm ZnO NPs

N: Represents Nanparticles S: represents Salt

Morpho-physiological parameters

Different physiological and morphological parameters were assessed and measured as previously reported [38]. Root length is the distance between the tip of the root and the portion of the plant just touching the surface soil was measured as the root length in centimetr (cm). All three replicates groups plants were measure their root lenths and average was used as final value. Sinmilarly, Plant height was evaluated and measured with the measuring tape. Plant height was measured from the soil surface to the tip of the plant of all three replicates and then average was calculated. The total number of plant pods was counted at 3–4 days intervals. After this averages were calculated. The pod length (cm) was measured with the measuring scale and then the average was calculated. Number of the seeds of ripened pods was calculated. To calculate the fresh grain weight of pea seeds, the known quantity of pea seeds was directly onto the scale, ensuring an even distribution. Weight was recorded and displayed on the scale. To calculate the dry grain weight of pea seeds fresh pea seeds were placed in an oven or drying apparatus set to a specific temperature (typically between 80 °C and 105 °C) for a predetermined drying period (usually 12–24 h). After drying seeds were cooled in a desiccator and weighed. The difference between the fresh weight and the dried weight gives dry grain weight.

Biochemical parameters

Different Biochmeical parameters were assessed and measured as previously reported [10].

Hydrogen peroxide (H_2O2) determination

For the determination of H_2O_2 procedure followed by Velikova et al., (2000). Green leaves (0.5 g) were extracted in 5 mL of 0.1% (w/v) TCA (trichloroacetic acid). After this mixture was placed in an ice bath. In the extract potassium phosphate buffer (0.5 mL) and potassium iodide (I mL) was inserted. Mixture was shaken and absorbance was measured at 390 nm by spectrophotometer.

Malondialdehyde (MDA)

The method followed by Carmak and Horst, (1991) to assess MDA content in leaf samples. 0.5 g plant sample was ground in 5 mL of 1.0% TCA (trichloroacetic acid), followed by centrifugation at 20,000 rpm. Next, 0.25mL

of the sample was mixed with 0.25mL of 0.5% TBA in 20% TCA. In a shaking water bath, the resulting mixture was heated at 100 °C for 1 h and then cooled on the ice. It was centrifuged at 10,000 × g for 10 min after cooling. By using a spectrophotometer, the OD (optical density) of the filtrate was determined at 600 nm and 532 nm.

Statistical analysis

The results for the different morphological parameters were displayed as (mean ± SE). The significant differences among the mean values were concluded using ANOVA. Assumptions for the raw data normality and variance homogeneity were calculated. A parametric data distribution was verified by a variance homogeneity test. Values were considered significantly different at a significance level of $p < 0.05$.

Results

ZnO NPs characterization

The scanning electron microscopy (SEM) micrograph of the ZnO NPs revealed a cottony shape Fig. 1(a). The round shaped ZnO NPs were looking effective for agriculture application due to nanosize and high sureface area [29, 38–40]. Spherical shaped nanoparticles especially the Zinc were previously studied in agriculture for reducing the abiotic stress [41, 42]. Previous research and current work in case of morphological looks same and mightbe very effective [43, 44]. The XRD (X-ray diffraction) spectrum of the green-synthesized ZnO NPs displayed a pointed band with a reflection at 20, indicating that the produced material is constituted with crystalline ZnO Fig. 1(b). The ZnO NPs crystalline size was estimated by the Scherrer equation, where D represents the particle size in nm (nanometers), k is the Scherrer constant (k = 0.94), β represents the FWHM (full width at half maximum) of the diffraction peak, X-ray wavelength is denoted by λ (1.54178 Å), and θ stands for the

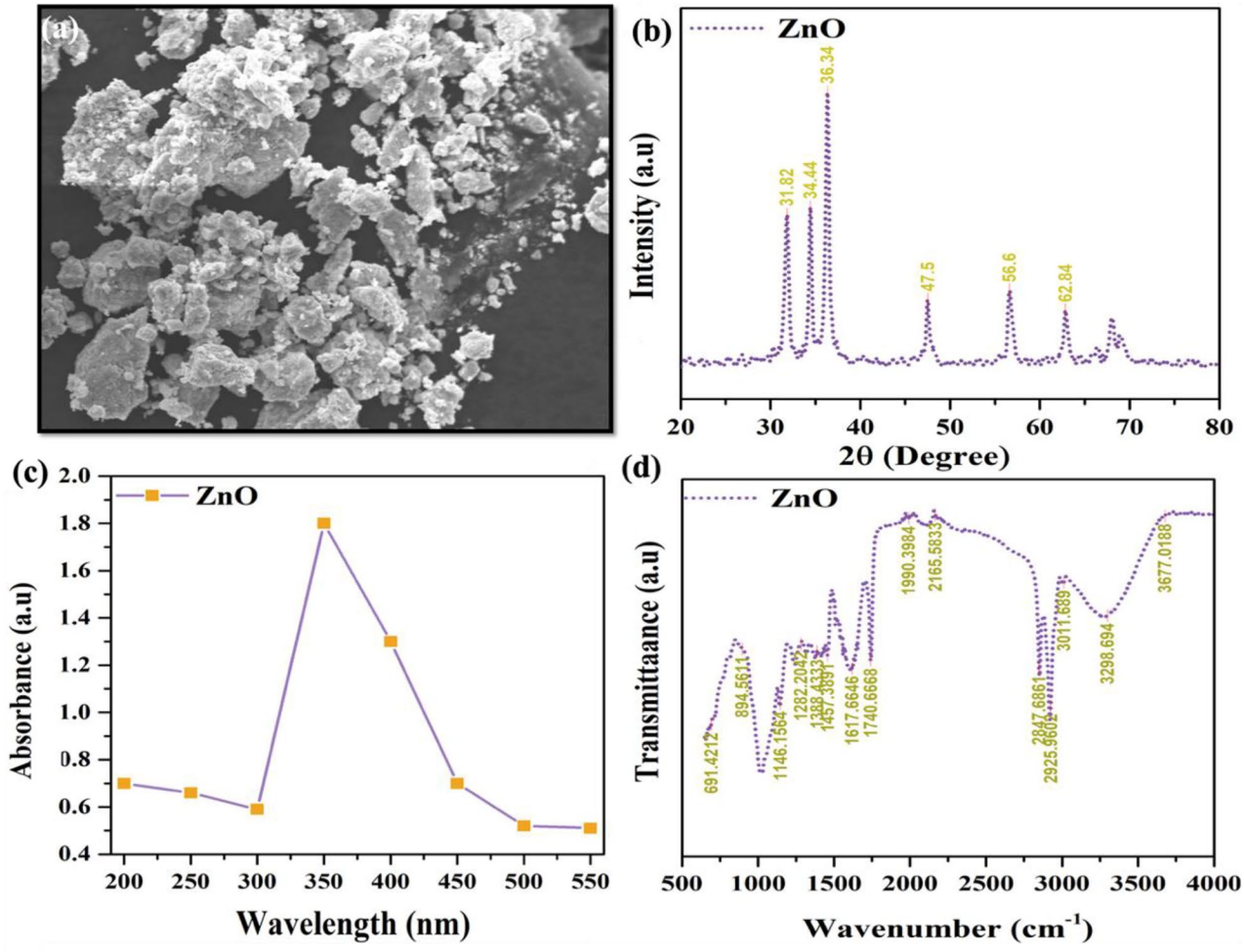

Fig. 1 Characterization of the ZnO NPs prepared by Sol gel Method. (**a**) Scanning electron microscopy analysis of ZnO NPs (**b**) Xray Diffraction of ZnO NPs (**c**) UV-Vis absorption analysis of ZnO NPs (**d**) Fourier-Transformed Infrared Spectrophotometer of prepared ZnO NPs

angle of diffraction. Ultimately, the average crystalline size of the ZnO NPs was determined to be 17.76 nm. This study showed good result comparative to previous reports regarding the characterization in case of structure [23]. Furthermore, the provided UV absorption data offers a glimpse into the optical behavior of ZnO NPs across a range of wavelengths. The UV-Vis absorption analysis of zinc oxide nanoparticles synthesized through chemical methods is analyzed at 300–800 nm. At 350 nm, NPs exhibit a substantial absorption peak, indicating their strong affinity for light in the ultraviolet spectrum Fig. 1(c). This peak suggests the involvement of the local density state of ZnO NPs. The absorption values gradually decrease as the wavelength increases, suggesting a diminishing ability to absorb light at longer wavelengths. This information is pivotal for understanding the nanoparticles' optical properties, which play a crucial role in various applications. For instance, the significant absorption observed in the UV range implies their potential application in UV sensors and detectors. Moreover, the gradual decrease in absorption towards visible light wavelengths hints at the nanoparticles' potential use in transparent coatings or films, where light transparency is desirable.

However, previous work also showed the same kind of presentation in the profiling of Uv spectra [45–47]. The FTIR bands shows charcteristics peaks at different points 691 cm^{-1}, 1461 cm^{-1}, 1671 cm^{-1}, 1740 cm^{-1}, 2925 cm^{-1}, 3298 cm^{-1} that confirm formation of metal oxygen bands and characteristics hydroxyl, amines, alcohol, protein and phytochemical metabolites bonds Fig. 1(d). FTIR spectra also shwed the involvement of reducing agent molecules from the chemical reaction which have same peaks as reported previously [26, 27, 48].

Growth parameters

ZnO NPs showed excellent characterization which means, these NPs can be very effective and might be good for their agriculture application therefore, a study was designed to see the morphological, biochemical, physiological and immune response paramteres were explored in salt stress conditions. Intially, Different morphological growth parameters were calculated including the shoot length, root length, fresh and dry grain weight. The results illustrated in Fig. 2(a). Notably, ZnO NPs application led to a substantial increase in root length, with the magnitude of this effect being more pronounced at higher NPs application rates. Data exposed that salinity stress induced the declines in all the growth criteria. The visible antagonistic impact of saltiness on pea was shown in terms of the marked reduction in root length 18.72% over control when treated with 100 mM salt Fig. 3(a-i). ZnO NPs resulted in higher root length under control and salinity-stressed conditions in pea plants relative to the controls. Plants experimented with 50 ppm ZnO NPs showed maximum increase of 9.72% in root length over the control Fig. 3(e) but when treated with high concentration as 100 ppm ZnO NPs there was a 12% decline in the growth of root Fig. 3(e). The combined effect of salt and nanoparticles proved a significant increase in the root growth of peas. Highest root length in our experiment was seen in the combined effect of 50 ppm ZnO NPs and 50 mM NaCl which was 71.37%. Increase over the control. While there was a decline of 21% when treated with 100ppm ZnO NPs along with 100 mM Nacl Fig. 3(i).

In case of shoot length, the heights of pea plants exhibited significant variation. The largest plants, at a maximum 33.2 cm height were seen in the group treated with S1 (50mM NaCl) Fig. 2(b). The second-highest height, measuring 32.6 cm, was recorded among plants treated with a combination of 50 ppm ZnO NPs and 50 mM NaCl (S1N1). In contrast, plants treated with CN2 (100 ppm ZnO NPs) demonstrated the least growth, reaching a height of 25.3 cm. The remaining plant variations displayed moderate performance concerning this specific trait.

The effect of ZnO NPs and salinity on fresh and dry weight of pea grains can vary depending on the specific experimental conditions, concentrations used, and duration of exposure. The collective application of ZnO NPs and exposure to salt stress can have a significant impact on the fresh and dry grain weight of pea plants.

The data presented highlighted distinct outcomes among the replicates subjected to various treatments. Notably, the replicates treated with treatment S1*N1 (50 ppm ZnO NPs + 50 mM NaCl) exhibited the highest fresh grain weight (0.38) Fig. 2(c). as well as the highest dry grain weight (0.08) Fig. 2(d). In contrast, the replicates treated with S2*N2 (100 ppm ZnO NPs + 100 mM NaCl) displayed the lowest values, with mean fresh grain weight of 0.2 and mean dry grain weight of 0.03. These findings underscore the impact of different treatments on the measured parameters. Interestingly, the study highlights that the most favorable concentration of ZnO NPs for promoting growth and yield in these crops is around 50 ppm. At concentrations above this, like 100 ppm, the effects on growth and yield become less positive. This suggests that there's an optimal range for reaping the benefits of ZnO NPs in agriculture. The fresh weight increased with the increasing concentration of zinc nanoparticles (Fig. 3). There were nine different treatments, including ZnO NPs, salt, and a control group. The highest weight (0.38 g) was seen with 50 ppm zinc nanoparticles and 50mM salt concentration. The lowest weight (0.20 g) was observed with 50 ppm zinc oxide nanoparticles and 100 mM salt.

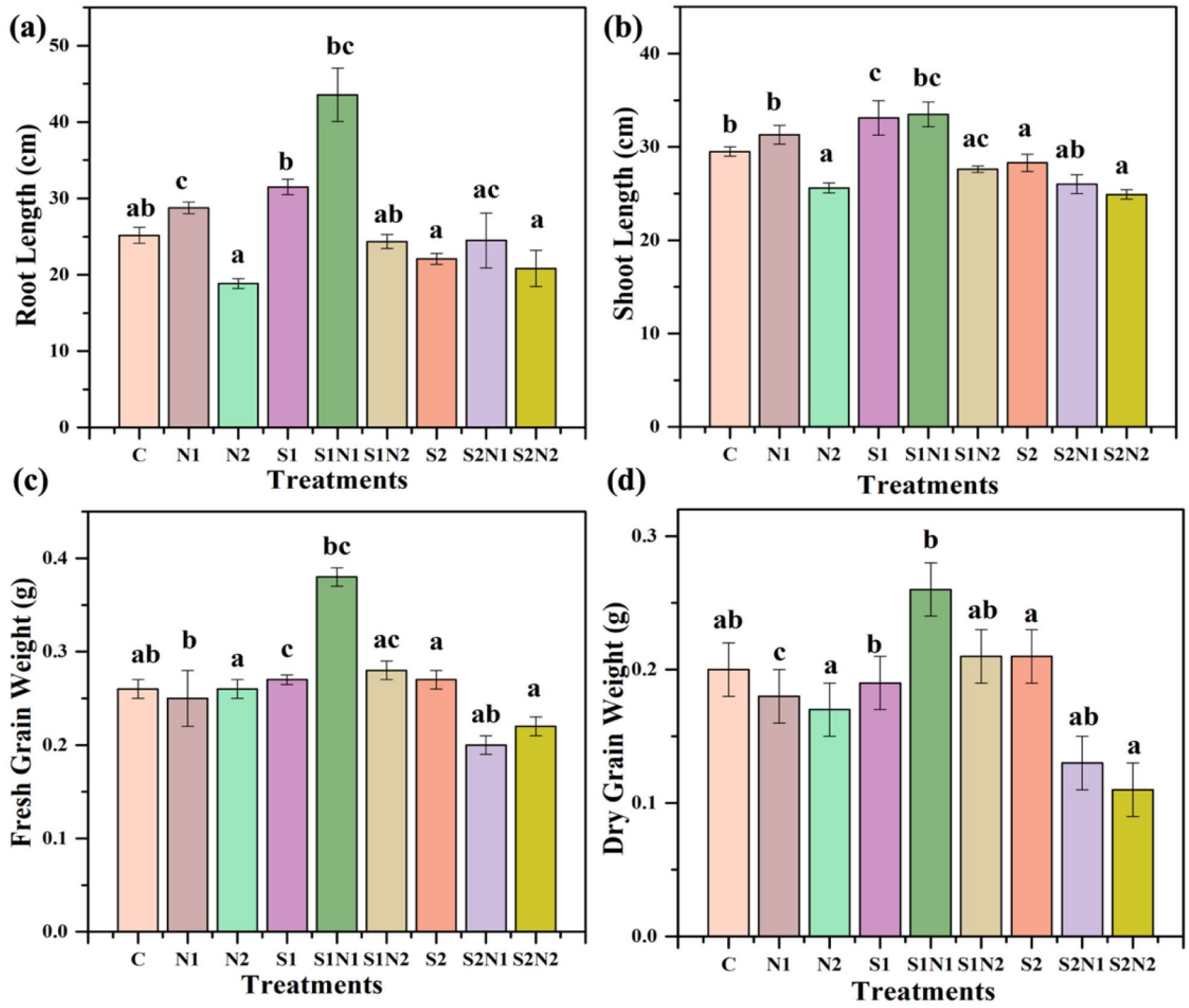

Fig. 2 Morphological characterization of pea plant with stress and ZnO NPs. (**a**) Root length (cm) (**b**) Shoot length (cm) (**c**) Fresh grain weight (**g**) and (**d**) Dry grain weight (**g**) were measured. Mean values of each plant parameters with different letters are significantly different according to ANOVA ($n = 5$, $p < 0.05$)

The growth of pea plants was better when zinc oxide nanoparticles were applied, in contrast with the control group. Plants treated with nanoparticles looked healthy and produced a greater number of leaves than those in the control group. The combined influence of zinc oxide NPs and salt stress on pea plants can impact the number of leaves Fig. 4(a). ZnO NPs, when applied in conjunction with salt stress, may exhibit a dual role in regulating leaf development.One way that these NPs might function is as a stress mitigator, assisting in reducing the negative consequences of salt stress and maintaining the integrity of the leaf. On the other hand, the NPs themselves might induce changes in leaf morphology and physiology.

The ZnO NPs application consistently increase the growth of pea plants contrasted to the control group, Fig. 4(b). ZnO NPs impact on the number of grains per pod in plants is a multifaceted phenomenon, contingent upon various factors. Zinc, as an important micronutrient, plays a pivotal role in plant development and growth, and NPs of zinc oxide have the potential to positively influence nutrient uptake, thereby enhancing seed development. High soil salinity can significantly impact the number of grains produced by pea plants. The presence of elevated salt concentrations in the soil poses multiple challenges to the growth and development of peas.Stunted growth and altered reproductive structures further contribute to a decreased number of flowers and, consequently, grains. The plants treated with these nanoparticles appeared healthy and yielded more grains than those in the control group. The average seed count per pod ranged from 0 to 5 indicates that the highest number of grains per pod was observed in plants

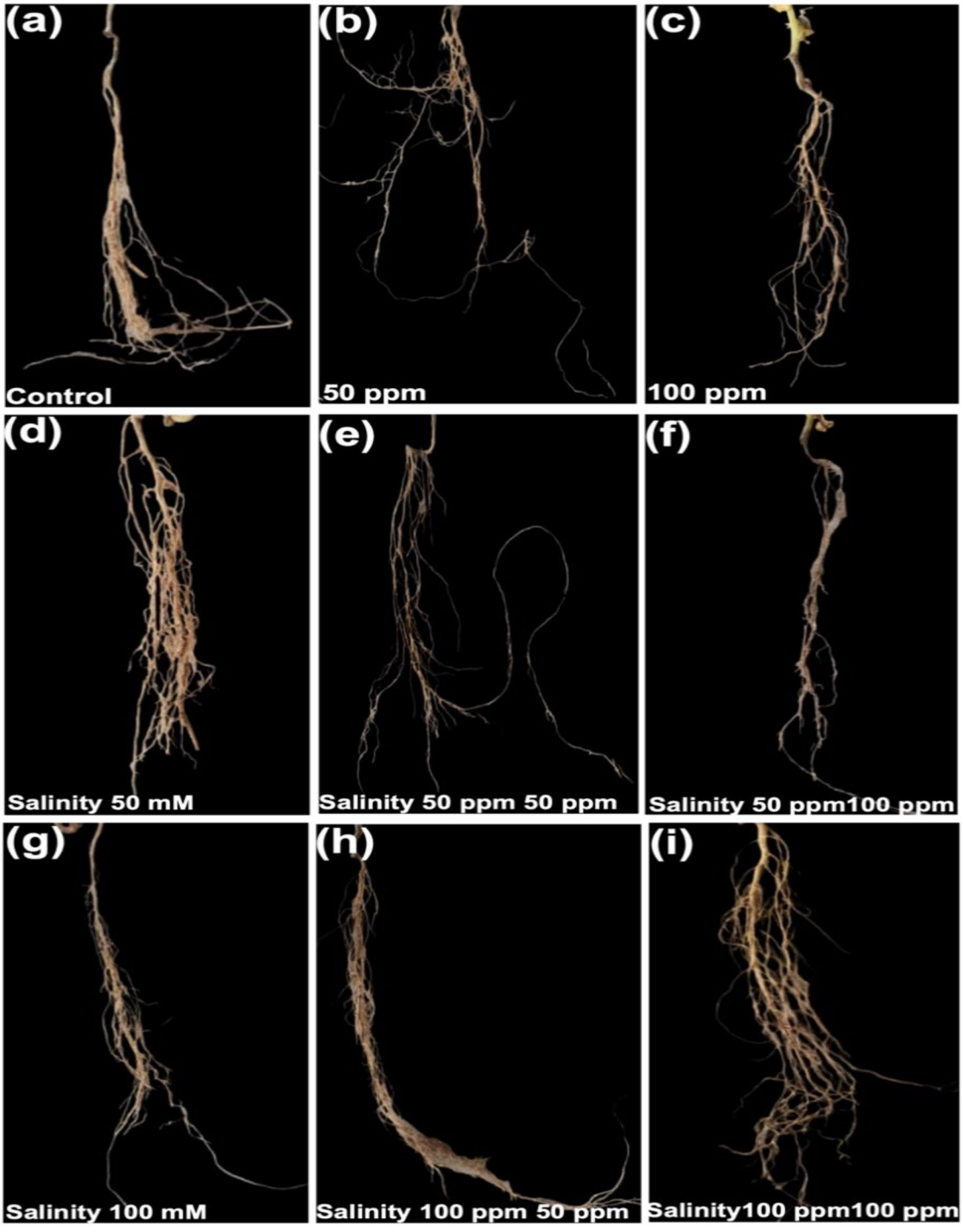

Fig. 3 Morphological of root in pea plant with stress and ZnO NPs (**a**) Control (cm) (**b**) root with 50 ppm NPs (**c**) root with 100 ppm NPs (**d**) root in salinity 50 mM (**e**) root in salinity 50 mM with 50 ppm NPs (**f**) root in salinity 100 mM with 100 ppm NPs (**g**) root in salinity 100 mM (**h**) root in salinity 100 mM with 50 ppm NPs (**i**) root in salinity 100 mM with 50 ppm NPs. Mean values ($n = 5$) in each point with different lettering are significantly different corresponding to ANOVA ($p < 0.05$)

treated with S1N1 (50 ppm ZnO NPs+50 mM NaCl) (5 grains per pod), and CN1 (50 ppm ZnO NPs) (3 grains per pod). On the other hand, plants treated with S2N2 (100 mM NaCl+100 ppm ZnO NPs) and S2 (100 mM NaCl) had the lowest seed count per pod (1 seed per pod). The remaining treatments displayed intermediate results in terms of number of grain per pod modifications. The comparative analysis of means and variances

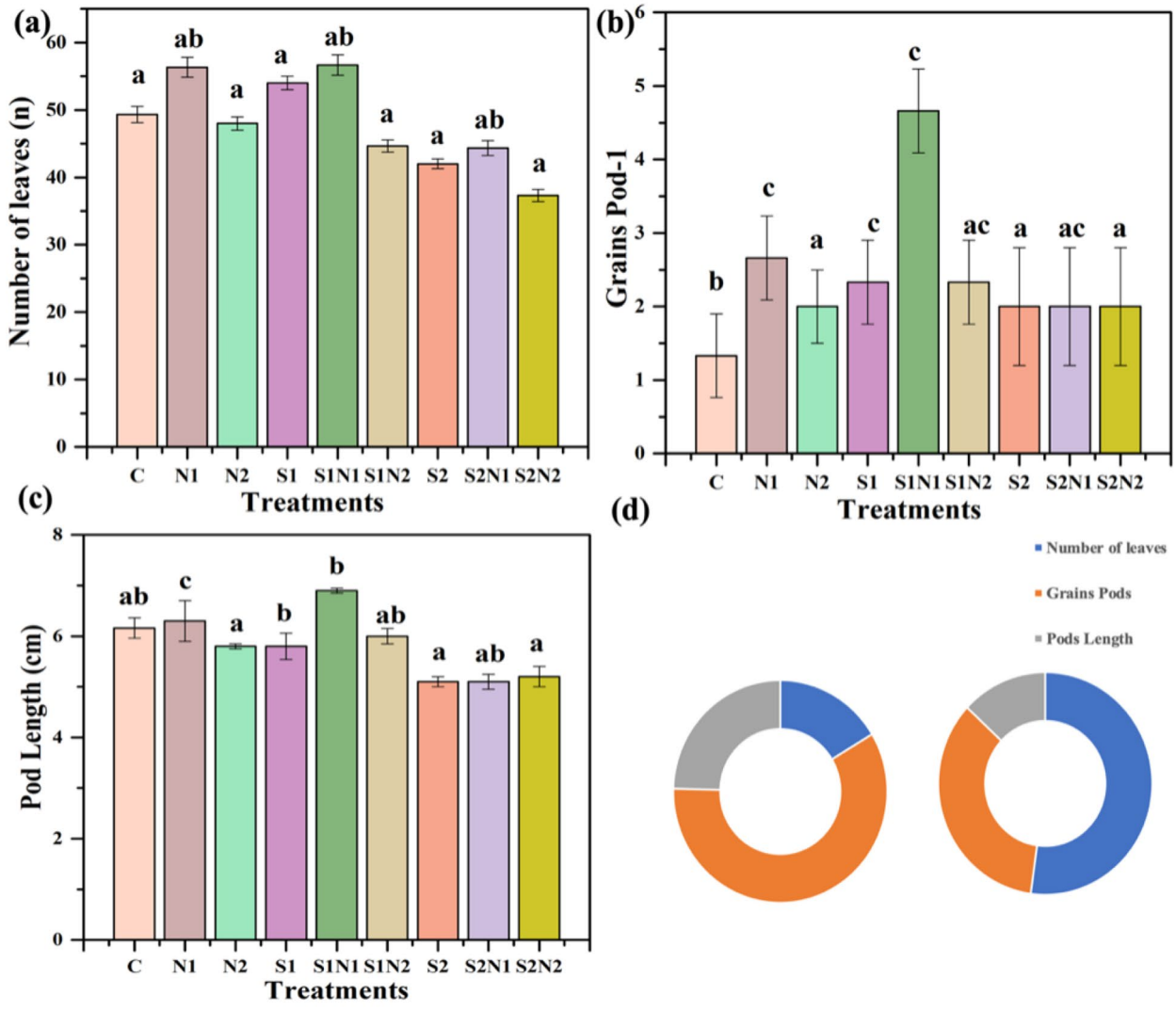

Fig. 4 Biochemical characterization of in pea plant with stress and ZnO NPs (**a**) number of leaves (**b**) grain pods (**c**) pod length (g) (**d**) ratio between grain pod, number of leaves and pod length. Mean values in each point with different letters are significantly different according to ANOVA ($n = 5$, $p < 0.05$)

across different treatment groups is aptly illustrated in Fig. 4(b). The combined impact of zinc oxide NPs and salt stress on pea plants can lead to changes in pod length. In current study the pod length data revealed significant variations across the different treatments. Upon comparing the treatment means, it was evident that the highest pod length approximately (7 cm) was found in the replicates of treatment of S1*N1 (50 ppm ZnO NPs+50 mM NaCl) followed by the (6.3 cm) in C*N1 (50 ppm ZnO NPs) Fig. 4(c). On the other hand plants treated with the treatment S2 (100 mM NaCl) showed the minimum (5 cm) length followed by the (5.16 cm) in the treatment of S2*N1(100 mM NaCl+50ppm ZnO NPs).The remaining treatments displayed intermediate results in terms pod length modifications.

Biochemical parameters

Glycine betaine

The results obtained from seed priming experiments using various concentrations of ZnO NPs are illustrated in the Fig. 5. These results show that when greater concentrations (100 ppm) of ZnO NPs were given (treatment CN2), there was a considerable rise in the amount of GB (20.23% higher than the control Fig. 5(a). In contrast, there was no significant difference observed when plants were treated with 50 ppm ZnO NPs (treatment CN1). A substantial increase in antioxidants such as GB was observed when Pea plants were grown in saline conditions. Specifically, there was a 48% increase in GB content when plants were exposed to a high level of salt (100mM NaCl, treatment S2), and a 14.34% increase when treated with 50 mM NaCl (treatment S1).

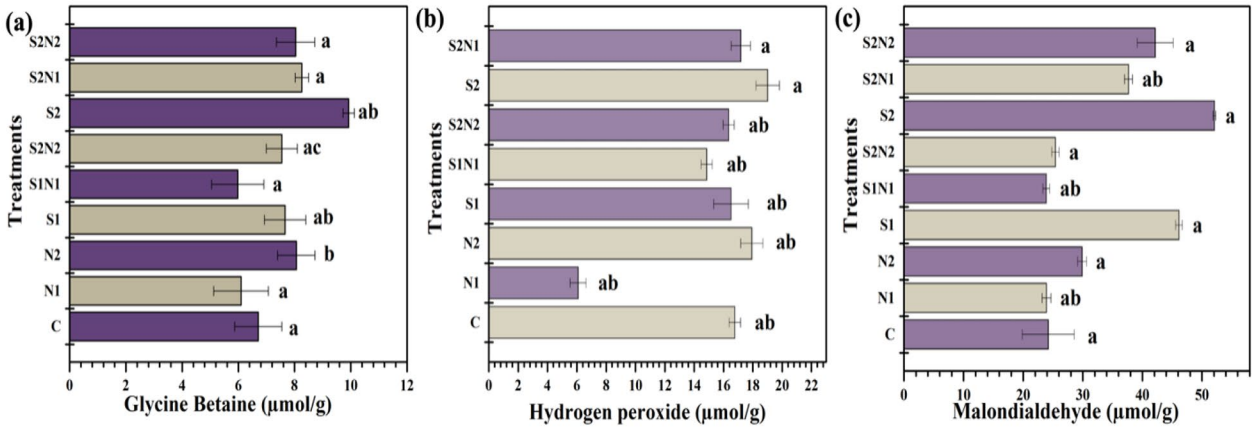

Fig. 5 Levels of Glycine betain (**a**), Hydrogen peroxide (**b**) and Malondialdehyde (**c**) in *P. sativum* L. subjected to different levels of salt and ZnO NPs. Mean values in each point with different letters are significantly different according to ANOVA ($n = 5$, $p < 0.05$)

Furthermore, the combined impact of ZnO NPs and salt stress yielded even more promising results. The most significant increase, approximately 23.17%, was observed when plants were subjected to the dual treatment of 100mM NaCl along with 50 ppm ZnO NPs (treatment S2*N1). This was followed by treatment S2N2 (100mM salt+100ppm ZnO NPs), which resulted in a 19.88% increase over the control. Conversely, treatment with S1N1 (50mM NaCl+50ppm ZnO NPs) led to a significant decrease in GB content (10.80% lower than the control).The remaining treatment combinations exhibited intermediate effects on this specific trait.

Hydrogen peroxide

Hydrogen peroxide in plants can serve various roles, including defense against pathogens, cell signaling, and regulation of physiological processes. However, maintaining the right balance of hydrogen peroxide is crucial, as excessive levels can be harmful and lead to oxidative stress.Hydrogen peroxide levels in pea plants treated with CN1 (50 ppm ZnO NPs) showed lower concentrations than those in the control group, by 9.98%. This indicates that ZnO NPs have the potential to mitigate hydrogen peroxide concentrations within plant tissues. As we increased the concentrations to 100ppm ZnO NPs (treatment C*N2) there was a significant increase 6.98% over the control in H_2O_2 seen Fig. 5(b).

Furthermore, the combined impact of ZnO NPs and salt stress showed even more promising results. The maximum decrease, approximately 11.47%, was achieved when plants were subjected to the dual treatment of 50 ppm ZnO NPs and 50 mM NaCl. In contrast, an increase in hydrogen peroxide content (2.42% over control) was noticed when plants were subjected to high concentrations of salt and ZnO NPs (100 ppm ZnO NPs+100 mM NaCl treatment S2*N2).

Notably, the levels of hydrogen peroxide gradually increased when plants were exposed to salt stress on its own.As for our experiment when plant treated with 100 mm Nacl (treatment S2) there was 13.35% increase in hydrogen per oxide over the control seen. This suggests that salt stress alone may enhancethe effect on hydrogen peroxide within pea plants.

The remaining treatment combinations exhibited intermediate effects on this specific trait. These findings highlight the substantial influence of ZnO NPs, especially when combined with salt.

Malondialdehyde (MDA)

In recent years, the role of nanotechnology in enhancing salt tolerance in various plant species has gained significant attention, as previously noted by Chen and Yada in 2011. Our new study found that plants treated with salt had more melodealdehyde (MAD) compared to untreated ones. Among the different salt treatments, the S2 treatment (100 Mm Nacl) showed the highest increase in MAD (115% more than control), followed closely by the S1 treatment (50 Mm Nacl) which had a 90% increase. When we added ZnO NPs along with salt stress, the S1N1 treatment (50 ppm ZnO NPs+50 mM NaCl) reduced MAD by 1.40% compared to control. But in all other combined treatments, MAD levels increased. For example, S1N2 increased by 4%, S2N1 by 52%, and S2N2 by 73%. Using ZnO NPs alone also improved MAD levels in pea plants. When plants were treated with only 50 ppm ZnO NPs, MAD decreased by 1.22% compared to normal Fig. 5(c). These findings show that ZnO NPs, especially combined with salt stress, affect MAD levels in pea plants.The relationship between ZnO NPs salt stress, and MAD content in pea plants is complex, and this study highlights their significant impact.

Discussion

According to literature, root is the most susceptible to the effects of salt stress. Salinity-stressed pea plants had shorter root lengths, corresponding to the findings in Fig. 3. Related results were recorded in *Tanacetum parthenium L*, and three varieties of green beans by Assimakopoulou et al. [49]. and *Phaseolus vulgaris* L [50]. In the current study, pea plants exposed to ZnO NPs in different doses (0, 50, and 100 mgL^{-1}) showed an improvement in plant growth under priming treatment with 50 mgL^{-1} ZnO NPs compared to the corresponding control; however, 100 mgL^{-1} ZnO NPs higher doze brought a reduction in growth. Similarly [51] discovered that (50 ppm) ZnO NPs increased the growth of broad beans, and Salem et al. [52]. discovered similer effects on the growth of tomatoes. Related results were shown by Singh et al. on wheat which depicted that plant height increased 18% over control on application of ZnO NPs [53]. Verma et al., (2023) also showed in their work on wheat that the plant height of wheat significantly increased with the initial concentration (upto 50 ppm) and decreased at higher doses at 100 ppm.

The comparative analysis of means and variances across different treatment groups is aptly illustrated in Fig. 4(c). However, the ratio and comparative studies between pod length, grain per pod and number of leaves were illustrated in Fig. 4(d). High salinity levels can lead to reduced growth, a decrease in the leaves number, as the plant may struggle to sustain its normal growth processes under such stress. In current study, it was observed that plants considered with 50 ppm of zinc oxide nanoparticles along with salt (NaCl) had the highest number of leaves (an average of 56 in 3 replicates). On the other hand, plants gave with 100 ppm of ZnO NPs along with salt had the lowest number of leaves (37). These results are similar to erliar reports found in maize plants [49, 54]. Additionally, Salama et al., (2019) discovered that the number of leaves in common bean plants increased as the amount of zinc oxide nanoparticles increased. However, Ahmed et al., (2022) found that very high concentrations, like 800 ppm, led to a reduction in the number of leaves [55].

The present inquiry was commenced to improve tolerance of the morphophysiological and biochemical mechanisms associated in salinity tolerance, and prompt stimulation of such mechanisms by exogenous treatments of ZnO NPs. The addition of nanoparticles showed ameliorative effects in both non-saline and saline conditions [56]. Application of ZnO NPs on NaCl-subjected *P. sativum L.* considerably decreased the ROS level, oxidized products like Glycine betaine, MDA, and Hydrogen peroxide. Treatment that was applied revealed a reduced negative impact of NaCl-stress on growth as shown in Fig. 6. It is one of the main factors restricting plant development and yield output, especially for leguminous plants, is soil salinity [57]. However, the biomarker expression due to ZnO NPs not only reduce the salrt stress effect but also improve the growth by stimulating the hormones and other biochemical molecules as indicated by the previous researcher [58, 59]. Reduced osmotic potential from salt-induced ion imbalance affects physiological, morphological, biochemical and other metabolic processes, which reduces growth overall [60, 61]. An increased or high amount of salt in the cytoplasm interferes with cell division, elongation, and other growth processes by challenging the vacuole's ability to compartmentalize [61, 62]. As per our findings plant treated with high level of salt (100 mm) increased the amount of GB content 48% over the control. Parallel results were documented incommon bean plants anxious by 100 mM of NaCl [50]. The effect of ZnO NPs on the glycine betaine content of pea plants can vary based on the concentration of NPs used and the specific conditions of the experiment. Glycine betaine is a compatible solute that helps plants cope with abiotic stress, including salinity and drought as already reported work. In some studies, it has been described that the treatment of zinc oxide nanoparticles can enhance the growth of osmoprotectants like glycine betaine in plants. When used in suitable amounts, nanoparticles can trigger stress responses in plants, prompting the synthesis of substances such as glycine betaine. These compounds aid the plant in withstanding unfavorable conditions.However, the specific effect on glycine betaine content in pea plants would depend on the concentration of ZnO NPs, the duration of exposure, and the plant's physiological response to these nanoparticles. When stress in applied Glycine betaine is produced in many crop plants, including *Beta vulgaris*, *Spinacia oleracea* [63], *Hordeum vulgare*, *Triticum aestivum*and, *Sorghum bicolor* [64]. As a result of water stress many physiological processes such as photosynthesis and protein synthesis are protectd by GB. Research has demonstrated that ZnO NPs exposure can affect plant physiology in both favorable and negative ways. When combined with salt stress, ZnO NPs have demonstrated the possibility to mitigate the adverse effects of salinity on pea plants as per our findings synergitic effect of ZnO NPs and salt stress S2N1 treatment with concentratins of (100 mm salt+50 ZnO NPs) produced a significant amount of GB in leaves of pea approximately 23.7% higher than control on the other hand treatment S1N1 (50 mM Nacl+50 PPM ZnO NPs) reduced the amount approximately 10.8% then control. Similar fndings were reported by (Gaafar et al. (2020) on salinitystressed soybean plant.Research suggests that the presence of ZnO NPs can enhance the accumulation of glycine betain in pea plants, potentially contributing to their improved salt tolerance [65]. ZnO NPs levels (50 and 100 mg L^{-1}).

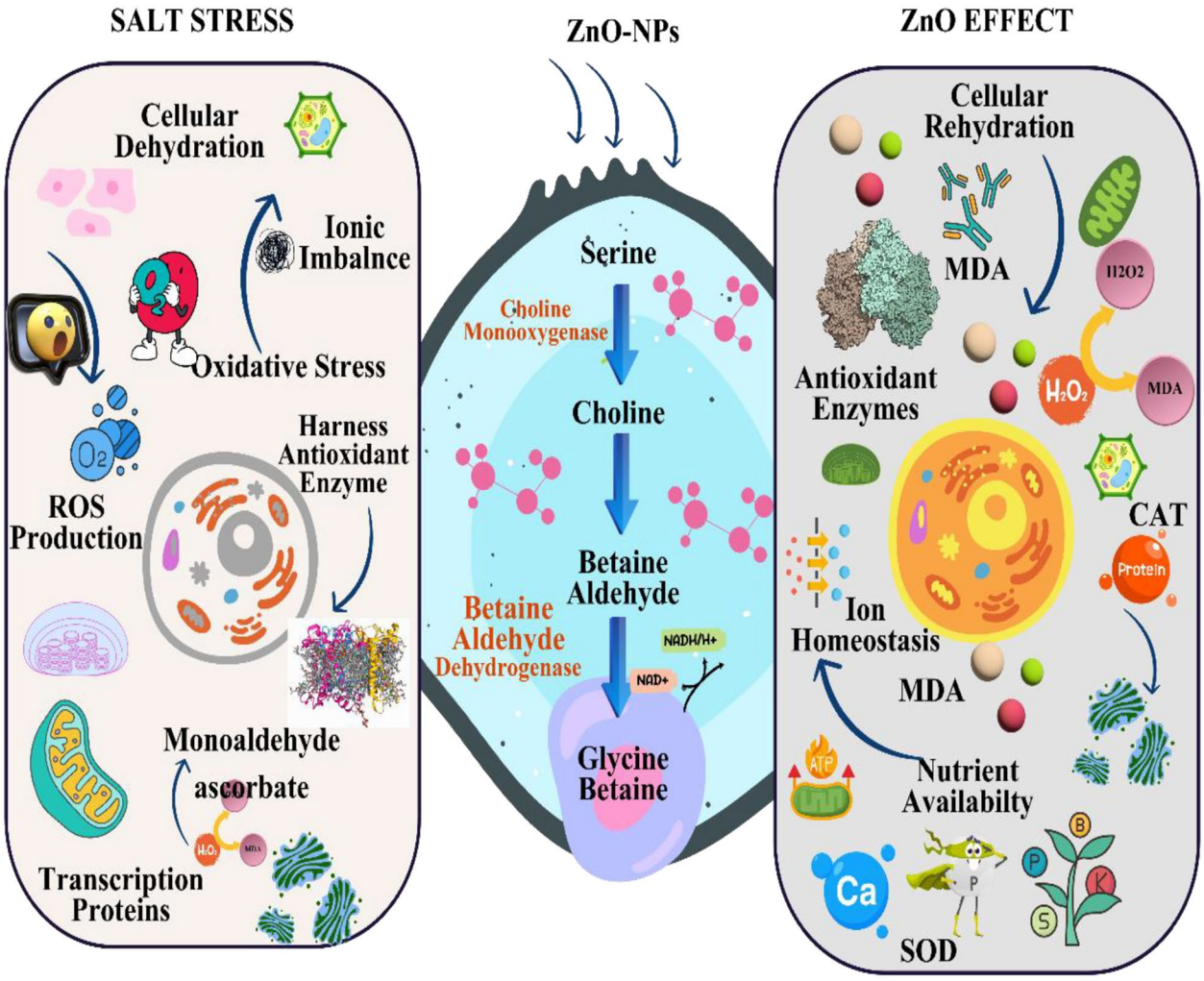

Fig. 6 Schemtic illustration of Mechanism underlying reaction occurring in *P. sativum L.* exposed to ZnO NPs and salt stress

The effect of ZnO NPs on the levels of malondialdehyde (MDA) in pea plants can be multifaceted.The outcome can differ based on factors like NPs concentration, exposure duration, and the plant's physiological condition.In some instances, when applied in suitable concentrations, ZnO NPs have shown a positive influence by reducing MDA levels as shown in mechanism Fig. 6. This reduction suggests that the nanoparticles might have antioxidative properties or trigger stress responses that help the pea plants cope with environmental challenges. However, when used in excessive concentrations, ZnO NPs may have the opposite effect, inducing oxidative stress and causing an increase in MDA levels. Furthermore, due to stress, some enzymes were expressed to regulate the homestasis which modulate antioxidant response of the plant to cope the toxicity. However, some antoxidatn ezymes also showed their expression in presence of nanoparticles as stress but work antagonstically [66, 67].

MDA concentration (act as an indicator of lipid peroxidation) was utilized to assess the lipid peroxidation in stressed plants. The plants introduced 100 mM NaCl showed a considerable increase in lipid peroxidation, with a jump of 115% when compared to the control plants. Additionally, the S1 treatment (50 mM NaCl) led to a 90% increase in lipid peroxidation. Similar findings were reported by Mogazy & Hanafy in *Vicia faba* plants, demonstrating a substantial increase in amount of MDA in response to high-level salt treatment [68].

In our study, the application of 50 ppm zinc oxide significantly reduced MDA content. This aligns with the conclusions of Yasmin et al. (2021), who reported a 65% decrease in MDA content in sunflowers when treated with ZnO NPs [69]. Furthermore, our learning

demonstrated that the combined treatment of salt and nanoparticles led to a substantial decrease in MDA content. Zinc oxide nanoparticles alleviated oxidative stress induced by salinity by enhancing plant defense mechanisms and antioxidant enzyme activities. These results are reliable with the findings of previous reports who monitored the alleviation of salinity stress in cotton plants with nano-zinc application [42, 70–73]. The impact of ZnO NPs combined with table salt stress on the hydrogen peroxide content of pea plants is intricate and contingent upon various factors. Under certain conditions, ZnO NPs can function as antioxidants, mitigating oxidative stress by scavenging reactive oxygen species like hydrogen peroxide. In these cases, the nanoparticles might counteract the increase in hydrogen peroxide induced by salt stress, demonstrating a protective effect. On the other hand, the result could potentially be neutral, with the ZnO NPs having little effect on the plant's capacity to fend off oxidative stress brought on by salt. Conversely, in situations where the concentrations of ZnO NPs are excessive, they could exacerbate oxidative stress, potentially leading to a significant rise in hydrogen peroxide content when combined with salt stress. Excessive exposure to anions can be detrimental to plants due to the rapid generation of ROS and the collection of Na (sodium) and Cl (chloride) ions, disrupting plant growth and physiological functions [74, 75]. In our study, pea plants exposed to salinity stress (100 mm salt, a 13.35% increase over control) exhibited a significant rise in hydrogen peroxide (H_2O_2) levels when compared to control plants. Parallel results were registered by Gupta and Pandey (2020) and Sofy et al. (2020) [76]. The elevated levels of free radicals can damage the plasma membrane, leading to cell membrane component degradation. Salt stress potentially compromises cellular membrane integrity and essential molecules like proteins and lipids. Although reactive oxygen species play a crucial role in plant responses to abiotic stress, their excessive production can be toxic and energetically costly to detoxify. Energy-intensive pathways aimed at ROS detoxification may become ineffective once energy reserves are depleted, leading to ROS toxicity. As a result, plants may experience oxidative stress due to increased concentrations of nanoparticles (NPs), such as ZnO NPs, which would decrease the activity of antioxidant enzymes.Our study demonstrated that 50 ppm ZnO NPs mitigated the oxidative stress in pea plants as compared to the control. However, treatment with 100 ppm ZnO NPs increased oxidative stress, consistent with the outcomes of Burman et al. (2013), who observed defensive effects of ZnO NPs on bio-membranes in the seedlings of chickpea, altering the membrane permeability and inducing oxidative stress [77]. The influence ZnO NPs and salt on H2O2 levels was investigated, and the results differed according to the amounts utilized.Treatment with 50 mm NaCl and 50 ppm ZnO NPs significantly decreased H_2O_2 levels in pea plants. However, with increasing concentrations, a substantial rise in malondialdehyde (MAD) content was observed. These outcomes align with Gaafar et al. (2020), who reported reduced H_2O_2 content in soybean plants under salinity stress exposed to 25 and 50 ppm ZnO NPs but a significant enhance in response to 100 and 200 ppm zinc oxide nanoparticles.

Conclusions

The findings of our study reveal that elevated salt levels 100 mM have detrimental effects on pea morphology, leading to reduced root length and stunted growth while hydrogen peroxide and MDA levels was increased. Conversely, ZnO NPs with concentration 50 ppm showed positive impacts on pea morphology and biochemical parameters. However, 100 ppm of ZnO NPs showed adverse effects on all assessed parameters, possibly indicating NPs toxicity at higher concentrations. Moreover, combined impacts of salt stress and ZnO NPs on peas showed concentrations dependent results. Among all these concnetrations, only S1*N1 demonstrated a positive impact on pea physiology, significantly increasing root length and improving other physiological parameters, while concurrently decreasing MDA and hydrogen peroxide levels. Conversely, all other treatments exhibited negative effects on the assessed parameters, potentially attributed to the high concentrations of both stressors.

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Author contributions

Ghazala Mustafa: Experimentation, Software, statistical analysis. Madhia Manzoor: First draft writing, Supervision. Sunbal Khalil C: Funding, supervision. Sana Batool: Experimentation, data collection. Mehrnaz Hatami: Writting, editing, Formal analysis. Murtaza Hasan: Conceptualization, supervision, Manuscript writing.

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Data availability

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate

All methods performed in this study were in compliance with the relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

All the authors stated and declared no conflict of interest.

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