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Research Article

Keywords: Drought stress, ZnO nanoparticles, nanopriming, wheat, osmotic stress, seedling vigor

Posted Date: September 15th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10939912/v1>

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Additional Declarations: No competing interests reported.

ZnO nanopriming improves early drought resilience of wheat by maintaining water status, membrane integrity, and seedling growth

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Abstract

Drought during germination and early seedling development can severely constrain wheat establishment, highlighting the need for seed-based strategies that enhance physiological resilience to water limitation. This study investigated whether zinc oxide nanoparticle priming (NZp) improves the physiological and growth responses of wheat (*Triticum aestivum* L. cv. Jom) exposed to polyethylene glycol (PEG)-induced osmotic stress. Seeds were hydroprimed (Hp) or primed with ZnO nanoparticles (NZp) or zinc sulfate (IZp) at 50 mg L⁻¹ and subsequently germinated under 0, -0.3, and -0.6 MPa osmotic potentials. Drought stress impaired seedling performance, with radicle fresh weight and plumule length decreasing by 15.3% and 37.8% and 26.4% and 60.4%, at -0.3 and -0.6 MPa, respectively, relative to the non-stressed condition. Under non-stress conditions, NZp increased relative water content, germination, plumule length, radicle fresh weight, plumule fresh weight, and seedling vigor index by 13.6%, 5.26%, 15.0%, 18.0%, 15.0%, and 16.4%, respectively, compared with Hp. Under moderate drought (-0.3 MPa), NZp increased soluble protein and radicle dry weight by 10.8% and 55.4% relative to Hp. Under severe drought (-0.6 MPa), NZp reduced malondialdehyde levels by 20.9% and 36.8% compared with Hp and IZp, respectively. Overall, ZnO nanopriming strengthened early wheat drought resilience by coordinating water-status maintenance, membrane protection, and seedling growth, with its benefits being particularly evident under non-stress and moderate drought conditions.

Keywords: Drought stress; ZnO nanoparticles; nanopriming; wheat; osmotic stress; seedling vigor

1. Introduction

Wheat (*Triticum aestivum* L.) is a major staple cereal underpinning global food security and nutrition, with more than 200 million hectares cultivated annually (Mathur et al. 2019; Zahra et al. 2021). Dryland wheat production is particularly vulnerable to water scarcity, and increasing drought frequency under climate change threatens productivity in water-limited environments (Farooq et al. 2014; Osman et al. 2022). Seed germination and early seedling establishment are among the most drought-sensitive developmental stages because they depend strongly on adequate hydration for metabolic activation, cell expansion, and tissue growth (Barnabás et al. 2008; Farooq et al. 2019). Improving seedling establishment under water deficit is therefore critical for sustaining wheat productivity under increasingly variable climatic conditions.

Drought restricts seed hydration, disrupts cellular metabolism, and impairs water relations, resulting in reduced relative water content (RWC), germination, and seedling vigor (Hassan et al. 2020). Water deficit also induces excessive accumulation of reactive oxygen species (ROS), which, beyond their signaling functions, can cause oxidative damage to lipids, proteins, and nucleic acids (Hasanuzzaman et al. 2020; Fallah et al. 2025). Enhanced lipid peroxidation, reflected by malondialdehyde (MDA) accumulation, compromises membrane integrity and stability, while inhibition of cell division and elongation restricts radicle and plumule development (Al-Quraan et al. 2021; El-Shazoly et al. 2025). Thus, maintaining cellular water status, membrane integrity, and antioxidant capacity is fundamental to drought resilience during early development.

Seed priming provides a cost-effective approach to improve germination and stress tolerance by initiating pre-germinative metabolic processes, including enzymatic activation, cellular repair, and reserve mobilization, without radicle protrusion (Farooq et al. 2019; Zhao et al. 2024). Nanopriming may further enhance these benefits through the high reactivity and surface area of nanoparticles, facilitating nutrient delivery, metabolic regulation, antioxidant defense, and ROS-mediated signaling (Yang et al. 2024; Zhao et al. 2024).

Zinc (Zn) is an essential micronutrient involved in enzyme activity, protein synthesis, membrane stability, photosynthesis, and antioxidant defense (Khanm et al. 2018; Hamzah Saleem 2022). Recent evidence indicates that ZnSO₄ and ZnO nanoparticles can enhance germination, seedling vigor, water status, antioxidant activity, and phenolic accumulation while reducing oxidative damage, with ZnO nanoparticles often showing greater efficacy than ZnSO₄ (Geremew et al. 2025). Importantly, priming-induced improvements in early root development may enhance subsequent soil exploration and water acquisition, potentially strengthening drought adaptation (Aswathi et al. 2022; Liu et al. 2022; Li et al. 2021).

However, whether ZnO nanopriming primarily accelerates early development or also enhances physiological preparedness for water deficit remains insufficiently understood. Controlled osmotic stress using polyethylene glycol (PEG) provides a useful approach for simulating the reduced water potential associated with soil drying during germination and early seedling growth (Verslues et al. 2006; Mahpara et al. 2022). Therefore, evaluating wheat responses to ZnO nanopriming under contrasting PEG-induced water potentials can provide mechanistic insight into its potential to improve early drought resilience.

In dryland wheat production systems, successful crop establishment depends strongly on the availability and timing of early-season rainfall. Short periods of insufficient soil moisture during germination and early seedling development can reduce stand establishment and constrain subsequent crop performance. Therefore, seed-based approaches capable of improving early seedling performance under transient water limitation may have particular relevance to wheat production in semi-arid environments.

We hypothesized that ZnO nanopriming would improve wheat germination and early seedling performance under osmotic stress by maintaining tissue water status, membrane integrity, and root development, and that its effectiveness would vary with stress intensity. However, it remains unclear whether nanoparticulate and ionic Zn provide comparable protection across contrasting levels of water limitation during the critical germination-to-seedling transition. In particular, the extent to which ZnO nanopriming differentially affects water status, membrane stability, protein accumulation, and root development under moderate versus severe osmotic stress remains insufficiently resolved. Therefore, this study compared ZnO and ZnSO₄ priming

at an equivalent Zn concentration to determine whether their effects on wheat germination and early seedling physiology vary with stress intensity.

2. Materials and Methods

2.1. Experimental site, design and treatments

The study was conducted in the Agronomy Research Laboratory, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran, using wheat (*Triticum aestivum* L.) cultivar ‘Jom’. Treatments were arranged factorially in a completely randomized design (CRD) with four replications. The experimental factors comprised drought stress, imposed at three osmotic potentials (0, -0.3, and -0.6 MPa), and seed-priming treatment, including hydropriming with distilled water, priming with zinc oxide nanoparticles (ZnO-NPs; 50 mg L⁻¹), and priming with zinc sulfate (ZnSO₄; 50 mg L⁻¹). Polyethylene glycol (PEG-6000) was used to impose controlled osmotic stress as a laboratory proxy for water limitation during germination and early seedling development. The amount of PEG-6000 required to achieve the target osmotic potentials was calculated according to the equation proposed by Michel and Kaufmann (1973). The factorial combination resulted in nine treatment combinations, each replicated four times.

2.2. Seed sterilization, priming, and stress application

Uniform, healthy, and undamaged seeds were selected manually before treatment. Seeds were surface sterilized with 2% (v/v) sodium hypochlorite solution for 2 min and then rinsed three times with sterile distilled water to remove any residual disinfectant. Excess moisture was removed by blotting the seeds on sterile filter paper.

The ZnO-NP and ZnSO₄ priming solutions were prepared to provide an equivalent Zn concentration of 50 mg Zn L⁻¹. Accordingly, 62 mg ZnO-NPs and 0.123 g ZnSO₄ were dispersed/dissolved in 1 L of sterile distilled water, respectively (Awasthi et al. 2017; Rather et al. 2022; Ochoa-Chaparro et al. 2025). The suspension was first vortexed for 5 min and then ultrasonicated for 15 min to achieve a homogeneous dispersion of nanoparticles and minimize aggregation. Seeds were soaked in the suspension for 15 h at 15°C in darkness. After soaking, seeds were removed, surface-dried on sterile filter paper, and immediately used for germination tests. Seeds were soaked in the solution for 15 h at 15°C under the same conditions used for

nanoparticle priming. Hydroprimed seeds were soaked in sterile distilled water for 15 h at 15°C and processed similarly.

PEG-6000 solutions corresponding to −0.3 and −0.6 MPa were prepared by dissolving 151.4 and 223.7 g PEG-6000, respectively, in 1 L of sterile distilled water with continuous magnetic stirring until complete dissolution.

After priming, 20 seeds were placed in 10-cm diameter Petri dishes lined with two sheets of Whatman No. 10 filter paper. Each dish received 10 mL of the corresponding PEG solution or distilled water for the control treatment. Petri dishes were sealed with parafilm to reduce evaporation and maintain a stable osmotic environment and were incubated in the growth chamber at $25 \pm 1^\circ\text{C}$. Germination was monitored daily, and all measurements were performed 7 days after sowing.

2.3. Measured traits

2.4.1. Germination percentage (GP)

Seeds were considered germinated when the radicle reached at least 2 mm in length (Espanany et al. 2016). Germination percentage was calculated on the seventh day using:

$$GP = \frac{N_i}{N} \times 100 \quad [1]$$

where N_i is the number of germinated seeds and N is the total number of seeds tested.

2.3.2. Seedling growth

Ten normal seedlings were randomly selected from each Petri dish to assess seedling growth. Radicle and plumule lengths were measured using a millimeter ruler and expressed as centimeters per seedling. Subsequently, the radicles and plumules were separated, gently washed with distilled water, and blotted with tissue paper to remove surface moisture. Fresh weights were immediately determined using an analytical balance with a precision of 0.001 g. For dry-weight determination, the samples were oven-dried at 60°C for 48 h until reaching a constant weight and then weighed. Radicle and plumule fresh and dry weights were expressed on a per-seedling basis.

2.3.3. Seedling vigor index (SVI)

Seedling vigor index was calculated according to Espanany et al. (2016):

$$SVI = GP \times \text{Seedling length} \quad [2]$$

where seedling length is the sum of radicle and plumule lengths (cm).

2.3.4. Relative water content (RWC)

Relative water content was determined following Martínez et al. (2007). Two seedlings were randomly sampled from each replicate, and uniform segments from the middle portion of the plumule were weighed immediately to obtain fresh weight (FW). Samples were floated on distilled water for 24 h in darkness at 4 °C to obtain turgid weight (TW). After gently blotting the surface, samples were weighed again and then dried at 70 °C for 24 h to determine dry weight (DW). RWC was calculated as:

$$RWC = \frac{FW - DW}{SW - DW} \times 100 \quad [3]$$

2.3.5. Membrane stability index (MSI)

Membrane stability index was estimated according to Awan et al. (2021). Plumule segments (2 cm) were placed in test tubes containing 10 mL of distilled water. Tubes were incubated at 25°C for 24 h, and the initial electrical conductivity (C_1) was measured using a calibrated conductivity meter. Samples were then autoclaved at 121°C for 20 min, cooled to room temperature, and the final conductivity (C_2) was recorded. The conductivity meter was calibrated with 0.01 M KCl before use. MSI was calculated as:

$$MSI = \left(1 - \frac{C_1}{C_2}\right) \times 100 \quad [4]$$

2.3.6. Lipid peroxidation

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) using the thiobarbituric acid (TBA) method of Heath and Packer (1968). Fresh plumule tissue (0.1 g) of four seedlings was homogenized in 1.5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and

centrifuged at $10,000 \times g$ for 10 min. Mix 1 mL of the supernatant with 2 mL of 20% TCA containing 0.5% TBA. The mixture was heated at 95°C for 30 min, rapidly cooled in an ice bath, and centrifuged again at $10,000 \times g$ for 5 min.

Absorbance of the supernatant was measured at 532 and 600 nm using a UV–Vis spectrophotometer. MDA concentration was calculated using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ (Narwal et al. 2009):

$$MDA = \frac{(A_{532} - A_{600}) \times V}{\epsilon \times d \times FW} \quad [5]$$

where V is the extract volume, d is the cuvette path length, and FW is the fresh weight of tissue.

2.3.7. Soluble protein content

Total soluble protein was determined by the Bradford assay (1976). Fresh plumule tissue (1 g) of four seedlings was homogenized in 10 mL of ice-cold 0.1 M potassium phosphate buffer (pH 7.5). The homogenate was centrifuged at $20,000 \times g$ for 30 min at 4°C , and the supernatant was collected for analysis.

For protein determination, 50 μL of the extract was mixed with 3 mL of Bradford reagent, and absorbance was read at 595 nm after 5 min. Protein concentration was estimated from a bovine serum albumin (BSA) standard curve and expressed as mg g^{-1} fresh weight.

2.4. Statistical analysis

Data were analyzed using SAS software (version 9.4). Analysis of variance (ANOVA) was performed for all measured traits. Residuals were examined for normality and homogeneity of variance before analysis. Data were subjected to two-way analysis of variance using a factorial model, with drought stress, seed-priming treatment, and their interaction considered as fixed effects. The Petri dish was considered the experimental unit. . For traits measured on multiple seedlings within each dish, measurements were averaged to obtain a single value per experimental unit before statistical analysis. When significant effects were detected, treatment means were separated using Fisher's least significant difference (LSD) test at $p < 0.05$. For

traits showing a significant stress $\times$ priming interaction, treatment effects were interpreted separately within each osmotic stress level rather than from marginal means.

3. Results

3.1. Relative water content

Drought stress, seed priming treatments, and their interaction significantly affected relative water content (RWC) ($p < 0.01$ for main effects and $p < 0.05$ for interaction; Table 1). Under non-stress conditions, ZnO nanoparticle priming (NZp) resulted in the highest RWC (95%), representing a 13.6% increase compared with Hp, while zinc sulfate priming (IZp) increased RWC by 8.2%. Under moderate drought stress (-0.3 MPa), both zinc-based priming treatments showed no significant difference in RWC. Under severe drought stress (-0.6 MPa), IZp exhibited the greatest improvement in water status, increasing RWC by 11% compared with Hp. These findings indicate that NZp was more effective in maintaining tissue hydration under optimal and moderate stress conditions, whereas IZp provided greater protection against severe water deficit (Fig. 1a).

3.2. Soluble protein content

Drought stress, seed priming, and their interaction significantly influenced soluble protein content ($p < 0.01$; Table 1). The response pattern varied depending on drought intensity and priming treatment. Under non-stress conditions, Hp exhibited the highest soluble protein content (2.70 mg g⁻¹ FW), although it was statistically comparable with NZp (2.63 mg g⁻¹ FW). In contrast, IZp markedly reduced soluble protein accumulation to 1.24 mg g⁻¹ FW, representing a 53.9% decrease relative to Hp. Under moderate drought stress (-0.3 MPa), NZp resulted in the highest soluble protein concentration (2.51 mg g⁻¹ FW), exceeding Hp and IZp by 10.8% and 6.57%, respectively (Fig. 1b).

Under severe drought stress (-0.6 MPa), soluble protein content declined across all treatments; however, the greatest reduction occurred in NZp seedlings (1.83 mg g⁻¹ FW), which was 22.3% lower than Hp and 17.9% lower than IZp. Overall, NZp promoted soluble protein accumulation under moderate drought conditions, whereas Hp and IZp maintained relatively higher protein levels under severe drought stress (Fig. 1b).

3.3. Membrane Lipid Peroxidation

Seed priming and its interaction with drought stress significantly affected malondialdehyde (MDA) concentration ($p < 0.01$), whereas the main effect of drought stress alone was not significant (Table 1). Under non-stress conditions, IZp resulted in the lowest MDA concentration ($0.61 \mu\text{mol g}^{-1} \text{FW}$), representing a 62% reduction compared with Hp ($1.60 \mu\text{mol g}^{-1} \text{FW}$). In contrast, NZp did not significantly differ from Hp. Under moderate drought stress (-0.3 MPa), NZp exhibited the highest MDA accumulation ($3.08 \mu\text{mol g}^{-1} \text{FW}$), which was 20.8% and 19.2% higher than Hp and IZp, respectively (Fig. 1c).

Under severe drought stress (-0.6 MPa), NZp significantly reduced MDA accumulation to $2.93 \mu\text{mol g}^{-1} \text{FW}$, corresponding to decreases of 20.9% and 36.8% compared with Hp and IZp, respectively. Conversely, IZp increased MDA concentration to $4.64 \mu\text{mol g}^{-1} \text{FW}$ (Fig. 1c). These results suggest that ZnO nanoparticle priming enhanced membrane protection primarily under severe drought stress by reducing lipid peroxidation.

3.4. Membrane stability

Drought stress significantly affected membrane stability ($p < 0.01$), while the effect of seed priming was significant at the 5% probability level (Table 1). Increasing drought severity progressively reduced membrane stability, with mean values decreasing from 64.8% under non-stress conditions to 61.9% and 60.3% under moderate (-0.3 MPa) and severe drought stress (-0.6 MPa), respectively (Fig. 2a). This decline indicates enhanced membrane disruption and cellular injury under limited water availability.

Across priming treatments, NZp produced the highest membrane stability (67.2%), followed by IZp (63.5%), whereas Hp showed the lowest value (56.3%) (Fig. 2a). The superior performance of NZp suggests that ZnO nanoparticles effectively enhanced membrane integrity and reduced drought-induced cellular damage.

3.5. Germination percentage

Drought stress and seed priming significantly influenced germination percentage ($p < 0.05$ and $p < 0.01$, respectively), and their interaction was also significant ($p < 0.01$; Table 1). The effects

of NZp and IZp on germination percentage were similar in all moisture conditions ($p > 0.05$). Under non-stress conditions, NZp produced the highest germination percentage (97.7%), representing increases of 5.26% compared with Hp ($p < 0.05$; Fig. 3a). Under moderate and severe drought stress conditions, NZp and Hp showed no significant difference ($p > 0.05$). The germination of IZp-treated seeds was lower than Hp under moderate drought stress conditions and higher than Hp under severe stress. Therefore, NZp was more beneficial for germination under favorable conditions, whereas IZp provided greater protection under severe drought stress (Fig. 3a).

3.6. Radicle length

Drought stress, seed priming, and their interaction significantly affected radicle length ($p < 0.01$; Table 1). Under non-stress conditions, NZp produced the longest radicles (13.3 cm), whereas IZp resulted in the shortest radicles (11.6 cm) (Fig. 3b). Under moderate drought stress (-0.3 MPa), there was no statistically significant difference in radicle length between NZp and IZp. Under severe drought stress (-0.6 MPa), radicle elongation declined in all treatments; however, NZp maintained higher radicle length (11.3 cm) than the Hp (10.6 cm). Thus, Zn-based priming, particularly NZp, enhanced early root development and partially mitigated drought-induced inhibition of radicle growth (Fig. 3b).

3.7. Plumule length

ANOVA revealed that drought stress and seed priming treatments significantly affected plumule length ($p < 0.01$), whereas their interaction was not significant (Table 2). Plumule elongation was progressively inhibited as drought severity increased. The highest plumule length was recorded under non-stress conditions (9.1 cm), while exposure to moderate drought stress (-0.3 MPa) reduced plumule length to 6.8 cm, representing a 26.4% decrease compared with the non-stressed condition ($p < 0.05$). Under severe drought stress (-0.6 MPa), plumule length declined further to 3.6 cm, corresponding to a 60.4% reduction relative to the control ($p < 0.05$; Fig. 4a).

Among seed priming treatments, NZp produced the greatest plumule length (6.9 cm), representing a 15.0% increase compared with Hp (6.0 cm). IZp also improved plumule growth, with an average plumule length of 6.6 cm, which was 9.9% higher than Hp; however, it remained significantly lower than NZp ($p < 0.05$; Fig. 4b). These results indicate that ZnO

nanoparticle priming was more effective in promoting plumule elongation and alleviating drought-induced inhibition of plumule growth.

3.8. Radicle fresh weight

Radicle fresh weight was significantly influenced by drought stress and seed priming treatments ($p < 0.01$), with increasing drought intensity resulting in a progressive reduction in root biomass (Table 2). Across drought levels, mean radicle fresh weight decreased from 0.67 g under non-stress conditions to 0.57 g under moderate drought stress (-0.3 MPa), representing a 15.3% reduction. Under severe drought stress (-0.6 MPa), radicle fresh weight declined further to 0.42 g, corresponding to reductions of 37.8% compared with the control and 26.5% compared with moderate stress conditions (Fig. 4c).

Seed priming significantly modified radicle biomass accumulation. NZp produced higher radicle fresh weight (0.59 g) than the Hp (0.50 g) (Fig. 4d). NZp increased radicle fresh weight by 18.0% relative to Hp across the tested stress levels (Fig. 4d).

3.9. Radicle dry weight

Radicle dry weight was significantly affected by drought stress, seed priming, and their interaction ($p < 0.01$; Table 2). Under non-stress conditions, radicle dry weight was 0.07, 0.08, and 0.07 g in Hp, NZp, and IZp treatments, respectively, and NZp was significantly higher than the other two treatments (Fig. 5 a).

Under moderate drought stress (-0.3 MPa), NZp resulted in the highest radicle dry weight (0.12 g), representing a 55.4% increase compared with Hp, whereas IZp increased radicle dry weight to 0.09 g ($p < 0.05$; Fig. 5a). Under severe drought stress (-0.6 MPa), both NZp and IZp maintained similar radicle dry weights (0.09 g), compared with 0.08 g in Hp (Fig. 5a).

3.10. Plumule fresh weight

Plumule fresh weight was significantly affected by drought stress, seed priming, and their interaction ($p < 0.01$; Table 2). Under non-stress conditions, NZp produced the highest plumule fresh weight (1.74 g), followed by IZp (1.62 g) and Hp (1.51 g). Compared with Hp, NZp increased plumule fresh weight by 15.0%, while IZp increased this trait by 7.16% ($p < 0.01$;

Fig. 5b). Under moderate and severe drought stress conditions, there was no significant difference between seed priming treatments for plumule fresh weight (Fig. 5b).

3.11. Plumule dry weight

Plumule dry weight was significantly influenced by drought stress, seed priming, and their interaction ($p < 0.01$; Table 2). Under non-stress conditions, NZp produced the highest plumule dry weight ($0.15 \text{ g seedling}^{-1}$), which was significantly greater than IZp ($0.13 \text{ g seedling}^{-1}$) and Hp ($0.13 \text{ g seedling}^{-1}$) (Fig. 6a). Under moderate and severe drought stress conditions, there was no significant difference between seed priming treatments for plumule dry weight (Fig. 6a).

3.12. Seedling vigor index

The results of ANOVA showed that the effect of drought stress, seed priming and the interaction effect of drought stress $\times$ priming on seedling vigor index were significant ($p < 0.01$; Table 2). Under no drought stress conditions (0 MPa), the highest seed vigor index (22.7) was observed in the priming treatment with NZp, which was significantly higher than Hp (19.5) and IZp (19.7). However, no significant difference was observed between Hp and IZp (Fig. 6b).

At -3 MPa stress, the seed vigor index in Hp decreased to 17.6, while its values in the NZp and IZp treatments were 20.1 and 19.4, respectively. At this stress level, NZp and IZp were not statistically significantly different from each other, but both had significantly higher seed vigor index than Hp (Fig. 6b). At -6 MPa, seed vigor index in Hp reached 12.5, while in NZp and IZp treatments it was 14.1 and 13.9, respectively. Based on the comparison of means, NZp and IZp were placed in the same statistical group, and no significant difference was observed between them, in contrast, both treatments had significantly higher seed vigor index than Hp (Fig. 6b).

4. Discussion

Drought stress constrained wheat seedling establishment by impairing tissue hydration, causing oxidative membrane damage, restricting germination, and inhibiting root and shoot development. However, the magnitude of these limitations depended strongly on the zinc

source and drought intensity. ZnO nanoparticle priming (NZp) provided the most consistent benefits for membrane stability, radicle development, and seedling vigor, whereas ZnSO₄ priming (IZp) showed specific advantages under severe drought, particularly for tissue hydration. Thus, the effectiveness of zinc priming appears to depend on the interaction among zinc availability, the physiological process involved, and stress intensity.

4.1. Water status and membrane protection under drought stress

The progressive decline in RWC with increasing drought severity indicates that decreasing external water potential substantially impaired tissue hydration. Maintaining cellular water status is essential for sustaining turgor, cell expansion, membrane function, and metabolic activity; consequently, RWC is an important indicator of early drought responses (Hassan et al. 2020). However, the response to Zn priming depended strongly on stress intensity. NZp produced the highest RWC under non-stress conditions, increasing it by 13.6% relative to Hp, whereas NZp and IZp were comparable under moderate drought. Under severe drought, IZp provided the greatest advantage, increasing RWC by 11% relative to Hp (Fig. 1a). Thus, zinc priming did not uniformly enhance water status, and the relative effectiveness of nanoparticulate and ionic Zn changed with stress severity. Under favorable conditions, the high surface area and reactive characteristics of ZnO nanoparticles may facilitate interactions with seed tissues and Zn availability during early development. Under severe drought, however, the rapid availability of Zn²⁺ from ZnSO₄ may be more advantageous for maintaining cellular processes associated with water balance. Similar improvements in plant water status following ZnO application have been attributed to enhanced root development, water acquisition, osmotic regulation, and activation of protective mechanisms (Amiri et al. 2024).

NZp did not reduce MDA under non-stress conditions and produced the highest MDA under moderate drought. In contrast, under severe drought, NZp reduced MDA by 20.9% relative to Hp and 36.8% relative to IZp (Fig. 1c). This shift suggests that the membrane-protective effect of NZp became more evident as oxidative pressure increased. Similar reductions in lipid peroxidation following ZnO application under drought have been associated with enhanced antioxidant protection and redox regulation (Rai-Kalal et al. 2021; Elsharkawy et al. 2025). The contrasting MDA response of NZp across stress intensities represents an important feature of the present study. Although ZnO nanopriming was associated with greater lipid peroxidation under moderate osmotic stress, it markedly reduced MDA under severe stress. This non-linear response indicates that the physiological consequences of nanoparticulate Zn cannot be

interpreted as uniformly protective across stress intensities and may depend on the balance between stress-induced oxidative signaling and cellular damage. Because ROS production and antioxidant capacity were not directly quantified, the mechanisms underlying this transition remain to be tested.

Despite the high MDA under moderate drought, NZp produced the highest mean membrane stability, whereas Hp had the lowest value (Fig. 1c). Because the drought $\times$ priming interaction was not significant for membrane stability, this advantage represents a relatively stable treatment effect across stress levels. Zinc can contribute to membrane protection through structural stabilization and its involvement in antioxidant defense, including Zn-dependent superoxide dismutase. Thus, the greater membrane stability under NZp likely reflects the combined effects of Zn-mediated structural protection and improved tolerance to oxidative perturbation. The contrasting responses of RWC and MDA under severe drought are particularly informative. IZp was more effective in maintaining tissue hydration, whereas NZp was more effective in limiting lipid peroxidation. Therefore, water conservation and oxidative protection appear to represent partially distinct components of drought adaptation, both of which contribute to maintaining seedling functionality.

4.2. Soluble protein metabolism and stress-dependent osmotic adjustment

Soluble proteins contribute to drought adaptation by supporting osmotic adjustment, stabilizing cellular structures, and maintaining metabolic and enzymatic functions during dehydration (Ozturk et al. 2021). They also include stress-responsive proteins involved in cellular protection and repair (Chen et al. 2021). The significant drought $\times$ priming interaction therefore indicates that Zn-mediated regulation of protein metabolism was strongly dependent on water availability.

Under non-stress conditions, Hp produced the highest protein concentration, although NZp was statistically comparable, whereas IZp reduced protein content by 53.9% relative to Hp (Fig. 1b). This indicates that greater Zn availability did not necessarily stimulate protein accumulation. Instead, the physiological response depended on whether additional Zn could be effectively utilized under the prevailing environmental conditions.

NZp showed its clearest advantage under moderate drought, where soluble protein concentration was 10.8% higher than Hp and 6.57% higher than IZp (Fig. 1b). This response

coincided with favorable effects of NZp on RWC, membrane stability, radicle development, and seedling vigor, suggesting coordinated maintenance of osmotic adjustment and cellular metabolism. Zn may support protein metabolism through its roles in enzyme activity, nucleic acid metabolism, protein synthesis, and cellular protection. Similar increases in protein accumulation following ZnO application have been reported in drought-stressed crops (Ajmal et al. 2023; Sadati et al. 2022).

However, this advantage disappeared under severe drought. Protein content declined in all treatments, with NZp producing values 22.3% and 17.9% lower than Hp and IZp, respectively (Fig. 1b). Severe dehydration may constrain substrates, energy metabolism, translation, and other processes required for protein synthesis beyond the capacity of nanoparticle-mediated Zn supply to compensate. Thus, NZp appears to support protein metabolism primarily under mild to moderate stress, whereas severe drought imposes stronger metabolic limitations.

4.3. Germination, metabolic activation, and seedling vigor

Germination depends heavily on water uptake and metabolic reactivation. Increasing drought severity reduced germination and seedling vigor, consistent with restricted imbibition, hydrolytic enzyme activity, reserve mobilization, respiration, and embryo growth under low water potential (Bhandari et al. 2023).

Under non-stress conditions, NZp produced the highest germination percentage (97.7%), representing a 5.26% increase relative to Hp, although it did not differ significantly from IZp (Fig. 3a). This response suggests that nanoparticulate Zn can support metabolic processes required during seed activation. Zinc is involved in membrane stabilization, enzyme activity, nucleic acid metabolism, and other processes required during germination, while the high surface area of nanoparticles may facilitate Zn–seed interactions.

Under moderate drought, NZp did not significantly improve germination relative to Hp, whereas IZp reduced germination below Hp (Fig. 3a). Under severe drought, IZp improved germination relative to Hp and remained statistically comparable with NZp. Thus, the effect of Zn priming on germination was strongly stress-dependent, with ionic Zn showing a specific advantage under severe water limitation. Differences in dissolution and Zn-release dynamics may contribute to these contrasting responses.

The seedling vigor index integrates germination and subsequent seedling development and therefore provides a broader measure of priming effectiveness. Both Zn treatments maintained significantly greater vigor than Hp under moderate and severe drought, while NZp produced the highest vigor under non-stress conditions (Fig. 6b). The maintenance of vigor despite limited effects on germination under some stress conditions indicates that Zn priming also acted through post-germinative processes, particularly preservation of membrane function and seedling growth. Similar improvements in germination and seed vigor following ZnO priming have been reported in wheat and peanut (Rai-Kalal et al. 2021; Ashwini et al. 2024).

4.4. Radicle development as a central component of drought adaptation

Radicle development is critical during early drought exposure because the root system determines the seedling's capacity to explore the growth medium and acquire water. Drought markedly reduced radicle length and biomass in the present study, reflecting the sensitivity of cell expansion to declining water potential. Reduced turgor, restricted cell elongation, and impaired metabolism can all contribute to inhibition of root growth under drought.

NZp provided the clearest advantage for radicle development. It produced the longest radicles under non-stress conditions and maintained greater radicle length than Hp under severe drought. Under moderate drought, NZp increased radicle dry weight by 55.4% relative to Hp (Fig. 5a). Thus, nanoparticulate Zn promoted both root elongation and dry matter accumulation when water availability was limited.

Zinc can influence root development through enzyme activation, nucleic acid metabolism, cell division, and growth regulation. Its effects on auxin metabolism and signaling may also contribute to root elongation and architecture (Hassan et al. 2020). The stimulatory effect of ZnO nanoparticles agrees with previous reports of enhanced root growth and biomass following ZnO application in wheat, rice, and leguminous crops (Abbas et al. 2023; Mazhar et al. 2023; Choudhury et al. 2025).

The strongest NZp effect occurred under moderate rather than severe drought. Under severe stress, NZp and IZp produced similar radicle dry weights, suggesting that extreme dehydration imposed limitations on cell expansion and carbon allocation and restricted the expression of Zn-mediated growth promotion. Therefore, the beneficial effect of NZp on root development

appears greatest when sufficient metabolic activity remains to convert improved Zn status into structural growth.

The parallel improvement in radicle development, RWC, membrane stability, and vigor suggests functional interdependence among these processes. The enhanced root development may have contributed to improved water relations; however, direct measurements of water uptake and root hydraulic conductivity are required to confirm this mechanism.

4.5. Plumule development and biomass under water limitation

Drought progressively inhibited plumule elongation, reducing plumule length by 26.4% under moderate and 60.4% under severe stress relative to non-stressed seedlings (Fig. 4a). This response reflects the sensitivity of shoot growth to reduced turgor and metabolic limitations under water deficit (Wach and Skowron 2022).

NZp produced the greatest mean plumule length, increasing it by 15.0% relative to Hp, while IZp increased it by 9.9% (Fig. 4b). Because the drought $\times$ priming interaction was not significant for plumule length, this advantage represents a general priming effect across stress levels. The positive effect of ZnO may involve improved Zn availability and the roles of Zn in enzyme activity, membrane stabilization, growth regulation, chlorophyll metabolism, and antioxidant protection. Previous studies have reported enhanced shoot growth and photosynthetic performance following ZnO application in safflower, mung bean, and peanut (Ghiyasi et al. 2023; Ajmal et al. 2023; Ashwini et al. 2024).

However, the biomass response differed from plumule length. NZp increased plumule fresh and dry weights under non-stress conditions, but no significant differences among priming treatments were detected under moderate or severe drought. Thus, the growth-promoting effect of NZp on shoot biomass was progressively constrained as water limitation intensified. This suggests that, under drought, water availability became a stronger limitation to biomass accumulation than Zn supply, even when Zn-mediated cellular protection was maintained.

4.6. Integrated physiological response of ZnO nanoparticle priming

The combined results indicate that drought imposed multiple constraints on wheat seedling establishment, while Zn priming alleviated several of these limitations through partially interconnected mechanisms. NZp showed the most consistent benefits for membrane stability,

radicle development, and seedling vigor, whereas IZp showed specific advantages for tissue hydration under severe drought.

Physiological response can be conceptualized as three interconnected processes: maintenance of water status, protection against oxidative and membrane damage, and preservation of developmental activity. NZp improved membrane stability and, under severe drought, markedly reduced MDA accumulation. It also promoted radicle growth and maintained greater seedling vigor. These responses suggest that ZnO nanoparticles created a physiological environment more favorable for continued cellular function and development under water limitation.

The contrasting performance of NZp and IZp indicates that Zn source and stress intensity determine which component of drought adaptation is most strongly expressed. NZp was particularly effective under non-stress and moderate drought, whereas IZp showed greater effectiveness for RWC and germination under severe drought. Differences in Zn dissolution, uptake, and timing of availability may contribute to these responses. Differences in Zn dissolution and availability may partly explain the contrasting responses of ZnO and ZnSO₄; however, Zn dissolution, uptake, and tissue accumulation were not quantified in the present study, and this interpretation therefore remains hypothetical.

The nonlinear MDA response further indicates that Zn-mediated protection was not constitutive. NZp had the highest MDA under moderate drought but the lowest MDA under severe drought. This may reflect a threshold-dependent activation of protective responses or differences in the balance between ROS signaling and oxidative injury. Because ROS and antioxidant enzyme activities were not directly measured, these possibilities remain hypotheses requiring further investigation.

Similarly, the observed responses are consistent with antioxidant defense, osmotic regulation, hormonal signaling, and Zn-dependent growth regulation, but these pathways were not directly quantified. Therefore, the present findings demonstrate physiological outcomes consistent with these mechanisms rather than establishing their causal sequence.

4.7. Overall physiological significance and implications

The results demonstrate that drought tolerance during early wheat establishment involves coordinated regulation of water relations, oxidative protection, membrane integrity, and seedling development. Zn priming alleviated several drought-induced limitations, but the magnitude and nature of protection depended on Zn formulation and stress intensity.

NZp provided the broadest physiological benefits, particularly for membrane stability, radicle development, plumule length, and seedling vigor. Its strongest effects generally occurred under non-stress and moderate drought, suggesting that nanoparticulate Zn is most effective when seedlings retain sufficient metabolic capacity to convert improved Zn status into growth and cellular protection. Under severe drought, IZp provided a specific advantage in maintaining tissue hydration, whereas NZp was more effective in reducing lipid peroxidation. These results therefore indicate complementary rather than universally hierarchical effects of the two Zn sources.

Mechanistically, the results support a model in which Zn priming enhances early physiological readiness and subsequently promotes cellular protection and developmental maintenance. Improved membrane integrity may preserve cellular compartmentation and metabolism, while sustained root development may support water relations and seedling establishment. However, the lack of direct measurements of antioxidant enzymes, ROS, osmolytes, Zn uptake, phytohormones, and gene expression limits the extent to which specific molecular pathways can be assigned to these responses.

Overall, ZnO nanoparticle seed priming appears promising for strengthening early wheat establishment under drought-prone conditions, particularly through coordinated maintenance of membrane integrity and root development. Nevertheless, the superior performance of ZnSO₄ for RWC under severe drought emphasizes that conventional ionic Zn should not be considered universally inferior. Optimization of Zn formulation and priming conditions according to drought intensity and the physiological target will therefore be important for developing effective drought-resilience strategies in wheat.

5. Conclusion

Drought stress substantially constrained wheat germination and early seedling development by disrupting tissue water status, increasing membrane damage, and restricting radicle and plumule growth. Zn priming alleviated these effects, but responses depended on Zn formulation and stress intensity. ZnO nanopriming provided the most consistent benefits under non-stress and moderate drought, improving tissue hydration, membrane stability, radicle development, and seedling vigor. Under severe drought, it also reduced lipid peroxidation, whereas ZnSO₄ improved tissue hydration and seedling biomass for some traits. These findings indicate that ZnO nanopriming supports early seedling performance through coordinated maintenance of water relations, cellular integrity, and growth. Thus, ZnO nanopriming shows promise for improving wheat establishment under water limitation. Field-scale validation is needed to confirm its agronomic effectiveness and feasibility under naturally variable drought conditions and to optimize its application for dryland wheat production.

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FIGURES AND TABLES

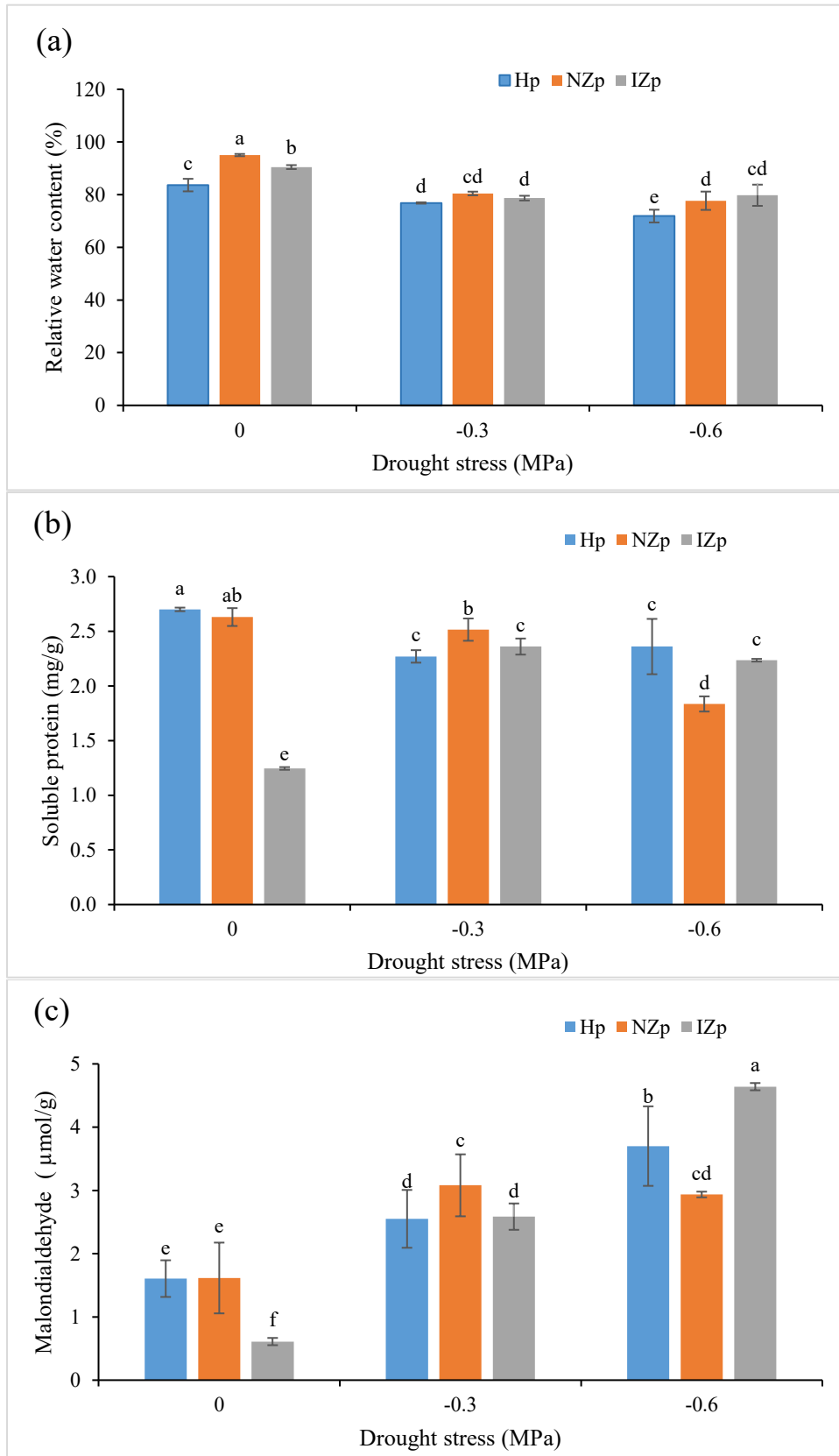


Fig. 1. Interaction effects of drought stress and seed priming treatments on (a) relative water content and (b) soluble protein content in wheat seedlings. Hp, hydropriming; NZp, nano-zinc oxide priming; IZp, zinc sulfate priming. Means ($\pm$ SD) with different lowercase letters are significantly different at $p < 0.05$ ($n = 4$).

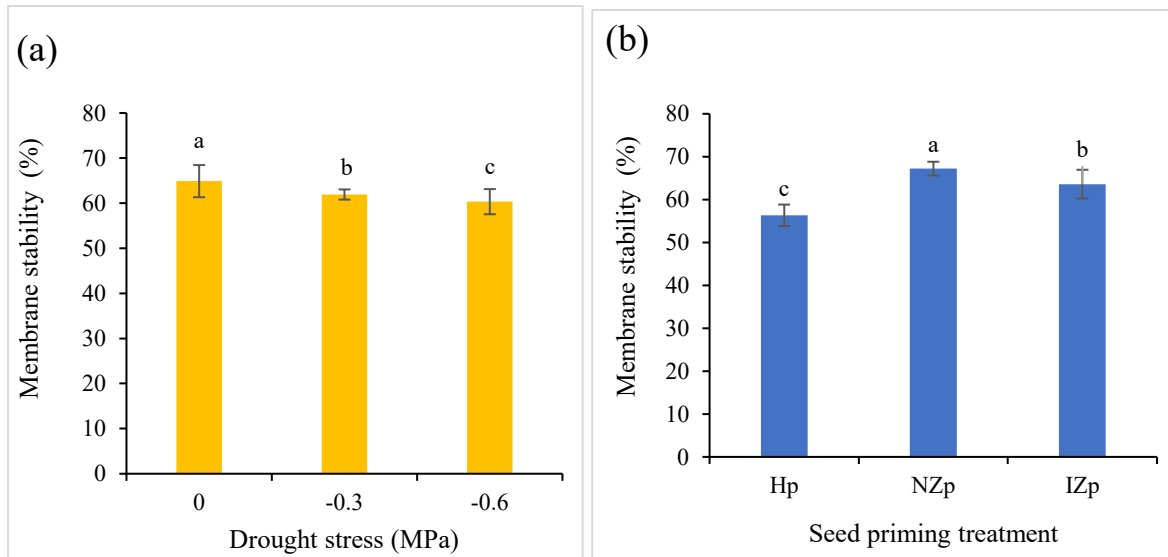


Fig. 2. Membrane stability of wheat seedlings in response to drought stress (a) and seed priming treatments (b). Hp, hydropriming; NZp, nano-zinc oxide priming; IZp, zinc sulfate priming. Means ($\pm$ SD) with different lowercase letters are significantly different at $p < 0.05$ ($n = 12$).

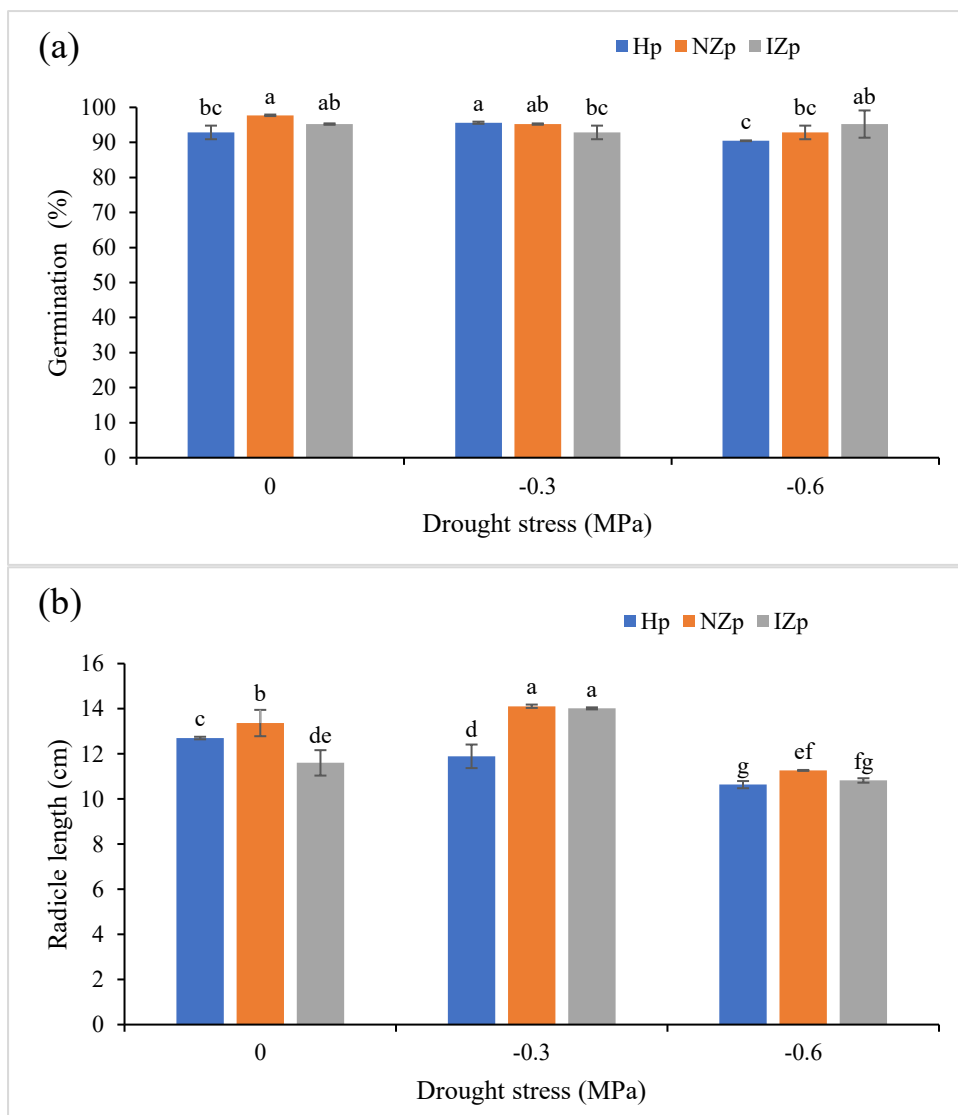


Fig. 3. Interaction effects of drought stress and seed priming treatments on (a) germination rate and (b) radicle length in wheat seedlings. Hp, hydropriming; NZp, nano-zinc oxide priming; IZp, zinc sulfate priming. Means ($\pm$ SD) with different lowercase letters are significantly different at $p < 0.05$ ($n = 4$).

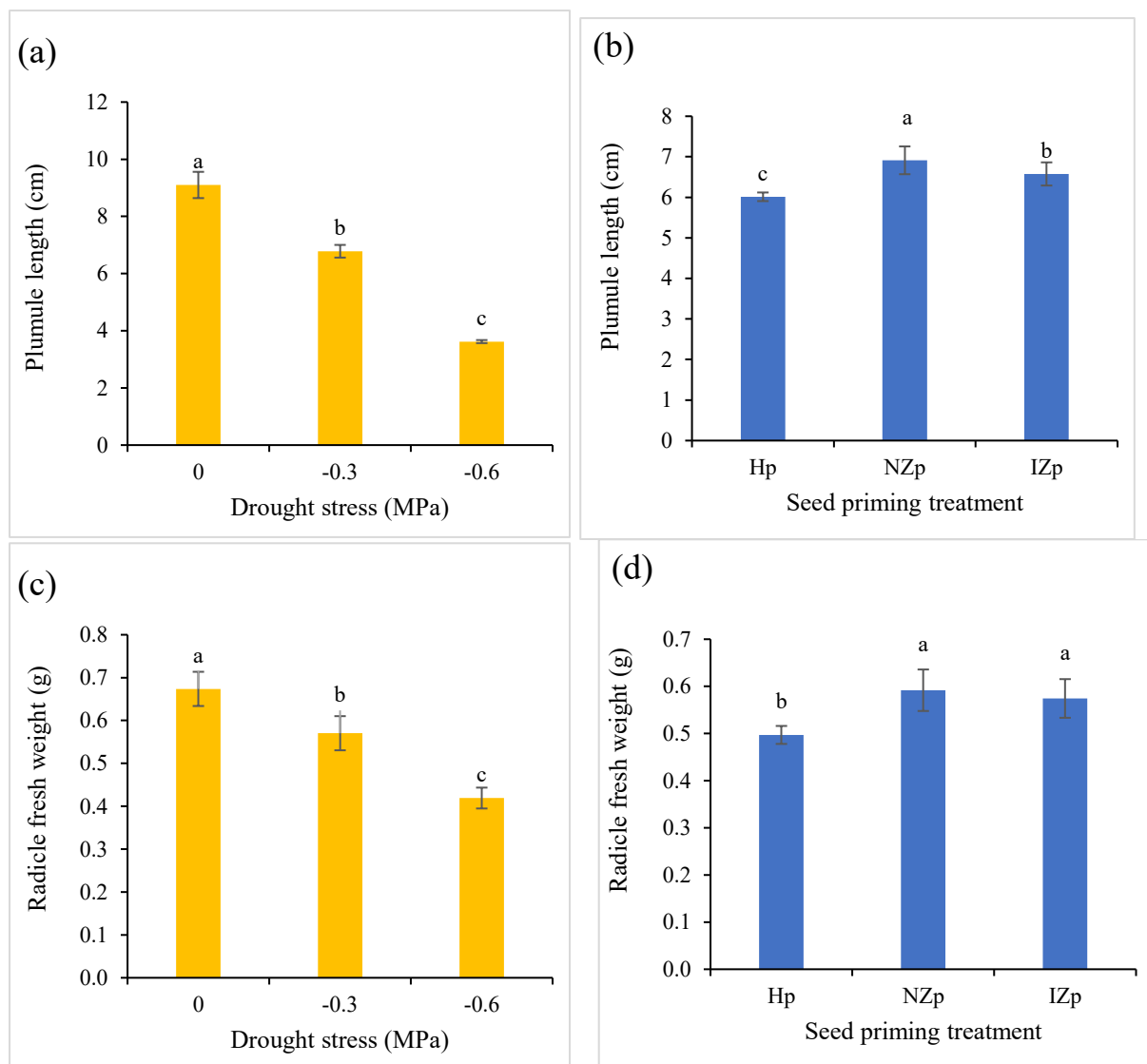


Fig. 4. Plumule length and radicle fresh weight of wheat seedlings in response to drought stress (a,c) and seed priming treatments (b,d). Hp, hydropriming; NZp, nano-zinc oxide priming; IZp, zinc sulfate priming. Means ($\pm$ SD) with different lowercase letters are significantly different at $p < 0.05$ ($n = 12$).

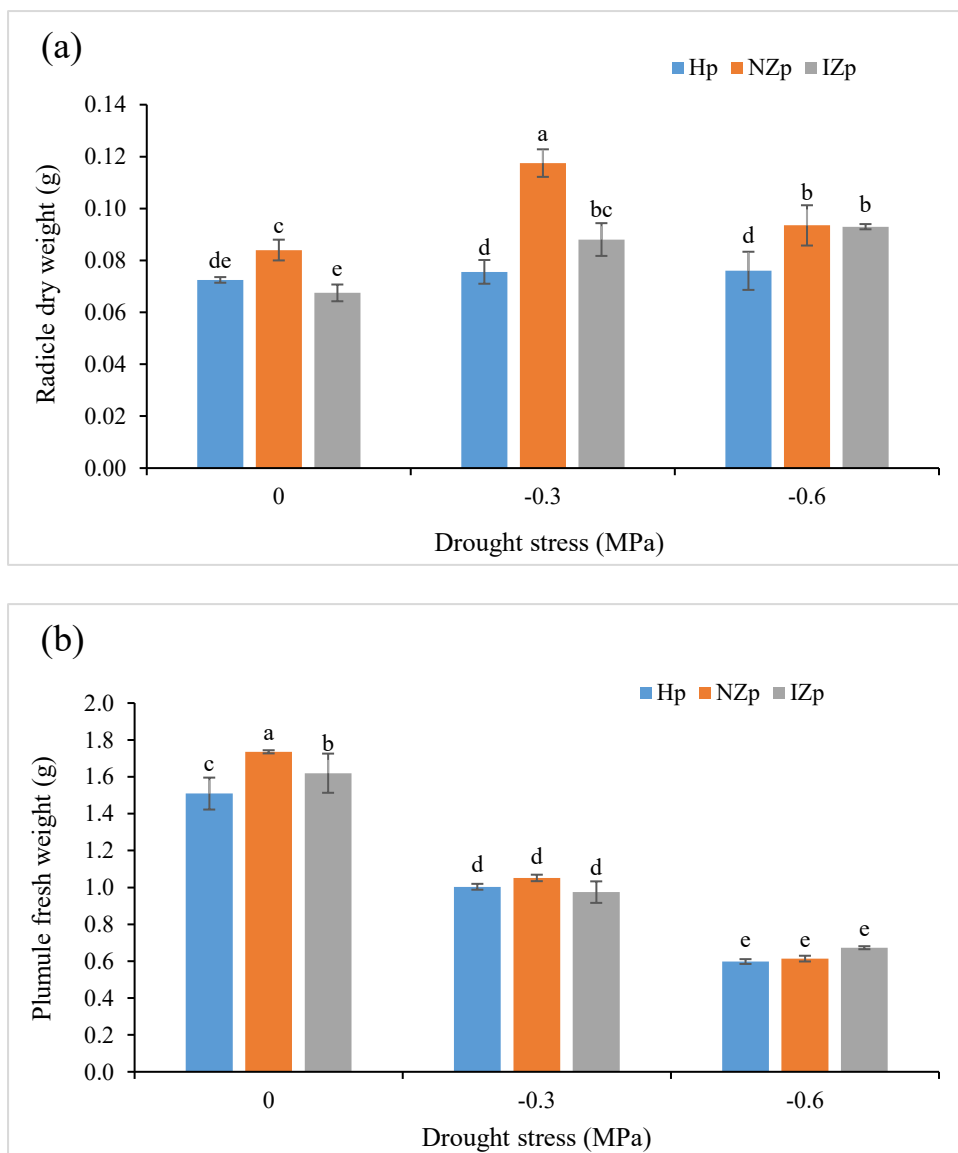


Fig. 5. Interaction effects of drought stress and seed priming treatments on (a) radicle dry weight and (b) plumule fresh weight in wheat seedlings. Hp, hydropriming; NZp, nano-zinc oxide priming; IZp, zinc sulfate priming. Means ($\pm$ SD) with different lowercase letters are significantly different at $p < 0.05$ ($n=4$).

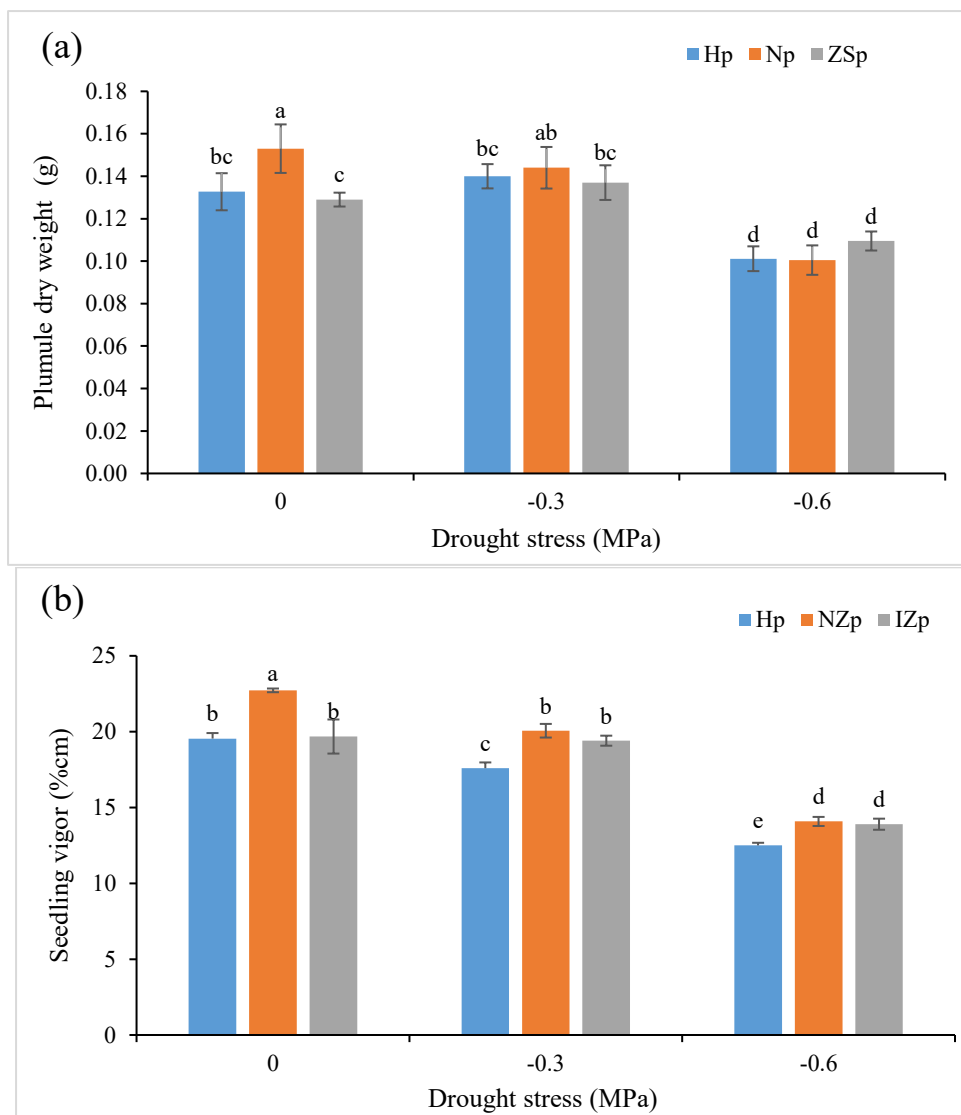


Fig. 6. Interaction effects of drought stress and seed priming treatments on a) plumule dry weight and (b,) seedling vigor index in wheat seedlings. Hp, hydropriming; NZp, nano-zinc oxide priming; IZp, zinc sulfate priming. Means ($\pm$ SD) with different lowercase letters are significantly different at $p < 0.05$ ($n=4$).

Table 1. Analysis of variance (mean squares) for drought stress and seed priming effects on relative water content, soluble protein content, membrane lipid peroxidation, membrane stability, germination rate, and radicle length in wheat seedlings.

Source of variation	DF	Relative water content	Soluble protein content	Membrane lipid peroxidation	Membrane stability	Germination percentage	Radicle length
Drought stress (DS)	2	161**	0.81**	0.20 ^{ns}	368**	16.2*	4.25**
Seed priming (SP)	2	606**	0.19**	18.7**	62.8*	18.6**	18.5**
DS × SP	4	25.1*	1.12**	2.29**	15.8 ^{ns}	19.7**	2.82**
Error	27	6.89	0.01	0.14	14.2	2.96	0.11
C.V (%)	-	3.21	4.59	14.6	6.03	1.82	2.68

ns, * and ** are non-significant, significant at 5 and 1% probability levels, respectively.

Table 2. Analysis of variance (mean squares) for drought stress and seed priming effects on plumule length, radicle fresh weight, radicle dry weight, plumule fresh weight, plumule dry weight, and seedling vigor in wheat seedlings.

Source of variation	DF	Plumule length	Radicle fresh weight	Radicle dry weight	Plumule fresh weight	Plumule dry weight	Seedling vigor index
Drought stress (DS)	2	2.48**	0.03 **	0.001**	0.03**	0.0002*	17.4**
Seed priming (SP)	2	90.7**	0.20**	0.001**	3.01**	0.005**	168**
DS × SP	4	0.35 ^{ns}	0.01 ^{ns}	0.0004**	0.02**	0.0002**	2.50**
Error	27	0.11	0.003	0.00001	0.004	0.0001	0.23
C.V (%)	-	5.16	9.88	5.18	5.94	5.93	2.73

ns, * and ** are non-significant, significant at 5 and 1% probability levels, respectively.

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