



NPS Based-seed Priming with ZnO: A Study to Optimize the Concentration and Duration for the Establishment of Barley Seedlings

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ABSTRACT

Background: Optimal seed germination and synchronized establishment of vigorous seedlings play a vital role in mitigating the initial challenges associated with germination and subsequent growth stages. The primary objective of this research was to determine the optimum concentration and duration of seed priming because more or less may not be effective in achieving the desired outcomes.

Methods: A laboratory experiment was conducted using a completely randomized design with four replications under controlled conditions in a BOD incubator. The temperature and humidity were maintained at 20°C and 70%, respectively, for the desired duration. The study aimed to evaluate the effects of nanoparticles (NP)-based ZnO on the establishment of healthy seedlings, employing priming durations of 6, 8, 10 and 12 hours and concentrations of 25, 50, 75 and 100 ppm.

Result: The findings revealed that among all the priming durations, 10 hours was the most effective. The treatments T₃ and T₄ (75 and 100 ppm of ZnO, respectively) were identified as the most suitable concentrations, achieving 100% seed germination along with higher fresh and dry weight of the shoot and roots. Additionally, these treatments attained 50% germination (T₅₀%) in 1.16 and 1.18 days, as compared to 3.95 days in the control group. It was followed by T₄ of 12 hours and T₃ of 8 hours, which recorded 96 and 92% of seed germination, respectively. An inverse relationship was observed between electrical conductivity (EC) and germination (%), as well as between 50% germination (T₅₀%) and germination performance. Furthermore, the total chlorophyll content (including chlorophyll a and b) was maximized in T₄ and T₃ at 10 hours of priming. The correlation matrix analysis indicated that the highest positive correlation was observed at 10 hours, whereas T₅₀% exhibited a negative correlation across all durations. The findings suggest that seed priming with (NP)-based ZnO with 75 and 100 ppm at 10 hours enhanced seed germination and promoted the establishment of more vigorous seedlings compared to the rest of the tested concentrations and durations.

Key words: Electrical conductivity, Seed germination, Seed priming concentration and duration, ZnO nanoparticles.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a widely cultivated cereal crop, valued for its versatile application in human consumption, animal feed and the brewing industry, which contributes to its high demand (Abebaw *et al.*, 2021; Geng *et al.*, 2022). However, both its production and quality are declining due to the shrinking cultivation areas and reduced consumer preference.

Barley is rich in dietary fiber, vitamins (Niacin, Thiamin and Pyridoxine) and essential minerals (Phosphorus, Manganese, Copper, Molybdenum and Selenium), which have long been recognized for their health benefits, particularly in improving digestion and aiding weight loss (Alka *et al.*, 2017). Moreover, it contains lignans, a class of antioxidants known for their potential role in reducing the risk of cancer (Narwal *et al.*, 2020 and Soleymani *et al.*, 2020). Seed viability, vigor and the successful establishment of healthy seedlings are crucial factors in maintaining plant population, directly impacting crop yield (Raja *et al.*, 2019; Massimi, 2018). Several techniques have been developed to mitigate seed viability and vigor loss, including hydration-dehydration treatments with and without agrochemicals (Tu *et al.*, 2023 and Adhikary; Mandal, 2019). However, due

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to the lack of research on the optimal concentration and duration of these treatments, their practical effectiveness for farmers remains limited. In recent years, nanotechnology has emerged as a promising field, offering unique molecular interactions (Wu *et al.*, 2017; Ranganathan and Groot, 2023). Zinc ranked as the 4th most important essential element, functions as a cofactor for numerous enzymes (Kushwaha *et al.*, 2021), plays a key role in

pigment biosynthesis (Dang *et al.*, 2024), and contributes to protein synthesis (Singh *et al.*, 2022) as well as inducing resistance against abiotic stress in plants (Akdemir, 2021; Singh *et al.*, 2020). The positive effects of ZnO NPs on pigment and protein synthesis have been well-documented (Donia and Carbone, 2023). However, some studies have also reported negative effects of (NP)-based seed priming, indicating the need for further investigation. Given these considerations, the present study aimed to optimize the concentration and duration of ZnO (NP)-based priming in barley to enhance seedling establishment and improve field performance.

MATERIALS AND METHODS

A laboratory experiment was conducted in the Department of Agronomy, School of Agriculture, Lovely Professional University in 2024. Genetically pure seeds of the Barley variety (PL-426) were procured from the Punjab Agricultural University, while zinc oxide nanoparticles (ZnO NPs) were obtained from Ad-Nano Technologies Pvt Ltd. The experiment was executed in a completely randomized design (CRD) with four replications under a BOD incubator (Model No-NU-151) at 18°C, wherein five different concentrations of ZnO NP-based priming (control, 25, 50, 75 and 100 ppm) and four different durations (6,8,10 and 12 hours) were used as treatments.

Measurement of electrical conductivity (EC, dS m⁻¹)

Before the execution of the trial, barley seeds were soaked in the respective ZnO NP solutions (25, 50, 75 and 100 ppm) for 6, 8, 10 and 12 hours. After the priming period, the respective solutions were used to measure the electrical conductivity (EC, dS m⁻¹) using an EC meter (LT-15). The primed seeds were then dried under a blower until they regained their original weight.

Seed germination and seedling growth

To ensure sterility, primed seeds were surface-sterilized using 0.1% mercuric chloride solution. Based on the duration (6, 8, 10 and 12 Hrs) and concentrations of seed priming (25, 50, 75 and 100 ppm), twenty-five seeds from each treatment were placed in each Petri dish and incubated in a BOD set at 20°C and 70% relative humidity. Germinated seeds were counted at regular 24-hour intervals up to 240 hours, while seed germination % and time required for 50% germination (T_{50}) were determined using the following formula. Additionally, at 240 hours, the fresh and dry weights of the roots and shoots were measured from five randomly selected seedlings.

Seed germination % =

$$\frac{\text{Total number of germinated seeds}}{\text{Total number of seeds placed}} \times 100$$

Time required for 50% germination (T_{50}) =

$$\frac{t_i + \{(N/2 - n_i)(t_i - t_j)\}}{(n_i - n_j)}$$

Where,

N= Total number of seeds that eventually germinated.

n_i and n_j = Cumulative counts of germinated seeds.

t_i and t_j = $n_i < N/2$ and $n_j > N/2$.

Estimation of total chlorophyll (mg g⁻¹)

The chlorophyll content was estimated following the Arnon (1949) method. The amount of total chlorophyll, chlorophyll (a) and (b) was determined as per the specific equation mentioned below. A gram of leaf sample was homogenized in 80% acetone using a mortar and pestle. The extract was centrifuged and the aliquot was transferred into a 100 ml volumetric flask. The final volume was maintained by 80% acetone. The optical density of extracted samples was measured at 645 nm and 663 nm using a visible spectrophotometer, with 80% acetone as the reference.

$$\text{Total chlorophyll (mg g}^{-1} \text{ fresh weight)} = \{20.2 (\text{OD } 645) + 8.02 (\text{OD } 663)\} \times (V/1000 \times W)$$

$$\text{Chlorophyll (a) (mg g}^{-1} \text{ fresh weight)} = \{12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)\} \times (V/1000 \times W)$$

$$\text{Chlorophyll (b) (mg g}^{-1} \text{ fresh weight)} = \{22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)\} \times (V/1000 \times W)$$

Where,

OD= Optical density.

V= Final volume of aliquate (ml).

W= Weight of sample taken for the chlorophyll extraction (g).

The data obtained from the present piece of research were subjected to statistical analysis to determine the significance of the treatments by using OPSTATE software developed by Sheoran *et al.* (1998).

RESULTS AND DISCUSSION

Seed germination and electrical conductivity

A laboratory-based study was conducted under controlled conditions to determine the optimum duration of ZnO NP-based seed priming for enhancing rapid seed germination and healthy seedling establishment in barley. Data presented in Fig 1 indicate that a 10-hour seed priming duration was the most effective. The highest germination percentages were recorded at 76%, 96% and 100% for the control, 25, 50, 75 and 100 ppm ZnO NP-based treatments, respectively. Additionally, an inverse relationship was observed between electrical conductivity (EC) and seed germination (%), where the lowest EC values (1.301, 1.326, 1.397 and 1.510 dSm⁻¹) corresponded with the highest germination percentages. Among the tested ZnO NP conc. T_3 (75 ppm) and T_4 (100 ppm) were the most effective in enhancing seed germination in barley.

Time taken for 50% germination (T_{50} %)

The data presented in Fig 2 illustrate the effectiveness of different durations and concentrations (Conc.) of ZnO NPs-based seed priming on the time taken for the 50% germination (T_{50} %) and overall germination (%). Among all durations, a 10-hour priming duration was the most effective

for $T_{50}\%$, compared to other durations. Among the ZnO NP Conc. tested at 10 hours, T_2 (50 ppm) recorded the least time for $T_{50}\%$ (1.11 days), followed by T_1 , T_3 and T_4 with values of 1.16, 1.16 and 1.18 days, respectively, whereas the control takes significantly more time (3.95 days). The overall trend in $T_{50}\%$ performance followed the sequence: 10hr > 12hr > 8hr > and 6 hr.

Fresh and dry weight of root and shoot

The fresh and dry weight of the root and shoot was assessed against seed priming duration and ZnO NP concentration. The data in Fig 3 indicated that the fresh and dry weight of both roots and shoots increased significantly at 10 hours of seed priming compared to 6, 8 and 12 hours. Among the tested treatments, the T_4 (100 ppm) was found to be the most effective, recording significantly the highest values of 1.92 g and 1.31 g of fresh weight of shoots and roots compared to a control (1.13 g and 0.66 g). A similar trend for the dry weight, whereas T_4 of 10 hours of seed priming noticed maximum values of 0.327 g and 0.277 g, which were followed by T_3 (0.292 g and 210 g), T_2 (0.245 g

and 0.190 g) and T_1 (0.228 g and 0.153 g) as depicted in Fig 4.

Total chlorophyll, chlorophyll (a and b)

The synthesis of total chlorophyll and Chlorophyll (a and b) was evaluated concerning priming duration and ZnO NP concentration. Data depicted in Fig 5 indicated that the chlorophyll synthesis remained relatively stable from 6 to 10 hours but exhibited a significant increase at 10 hours of priming. Among the ZnO NP-based priming treatments, the T_4 (100 ppm) was most effective, recording significantly the highest values of total chlorophyll and chlorophyll (b) 0.876 and 0.200 mg g⁻¹, while chlorophyll (a) was in T_3 of the same duration 0.690 mg g⁻¹ as compared to control 0.547, 0.434 and 0.113 mg g⁻¹.

Correlation studies among the parameters

Correlation matrix analysis was carried out to assess the influence of seed priming duration and ZnO NP concentration. On the studied parameters. Each priming duration was analyzed separately. As shown in Fig 6a, most

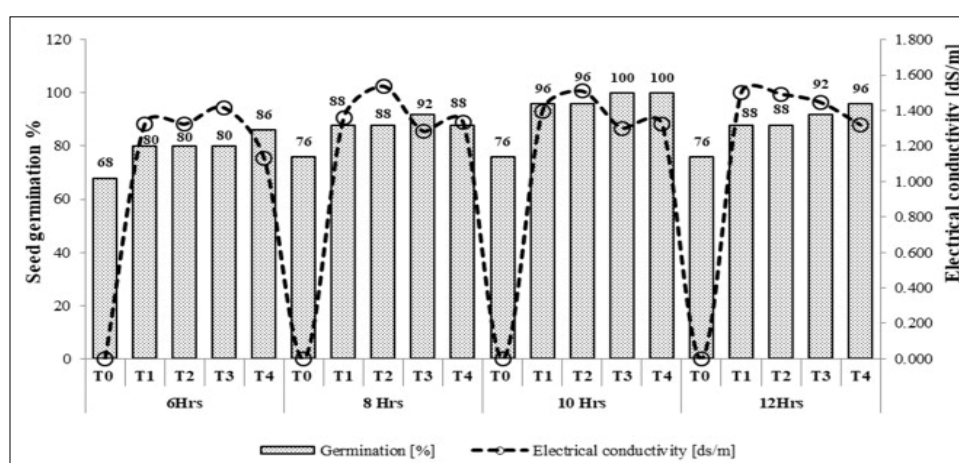


Fig 1: Influence of Nps-based seed priming with ZnO on seed germination and electrical conductivity in barley.

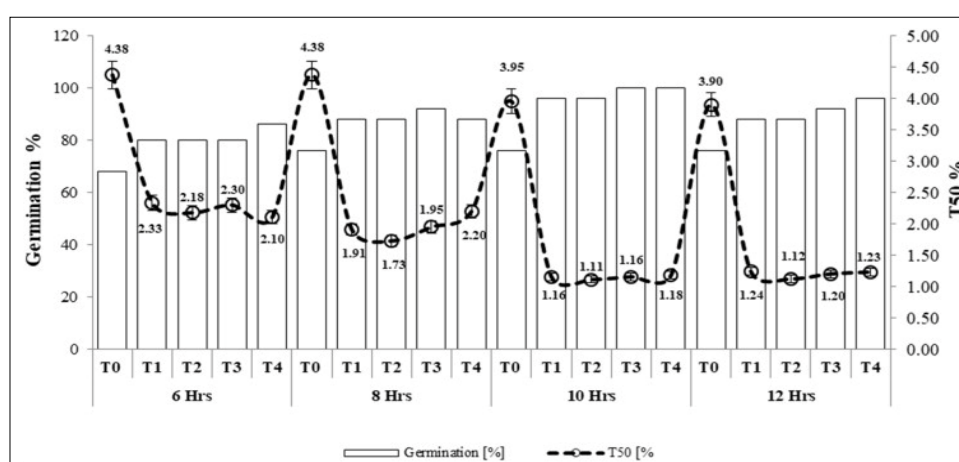


Fig 2: Influence of Nps-based seed priming with ZnO on seed germination and time taken for 50% germination ($T_{50}\%$) in barley.

of the studied parameters exhibited a highly significant and positive correlation with each other, except for the $T_{50}\%$ which was negatively correlated with most of the parameters. A similar trend was depicted in Fig 6b, where $T_{50}\%$ exhibited a strong negative correlation, while chlorophyll showed a partial correlation. In Fig 6c, only $T_{50}\%$ displayed a high degree of negative correlation with the rest of the parameters. Similarly, Fig 6d indicates that $T_{50}\%$

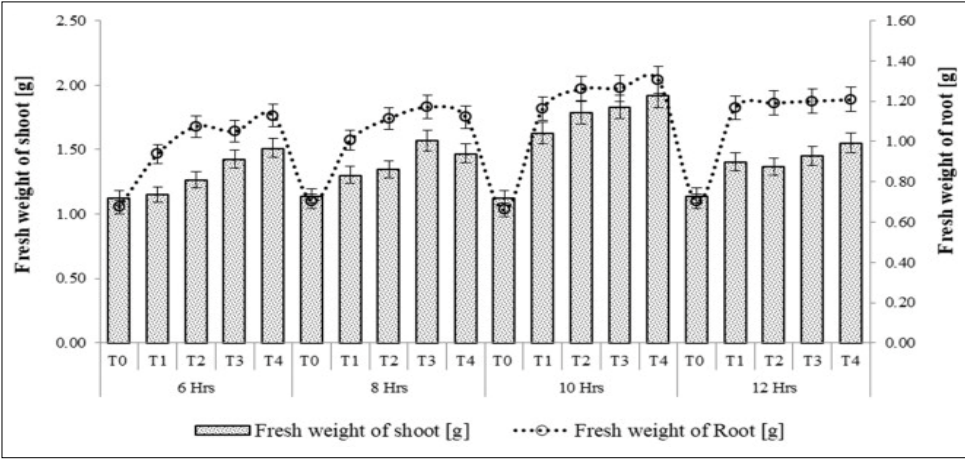


Fig 3: Influence of Nps-based seed priming with ZnO on fresh weight of shoot and root in barley.

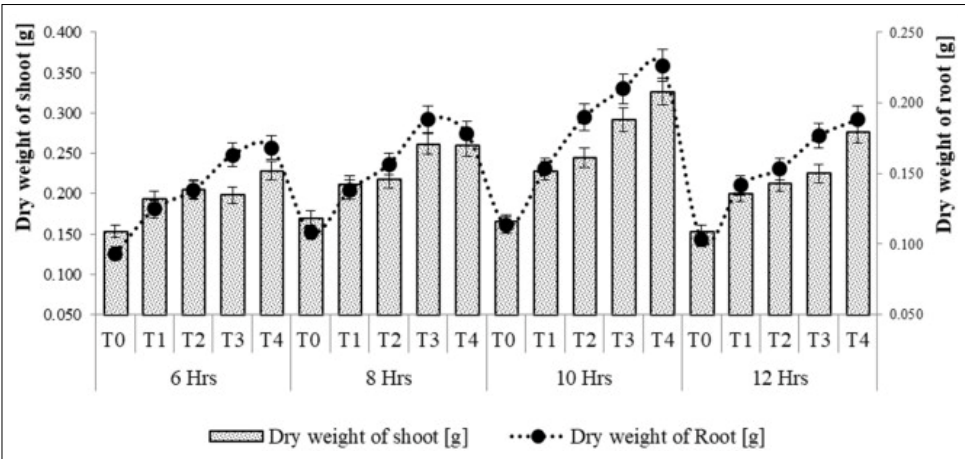


Fig 4: Influence of Nps-based seed priming with ZnO on dry weight of shoot and root in barley.

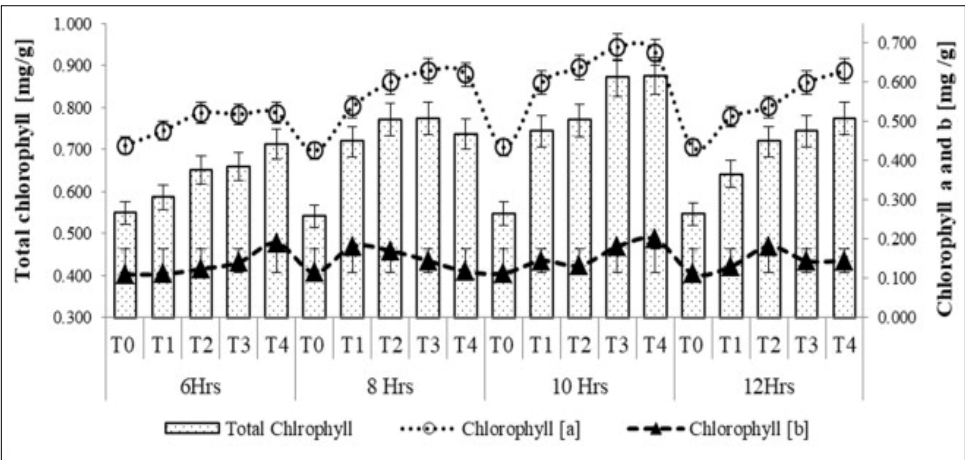


Fig 5: Influence of Nps-based seed priming with ZnO on the synthesis of total chlorophyll, chlorophyll a and b in barley.

remains with a strong negative correlation, whereas chlorophyll b revealed a partial correlation with the remaining parameters.

The rapid, uniform and healthy establishment of seedlings over the field provides additional opportunities to enhance the yield potential. Seed priming has emerged as an effective strategy to promote robust morpho-physiological growth; however, optimizing the specific duration and concentration of priming chemicals remains critical. To address the issue, the present study aimed to evaluate the

impact of ZnO NP-based seed priming by testing different priming durations and ZnO concentrations. Among the tested durations of priming, 10 hours resulted in the fastest germination rate, while simultaneously recording the lowest EC of the priming solution, compared to 6, 8 and 12 hours. These lower EC values suggest minimal leakage of leachates, indicating reduced membrane damage and enhanced metabolic efficiency. Among the priming treatments, T_3 (75 ppm) and T_4 (100 ppm) ZnO NPs were found to be the most effective in improving seed germination

	Seed germination	T50 [days]	EC	Fresh weight of shoot	Fresh weight of root	Dry weight of shoot	Dry weight of root	Total chlorophyll	Chlorophyll [a]	Chlorophyll [b]
Seed germination	● 1.000									
T50 [days]	○ -0.999	● 1.000								
Electrical conductivity	● 0.998	○ -0.995	● 1.000							
fresh weight of shoot	● 0.563	○ -0.557	● 0.608	● 1.000						
fresh weight of root	● 0.945	○ -0.954	● 0.950	● 0.724	● 1.000					
Dry weight of shoot	● 0.979	○ -0.987	● 0.975	● 0.610	● 0.986	● 1.000				
Dry weight of root	● 0.838	○ -0.832	● 0.869	● 0.920	● 0.907	● 0.850	● 1.000			
Total chlorophyll	● 0.790	○ -0.802	● 0.810	● 0.886	● 0.940	● 0.870	● 0.938	● 1.000		
Chlorophyll [a]	● 0.835	○ -0.852	● 0.845	● 0.793	● 0.969	● 0.920	● 0.894	● 0.985	● 1.000	
Chlorophyll [b]	● 0.528	○ -0.523	● 0.574	● 0.999	● 0.698	● 0.579	● 0.903	● 0.873	● 0.776	● 1.000

Fig 6a: Influence of Nps-based seed priming with ZnO on correlation studies at 6 hrs of duration.

	Seed germination	T50 [days]	EC	Fresh weight of shoot	Fresh weight of root	Dry weight of shoot	Dry weight of root	Total chlorophyll	Chlorophyll [a]	Chlorophyll [b]
Seed germination	● 1.000									
T50 [days]	○ -0.660	● 1.000								
Electrical conductivity	● 0.922	○ -0.840	● 1.000							
fresh weight of shoot	● 0.771	○ -0.344	● 0.718	● 1.000						
fresh weight of root	● 0.928	○ -0.678	● 0.926	● 0.913	● 1.000					
Dry weight of shoot	● 0.866	○ -0.349	● 0.727	● 0.958	● 0.916	● 1.000				
Dry weight of root	● 0.830	○ -0.425	● 0.749	● 0.982	● 0.942	● 0.981	● 1.000			
Total chlorophyll	● 0.971	○ -0.802	● 0.969	● 0.763	● 0.957	● 0.820	● 0.827	● 1.000		
Chlorophyll [a]	● 0.932	○ -0.653	● 0.885	● 0.901	● 0.988	● 0.936	● 0.954	● 0.953	● 1.000	
Chlorophyll [b]	● 0.501	○ -0.737	● 0.624	○ -0.080	● 0.299	○ 0.006	○ -0.022	● 0.534	○ 0.253	● 1.000

Fig 6b: Influence of Nps-based seed priming with ZnO on correlation studies at 8 hrs of duration.

	Seed germination	T50 [days]	EC	Fresh weight of shoot	Fresh weight of root	Dry weight of shoot	Dry weight of root	Total chlorophyll	Chlorophyll [a]	Chlorophyll [b]
Seed germination	● 1.000									
T50 [days]	○ -0.994	● 1.000								
Electrical conductivity	● 0.949	○ -0.977	● 1.000							
fresh weight of shoot	● 0.976	○ -0.964	● 0.919	● 1.000						
fresh weight of root	● 0.988	○ -0.990	● 0.966	● 0.989	● 1.000					
Dry weight of shoot	● 0.881	○ -0.834	● 0.716	● 0.925	● 0.868	● 1.000				
Dry weight of root	● 0.886	○ -0.847	● 0.756	● 0.953	● 0.898	● 0.977	● 1.000			
Total chlorophyll	● 0.966	○ -0.934	● 0.844	● 0.973	● 0.948	● 0.965	● 0.963	● 1.000		
Chlorophyll [a]	● 0.983	○ -0.963	● 0.903	● 0.986	● 0.978	● 0.920	● 0.944	● 0.988	● 1.000	
Chlorophyll [b]	● 0.779	○ -0.715	● 0.554	● 0.796	● 0.728	● 0.958	● 0.881	● 0.893	● 0.813	● 1.000

Fig 6c: Influence of Nps-based seed priming with ZnO on correlation studies at 10 hrs of duration.

	Seed germination	T50 [days]	EC	Fresh weight of shoot	Fresh weight of root	Dry weight of shoot	Dry weight of root	Total chlorophyll	Chlorophyll [a]	Chlorophyll [b]
Seed germination	● 1.000									
T50 [days]	○ -0.891	● 1.000								
Electrical conductivity	● 0.842	○ -0.993	● 1.000							
fresh weight of shoot	● 0.995	○ -0.879	● 0.837	● 1.000						
fresh weight of root	● 0.919	○ -0.997	● 0.985	● 0.911	● 1.000					
Dry weight of shoot	● 0.956	○ -0.748	● 0.680	● 0.950	● 0.793	● 1.000				
Dry weight of root	● 0.980	○ -0.838	● 0.770	● 0.958	● 0.865	● 0.956	● 1.000			
Total chlorophyll	● 0.951	○ -0.858	● 0.792	● 0.916	● 0.880	● 0.933	● 0.979	● 1.000		
Chlorophyll [a]	● 0.969	○ -0.796	● 0.722	● 0.947	● 0.827	● 0.959	● 0.997	● 0.970	● 1.000	
Chlorophyll [b]	● 0.515	○ -0.698	● 0.681	● 0.455	● 0.682	● 0.478	● 0.532	● 0.687	● 0.490	1.000

Fig 6d: Influence of Nps-based seed priming with ZnO on correlation studies at 12 hrs of duration.

and reducing EC. A similar finding was confirmed by the study of Nawaz *et al.* (2011), who indicated that the improved seed germination is associated with lower EC (dS m⁻¹) in tomato. Additionally, an optimum seed priming duration can limit the release of leachates, thereby accelerating the metabolic processes involved in seed germination of the canola crop (Jamil *et al.*, 2009). As illustrated in Fig 1, the maximum germination percentage was recorded at 75 and 100 ppm ZnO at 10 hours of priming duration, aligning with the findings of (Li *et al.*, 2021 and Meher *et al.*, 2020). Moreover, ZnO NP priming promoted early seed germination, as evident in Fig 2, in terms of T₅₀% which was further supported by Adhikary *et al.*, (2022) in rice. This enhancement in germination can be attributed to the appropriate priming duration and enhances α-amylase and total amylase activity (Li *et al.*, 2021). The use of ZnO as a seed priming treatment acts as a biostimulant, thereby promoting fresh and dry biomass accumulation in roots and shoots, consequently leading to healthy and vigorous seedling growth (Raja *et al.*, 2019 and Li *et al.*, 2021). A similar positive effect on seedlings was depicted in Fig 3 and 4, emphasizing the significance of optimal ZnO NP concentration and priming duration. The synthesis of chlorophyll is a key physiological parameter that determines the plant's ability to produce carbohydrates for subsequent growth (Donia and Carbone, 2023). As illustrated in Fig 5, maximum total chlorophyll and chlorophyll (b) were recorded in T₄ of the 10 hours of duration, while chlorophyll (a) was highest in T₃. Similar findings on ZnO-induced enhancement in chlorophyll synthesis have been reported in wheat (Abbas *et al.*, 2023), rice (Adhikary *et al.*, 2022) and beans (Carbone *et al.*, 2023). Zinc is a crucial micronutrient that regulates multiple metabolic processes in plants, including activation of enzymes, hormonal signaling (Hamzah *et al.*, 2022), protein synthesis, membrane integrity (Kareem *et al.*, 2022) and photosynthesis. It is useful in chlorophyll biosynthesis (Dang *et al.*, 2024), where it enhances nitrogen availability and activates ribulose-1,5-bisphosphate (RuBisCO), a key enzyme in photosynthesis. The use of nanoparticles of ZnO as a seed-priming treatment enhances the bioavailability of

Zn, thereby promoting healthy and vigorous plant growth (Rasouli *et al.*, 2022; Donia and Carbone, 2023). As indicated in Fig 6a to 6d, most of the studied parameters had a positive correlation, except for time of 50% germination, which was negatively correlated with the seedling vigor and physiological traits. The best correlation was observed at 10 hours of seed priming, reinforcing the effectiveness of this duration in improving seed germination and plant establishment.

CONCLUSION

The findings of NP-based barley seed priming with ZnO revealed that among all tested durations, 10 hrs was the optimal priming duration. Among the ZnO treatments, 75 ppm and 100 ppm resulted in significantly the highest values of seed germination, reduced T₅₀% and increased dry weight of shoot and root, total chlorophyll content and chlorophyll (a) and (b) synthesis. Additionally, EC was minimized in these treatments, indicating reduced leachate release and greater metabolic efficiency. The positive effect of ZnO NP-based seed priming in barley might be attributed to its nanoscale properties, which facilitate the entry of elements and enhanced uptake, thereby triggering the key metabolic process involved in seed germination and subsequent growth phases for the healthy establishment and overall crop performance in barley cultivation.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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