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Phytohormonal Priming by Gibberellic Acid for Enhance Tolerance of (*Chenopodium quinoa* L.) and their Oxidative Homeostasis to ZnO NPs Phytotoxicity

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ABSTRACT

The seed priming approach represents a viable, economically accessible, and sustainable management strategy for agricultural practices. This study investigates the ameliorating effects of 0.1 mmol gibberellic acid (GA3) as a phytohormonal priming agent on the growth and antioxidant system of quinoa (*Chenopodium quinoa* L.) under the phytotoxic effects of zinc oxide nanoparticles (ZnO NPs) at concentrations of 0, 50, and 100 mg L^{-1}. Zinc oxide nanoparticles are known to inhibit growth traits in quinoa, including fresh and dry mass as well as chlorophyll a content. This growth inhibition was associated with alterations in metabolic pools, specifically reductions in soluble carbohydrates and soluble proteins in the shoots. Furthermore, both enzymatic antioxidants and phenolic compounds, which serve as non-enzymatic antioxidants, were significantly inhibited by ZnO NPs but were stimulated by GA3 priming. In conclusion, the hazardous effects of ZnO NPs can be at least partially mitigated by priming quinoa seeds with gibberellic acid (GA3), highlighting the potential of this strategy to enhance plant resilience against environmental stressors.

Keywords: Antioxidant enzymes; Gibberellic acid (GA3); Growth inhibition; Oxidative Homeostasis; Phytohormonal Priming; Quinoa plant; Zinc Oxide Nanoparticles (ZnO NPs).

INTRODUCTION

In recent years, the application of nanomaterials in agriculture has gathered significant attention due to their potential benefits and associated risks. Nanotechnology has emerged as a transformative scientific trend, offering innovative solutions across multiple fields, including agriculture (Aziz *et al*., 2016; Prasad *et al*., 2016; Tripathi *et al*., 2017). The unique physicochemical properties of nanoparticles, which differ markedly from those of bulk particles, enable their use as effective chemical delivery agents in plants, allowing for targeted interventions at specific cellular organelles (Nair *et al*., 2010; Subbaiah *et al*., 2016; Wang *et al*., 2016).

Previous studies have shown both the beneficial and harmful effects of nanoparticles on plants, which depend on factors such as nanoparticle type, size, specific surface area, and plant species (Miller *et al*., 2012; Shweta *et al*., 2016; Singh *et al*., 2016). This targeted approach can enhance the efficacy of agrochemicals, improve nutrient uptake, and potentially reduce the environmental impact of traditional agricultural practices. However, the introduction of engineered nanoparticles into agricultural systems raises concerns regarding their environmental persistence and toxicity, demanding a systematic evaluation of their long-term effects on ecosystem health and human safety. Understanding both the advantages and disadvantages of nanotechnology in agriculture is crucial for developing sustainable practices that maximize crop productivity while minimizing ecological risks. Meanwhile, plants play a crucial role in the ecosystem by serving as both significant components and potential pathways for nanoparticle transport and bioaccumulation within food chains (Zhu *et al*., 2008; Wang *et al*., 2012; Zafar *et al*., 2016).

Zinc oxide nanoparticles (ZnO NPs) stand out among the various metal nanoparticles frequently used in commercial applications. ZnO NPs are extensively employed in the production of paints, plastics, lubricants, pigments, cement, foods, rubber, and batteries (Monica and Cremonini, 2009). Consequently, the widespread use of ZnO NPs in previous products increases their discharge into the environment, which can have severe consequences on plant productivity. The adverse effects of ZnO NPs phytotoxicity have been investigated in several plant species, including broad bean (Manzo *et al*., 2011), zucchini (Stampoulis *et al*., 2009), and wheat (Du *et al*., 2011). Several studies have also examined the toxicity of ZnO NPs on various plant species, such as: *Cicer arietinum*, *Arabidopsis thaliana*, *Pisum sativum*, and *Zea mays* (Burman *et al*., 2013; Mukherjee *et al*., 2016; Subbaiah *et al*., 2016; Wang *et al*., 2016). Therefore, the findings from previous research indicate that ZnO NPs have significant negative effects on plant productivity, highlighting the need for the development of strategies to mitigate their toxicity in plants.

In this context, the application of external phytohormones and modulators of their homeostasis can be employed to mitigate the effects of various abiotic stresses on plants (Campos *et al*., 2023). For instance, the external application of gibberellic acid (GA3) has shown its potential to alleviate negative impacts of environmental stressors, leading to improved growth, enhanced photosynthetic efficiency, increased enzyme activity, altered gene expression, enhanced nutrient

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uptake, and higher yields in different crops facing unfavorable conditions. GA3 also can interact with other plant hormones, influencing various metabolic processes within plants (Hernández-García *et al*., 2021; Shah *et al*., 2023). The protective effects of GA3 can be achieved through different application methods, including foliar spray (Elahi *et al*., 2022; Guo *et al*., 2022), substrate addition (Hamayun *et al*., 2010), and seed priming (Rhaman *et al*., 2020).

Seed priming is a pre-sowing technique that involves hydrating and dehydrating seeds before germination, without allowing the radicle to emerge (Nawaz *et al*., 2013). This technique has proven to be a promising application for sustainable agriculture, inducing rapid and uniform germination, promoting high seedling vigor, facilitating better plant establishment, improving crop yield, and enhancing tolerance to abiotic stresses (Amir *et al*., 2024). In sustainable agriculture, various methods of seed priming have been successfully utilized, including water priming (hydropriming), the application of plant growth regulators (phytohormonal priming), and the use of osmoprotectants and inorganic salts (osmopriming) (Dawood, 2018). Previous studies have highlighted the significant potential of seed priming with GA3 in enhancing germination, early growth, and tolerance mechanisms in different plants under stressful conditions. These conditions include water deficit (Fregonezi *et al*., 2024; Du *et al.,* 2022), low temperature (Xia *et al*., 2023), nickel toxicity (Bhat *et al*., 2023), and salinity (Tsegay and Andargie, 2018).

Quinoa (*Chenopodium quinoa* L.) exhibits remarkable adaptability to challenging climatic and soil conditions. Its abundance of gluten-free protein, complex carbohydrates, high fiber content, carotenoids, vitamins, and overall nutritional value have positioned quinoa as a viable alternative to enhance food security (Gholami *et al*., 2022). Additionally, quinoa is known for its potential protective effects against various diseases, particularly cardiovascular disease and cancer (Nowak *et al*., 2016). In light of these factors, the present study hypothesizes that gibberellic acid (GA3) could mitigate the negative effects of zinc oxide nanoparticles (ZnO NPs) phytotoxicity by enhancing antioxidant activity, thereby promoting improved growth in quinoa plants.

MATERIAL AND METHODS

Preparation of zinc oxide nanoparticles (ZnO NPs)

ZnO NP used in this study prepared by the technique of ball milling. Its nanoparticle size is (37±2.6) nm of commercial zinc oxide obtained from Chem. lab nv company, Belgium (density of 5.61 g/cm3, 99.5% purity, 81.37 g/mol and) (Othman *et al*., 2018). The tested suspension prepared by sonicated for 30 min of 100 mg/L Zn ONP ensure dispersion.

Sand matrix preparation and plant cultivation

To prepare the sand for the experiment, it was sieved and then soaked in a solution of 1% hydrochloric acid (HCl) overnight. Afterward, it was thoroughly rinsed with distilled water and left to dry before being used. Plastic pots lined with polyethylene bags were filled with 700 grams of the treated sand to create a solid matrix that mimics environmental relevant conditions. This was done to observe the impact of nanoparticles (NPs) on plants. The seeds of *Chenopodium quinoa* L. were generously provided by the Agricultural Research Center in Giza, Egypt. To ensure their cleanliness, the seeds were sterilized in a 10% hydrogen peroxide solution for 20 min and then washed extensively with distilled water. The seeds were soaked in a solution containing 0.1 mM gibberellic acid (GA3) for 12 hrs and then planted in the soil. In the experiment, 20 seeds were sown in quartz sand treated with different concentrations of ZnO NPs (0, 50, and 100 mg L^{-1}). The soil was kept at its full field capacity (100%) with approximately 11% water content. The control group consisted of seeds planted using only distilled water. After germination, the 10 healthiest and most uniform seedlings were selected. Each treatment was replicated in six plastic pots for accurate data collection.

Growth yield and harvest

At the end of the one-month experiment, the shoots that had grown were carefully separated from the roots. The roots were gently rinsed with distilled water to eliminate any adhering soil. To determine the fresh weight (FW), the shoots and roots were weighed separately. Subsequently, they were placed in an oven at 70°C for 72 hrs to remove all moisture and ascertain the dry weight (DW). Additionally, a portion of the freshly harvested shoots and roots was promptly stored at -80°C for future analyses.

Preparation of plant extract

Whole fresh quinoa samples (roots and shoots) were ground with 5 mL of extraction buffer using a chilled mortar and pestle. The extraction buffer consisted of 1 mM sodium EDTA and 3 mM $MgCl_2$ in 50 mM Tris-HCl (pH 7). The resulting mixture was then centrifuged at 5,000 rpm for 10 min at 4°C. The supernatant obtainned from this centrifugation was used for the determination of soluble metabolites and enzymatic antioxidants, following the methods described by Padmaja *et al*. (2011). This procedure ensures the effective extraction of compounds for subsequent analysis.

Photosynthetic pigments

The determination of photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, was conducted spectrophotometrically at wavelengths of 663 nm, 644 nm, and 452 nm, respectively. The concentrations of these pigments were calculated as mg g^{-1} fresh weight (FW) following the method described by Lichtenthaler (1987). Fresh leaf samples were extracted in 95% ethyl alcohol until they became colorless at 60°C. After extraction, the total volume was adjusted to 10 mL with ethyl alcohol to ensure accurate measurement of pigment concentrations.

Soluble metabolites

Soluble carbohydrates

The soluble carbohydrates content was measured according to Fales (1951). The formed blue green color was recorded at 620 nm. Soluble carbohydrates were calculated using glucose to prepare calibration curve as mg g^{-1} FW.

Soluble proteins

The soluble protein content determined according to Lowry *et al*. (1951) with the method of Folin reagent. Soluble proteins were calculated using bovine serum albumin (BSA) to prepare standard curve as mg g^{-1} FW.

Free amino acids

The free amino acid content was determined according to Friedman (2004). Free amino acids were calculated using glycine to prepare standard curve as mg g^{-1} FW.

Non enzymatic antioxidants (free phenolics)

The phenolic contents were assayed using Folin-Ciocalteu's phenol reagent method was used as described by Kofalvi and Nassuth, (1995) and El-Sharkawy and ElSharawy (2024). Phenolics were quantified using a gallic acid standard curve, expressed as µg g^{-1} FW.

Enzymatic antioxidants

Superoxide dismutase (EC 1.15.1.1)

Superoxide dismutase (SOD) activity of was detected by following the adenochrome (autoxidation of epinephrine) as described by Misra and Fridovich, (1972). Activity was presented as (UE mg^{-1} protein).

Catalase (EC 1.11.1.6)

Catalase (CAT) activity was recorded spectrophotometrically by following the decrease in absorbance in H_2O_2 at A_{240} nm. The method reported by Aebi (1984) and Essa *et al*. (2023).

Peroxidase POD (EC 1.11.1.7)

Peroxidase enzyme (POD) activity was determined spectrophotometrically by following the increase in absorbance at A_{470} nm, as cited by Tatiana *et al*., (1999).

Ascorbate peroxidase APX (EC 1.11.1.11)

Ascorbate peroxidase enzyme (APX) activity was assayed by detecting decrease in absorbance following the oxidation of ascorbic acid at A290 nm as cited by Jiang and Zhang, (2002).

Statistical analysis

The experiments were analyzed using a one-way ANOVA framework and conducted with a completely randomized (CR) design. The data collected for analysis comprised three replicates from six measurements obtained from two independent experiments. The SPSS statistical software version 11.0 was employed to perform the analysis of variance (ANO-VA). To determine significant differences between means, Duncan's multiple range test was conducted as a post hoc analysis, with a significance level set at $p \leq 0.05$. Principal Component Analysis (PCA) was utilized for variance regression ordination to analyze the assessed attributes. A heatmap was generated using the ggplot2 package, and the visualization of correlation matrices was performed using the corrplot package in R software (RStudio). Prior to analysis, the data (mean values) were normalized to a standard range of ±1 to ensure accurate interpretation of the results.

RESULTS

Results in Figures (1 and 2) demonstrated the effects of GA3 priming on some growth parameters, including fresh and dry weights of the shoots and roots of quinoa plants grown under ZnO NPs levels (0, 50, and 100 mg L^{-1}). Reduced growth parameters of quinoa plants grown without GA3 priming as a consequence of ZnO NPs applications (Figure 1and 2). On the other hand, a significant increase was observed in both the fresh and dry weights of the shoots and roots of quinoa plants treated with various concentrations of GA3 (50 and 100 mg L^{-1}). Additionally, a recognized improvement in the fresh and dry weights of the roots was recorded for plants subjected to GA3 priming at a concentration of 50 mg L^{-1} ZnO nanoparticles (NPs) compared to the corresponding treatment without GA3 (Figures 1 and 2).

Results presented in Figure (3) illustrate the effects of ZnO nanoparticles (NPs) and GA3 priming on photosynthetic pigments, specifically chlorophyll a, chlorophyll b, and carotenoids. A reduction in chlorophyll content was observed in quinoa plants due to the phytotoxic effects of ZnO NPs at concentrations of 50 and 100 mg L^{-1}. Conversely, the application of GA3 enhanced chlorophyll a levels at a concentration of 100 mg L^{-1} ZnO NPs (Figure 3a). Additionally, an increase in chlorophyll b and carotenoid contents was observed in quinoa plants with rising levels of ZnO NPs when combined with GA3 priming (Figures 3b and 3c).

The activity of SOD in shoot of quinoa plants grown under ZnO NPs applications showed induction or no change at 50 and100 mg L^{-1} ZnO NPs levels, respecttively (Figure 4a). While, SOD activity reduced in root of quinoa plants as a consequence of ZnO NPs applications (Figure 4b). External application of GA3 induced SOD activity at 100 mg L^{-1} ZnO NPs level in roots.

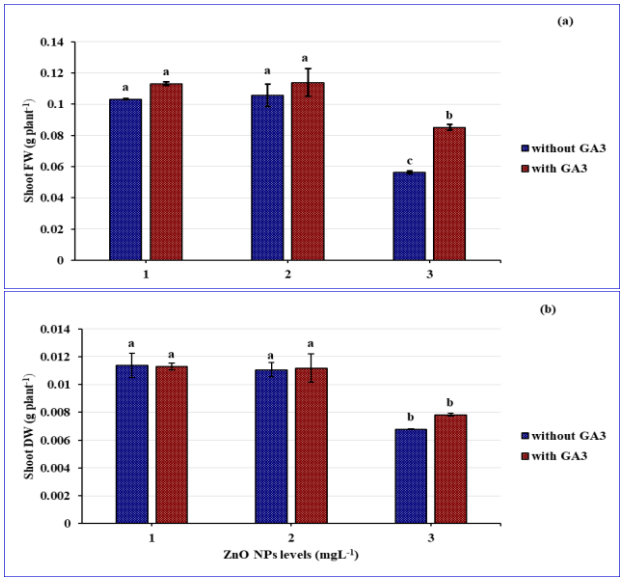

Figure (1): Effect of ZnO Nanoparticle Levels and GA3 Application (0.1 mM) on growth parameters of *Chenopodium quinoa* L. (a), Shoot fresh weight (FW) and (b) dry weight (DW). ZnO Nanoparticle Levels: 1, 0; 2, 50; 3, 100 mg L^{-1}. Column with different letters indicate significant differences at $p \leq 0.05$, based on Duncan's Multiple Range Test. The vertical bars represent the standard error (± SE).

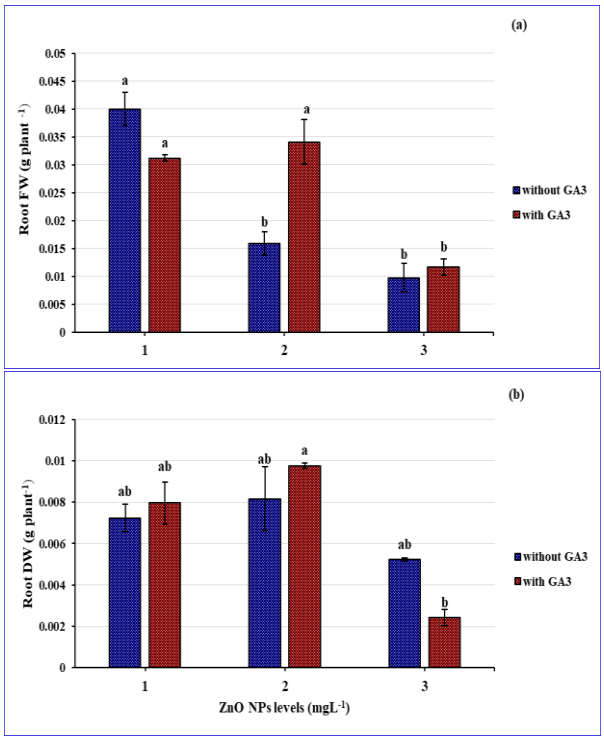

Figure (2): Effect of ZnO Nanoparticle Levels and GA3 Application (0.1 mM) on growth parameters of *Chenopodium quinoa* L. (a), Root fresh weight (FW) and (b), Root dry weight (DW). ZnO Nanoparticle Levels: 1, 0; 2, 50; 3, 100 mg L^{-1}. Values represent means of six replicates, from two independent experiments. Column with different letters indicate significant differences at $p \leq 0.05$, based on Duncan's Multiple Range Test. The vertical bars represent the standard error (± SE).

Results presented in Figures (5) demonstrate the effects of GA3 priming and ZnO nanoparticles (NPs) on ascorbate peroxidase (APX) enzyme activity in the shoots (Figure 5a) and roots (Figure 5b) of quinoa plants. The phytotoxicity of ZnO NPs induced APX activity in the shoots but no significant change was recorded in the roots. In contrast, GA3 application significantly ($p \leq 0.05$) enhanced APX activity in both the shoots and roots at a concentration of 50 mg L^{-1} ZnO NPs. Figure 6 illustrates the influence of different levels of ZnO nanoparticles (NPs) and seed priming with GA3 on catalase (CAT) enzyme activity. In the absence of GA3 priming, CAT activity significantly increased ($p \leq 0.05$) in both the roots and shoots due to the application of ZnO NPs at concentrations of 50 and 100 mg L^{-1}, respectively. Conversely, GA3 application further enhanced CAT activity in both the shoots and roots of quinoa plants at a concentration of 100 mg L^{-1} ZnO NPs (Figures 6a and b).

The activity of peroxidase (POD) enzyme in the shoots and roots of quinoa plants is shown in Figure (7). Generally, POD activity decreased under phytotoxicity of ZnO NPs in shoot and root. External application of GA3 as priming agent significantly increased POD activity in shoot and root of quinoa plants grown under 100 mg L^{-1} of ZnO NPs level.

Data presented in Table (1) demonstrate the effects of GA3 priming on the soluble carbohydrate content of quinoa plants subjected to ZnO nanoparticle (NP) phytotoxicity. The application of ZnO NPs resulted in a

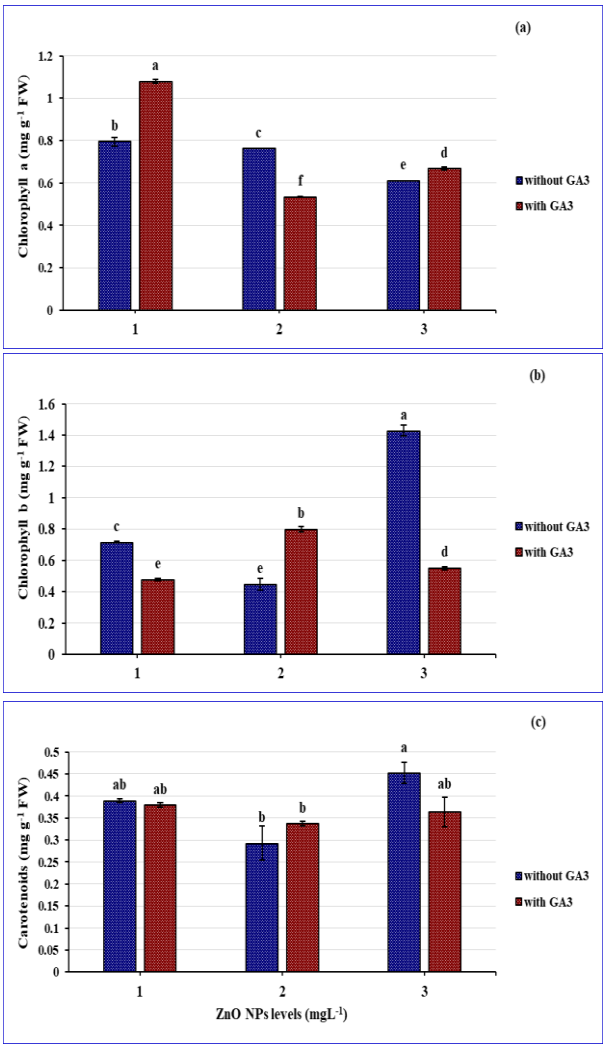

Figure (3): Effect of ZnO Nanoparticle Levels and GA3 Application (0.1 mM) on pigmentation of *Chenopodium quinoa* L. (a), Chlorophyll a; (b), Chlorophyll b and (c), Carotenoid content. ZnO Nanoparticle Levels are: 1, 0; 2, 50; 3, 100 mg L^{-1}. Values represent means of six replicates, from two independent experiments. Column with different letters indicate significant differences at $p \leq 0.05$, based on Duncan's Multiple Range Test. The vertical bars represent the standard error (± SE).

reduction of soluble carbohydrates in the shoots, while an increase was observed in the roots. However, GA3 priming altered this response; it significantly enhanced the soluble carbohydrate content in the shoots at elevated concentrations of ZnO NPs. This suggests that GA3 may mitigate the negative effects of ZnO NPs on carbohydrate metabolism in quinoa plants, thereby potentially improving their physiological resilience under stress conditions (Table 1).

Soluble proteins data showed trends similar to soluble carbohydrates particularly in shoot (Table 1). On the other hand, data of free amino acids showed opposite response to soluble carbohydrates, an enhancement in shoot and a reduction in roots were recorded under ZnO NPs toxicity. Application of GA3 induced free amino acids content in both shoot and root as compared to corresponding treatment under ZnO NPs toxicity without GA3 application (Table 1).

Phenolic content in shoot and root of quinoa plants were affected negatively by phytotoxicity of ZnO Nps.

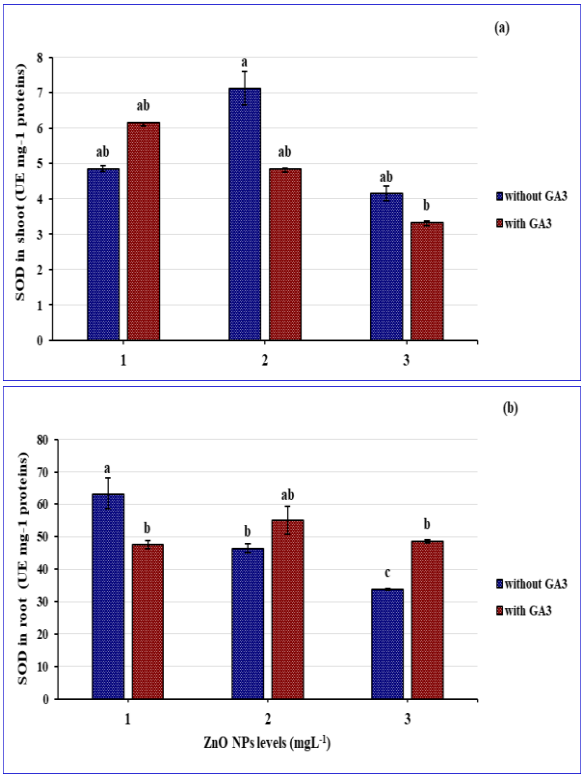

Figure (4): Effect of ZnO Nanoparticle Levels and GA3 application (0.1 mM) on antioxidant enzyme, Superoxide dismutase enzyme **(**SOD), of *Chenopodium quinoa* L. (a), SOD in Shoot; (b), SOD in root. ZnO Nanoparticle Levels are: 1, 0; 2, 50; 3, 100 mg L^{-1}. Values represent means of six replicates, from two independent experiments. Column with different letters indicate significant differences at $p \leq 0.05$, based on Duncan's Multiple Range. The vertical bars represent the standard error (± SE).

(Table 1). On the other hand, a significant increase was recorded in phenolics content in shoot and root at 100 mg L^{-1} ZnO NPs level as a consequence of GA3 priming compared to corresponding treatment without GA3 application (Table 1). Generally, the obtained results indicate that without GA3 priming, increasing levels of ZnO NPs usually lead to decreased levels of soluble carbohydrates and proteins in both shoots and roots. Meanwhile, the application of GA3 appears to mitigate some negative effects of high ZnO NP concentrations on protein and amino acid content, particularly in shoots. The results also suggest a complex interaction between GA3 priming and ZnO NP levels that influences biochemical parameters differently depending on the treatment conditions.

Principal component analysis (PCA)

To assess the relationships among treatments with and without GA3 priming under different levels of ZnO nanoparticles (NPs), a principal component analysis (PCA) was conducted on the experimental dataset. This dataset comprised 23 variables and 6 treatments. The primary objective of the PCA was to enhance discrimination power and group traits based on their interrelationships. The analysis revealed that the first two principal components (PCs) accounted for the highest percentage of variance in the data. Consequently, a PCA-based biplot (Figure 8) was generated using these components. This biplot provides a visual representation of both positive and negative correlation

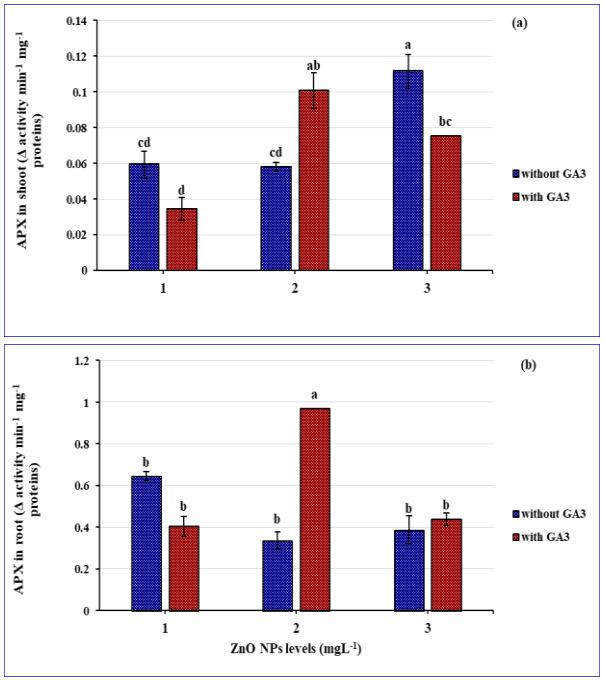

Figure (5): Effect of ZnO Nanoparticle Levels and GA3 Application (0.1 mM) on antioxidant enzyme, Ascorbate peroxidase enzyme APX in shoot (5a) and APX in root (5b). ZnO Nanoparticle Levels are: 1, 0; 2, 50; 3, 100 mg L^{-1}. Values represent means of six replicates, from two independent experiments. Column with different letters indicate significant differences at $p \leq 0.05$, based on Duncan's Multiple Range Test. The vertical bars represent the standard error (± SE).

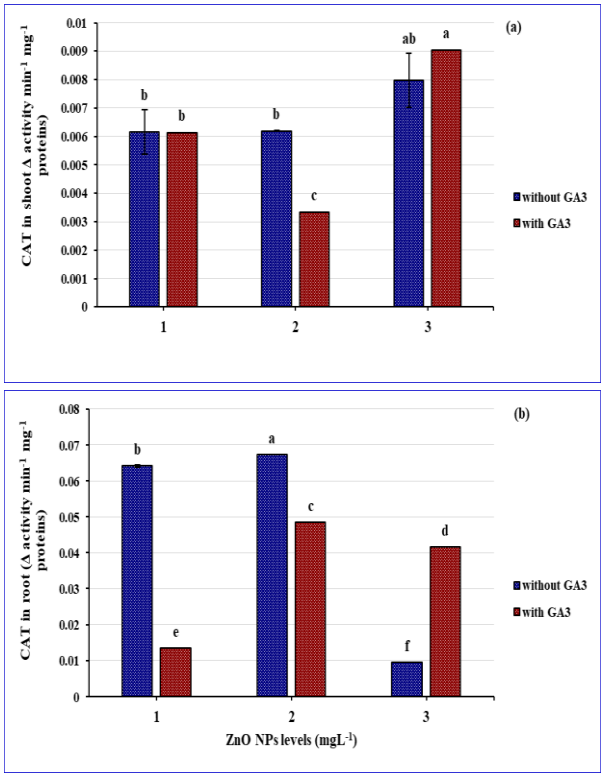

Figure (6): Effect of ZnO Nanoparticle Levels and GA3 Application (0.1 mM) on antioxidant enzyme, catalase enzyme (Δ activity min-1 mg-1 proteins). a, CAT in shoot and b, CAT in root. ZnO Nanoparticle Levels are: 1, 0; 2, 50; 3, 100 mg L^{-1}. Values represent means of six replicates, from two independent experiments. Column with different letters indicate significant differences at $p \leq 0.05$, based on Duncan's Multiple Range Test. The vertical bars represent the standard error (± SE).

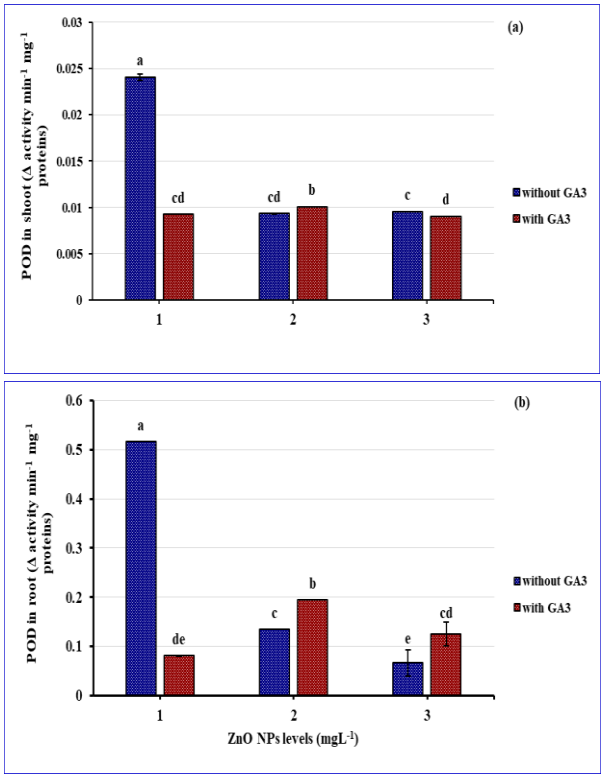

Figure (7): Effect of ZnO Nanoparticle Levels and GA3 Application (0.1 mM) on peroxidase enzyme (POD) in *Chenopodium quinoa* L. POD activity is measured as Δ activity min^{-1} mg^{-1} proteins. a, POD in shoot and b, POD in root. ZnO Nanoparticle Levels are: 1, 0; 2, 50; 3, 100 mg L^{-1}. Values represent means of six replicates, from two independent experiments. Column with different letters indicate significant differences at $p \leq 0.05$, based on Duncan's Multiple Range Test. The vertical bars represent the standard error (± SE).

among the evaluated traits, facilitating a clearer understanding of how different treatments and ZnO NP levels interact. Generally, PCA serves as a valuable tool for identifying patterns and relationships within complex datasets, enabling researchers to draw meaningful conclusions from their results.

The biplot provides a visual representation of the positive and negative correlations among the assessed traits. The closer the traits are to each other on the biplot, the more similar their trends are. In the right-hand half of Figure (8), there was a noticeable contrast between chlorophyll b, carotenoids, shoot soluble metabolites (proteins and amino acids), CAT, and APX in the shoot. On the other hand, all growth markers (fresh and dry weights), chlorophyll a, root soluble metabolites (proteins, carbohydrates, amino acids), antioxidant enzymes (POD, APX, SOD, CAT) in roots, and phenolic in both shoot and root were more prominent in the left-hand half. The plot also displays relationships among variables and the distribution of observations across six groups repre-senting control, level of ZnO NP (mg L^{-1}) of 50, 100, 0 mg L^{-1} ZnO NPs+GA3 priming, e, 50 mg L^{-1} ZnO NPs+GA3 priming and 100 mg L-1 ZnO NPs +GA3 priming (a, b, c, d, e, f, respectively). Individual observations are represented as data points, while ellipses illustrate group clustering, reflecting within-group variability and between-group separation. For example, groups "d" and "f" exhibit tight clustering, indicating high within-group similarity. In contrast, groups "a" and "c" are more dispersed, suggesting greater variability within those groups.

The first axis of the PCA captured approximately 35.9% of the cumulative variance, while the second axis captured 18.8%. The right-hand half of Figure (8) was greatly influenced by treatments involving 100 mg L^{-1} ZnO NPs, with or without GA3 priming. Meanwhile, the left-hand half was largely influenced by the control treatment (upper left) and treatments involving 50 mg L-1 Zn ONPs, with or without GA3 priming (down left).

Hierarchical clustering pattern

A cluster analysis was performed, resulting in a dendrogram with different sub-clusters for all the studied treatments and evaluated attributes (Figure 9). Most treatments showed positive linkages with traits such as soluble proteins in the shoot, soluble proteinsin the roots, soluble carbohydrates in the shoots, and SOD in the roots, which separated them from other parameters and formed distinct clusters. The remaining parameters formed distinct sub-clusters, indicating a negative relationship for the majority of the treatments. These parameters included dry weight, fresh weight, photosynthetic pigments, amino acids, CAT, POD, APX, and phenolics

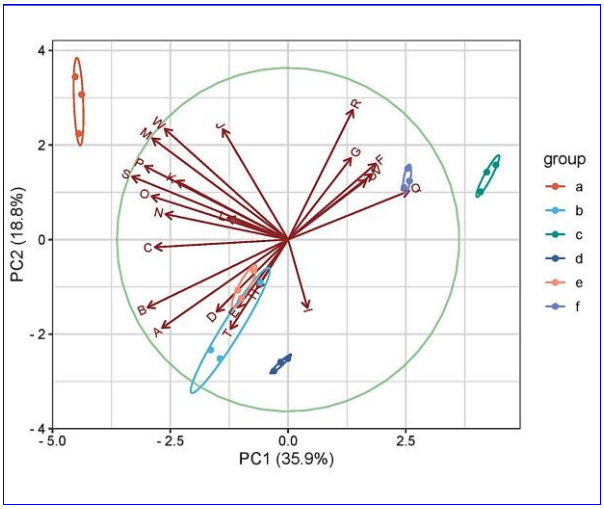

Figure (8): Principal Component Analysis (PCA) illustrates the correlations of various studied attributes on *Chenopodium quinoa* L., under different treatments of zinc oxide nanoparticles (ZnO NPs) with respect to the first two principal component axes. The horizontal and vertical arrows indicate the direction of influence for ZnO NPs and gibberellic acid (GA3) priming treatments, respectively. Treatment groups are defined as follows: a, Control (0 mg L^{-1} ZnO NPs); b, 50 mg L^{-1} ZnO NPs; c, 100 mg L^{-1} ZnO NPs; d, 0 mg L^{-1} ZnO NPs + GA3; e, 50 mg L^{-1} ZnO NPs + GA3 and f, 100 mg L^{-1} ZnO NPs + GA3. The parameters represented in the plot are: A, Shoot fresh weight; B, Shoot dry weight; C, Root fresh weight; D, Root dry weight; E, Chlorophyll a content; F, Chlorophyll b content; G, Carotenoids content; H, Carbohydrates in roots; I, Amino acids in roots; J, Proteins in roots; K, phenolics in roots; L, Ascorbate peroxidase (APX) activity in roots; M, Peroxidase (POD) activity in roots; N, Catalase (CAT) activity in roots; O, Superoxide dismutase (SOD) activity in roots; P, Carbohydrates in shoots; Q, Amino acids in shoots; R, Proteins in shoots; S, Phenolics in shoots; T, SOD activity in shoots; U, APX activity in shoots; V, CAT activity in shoots; W, POD activity in shoots.

Table (1): Effects of GA3 application, as priming agent, under different levels of ZnO NPs (0, 50 and 100 mg L^{-1}) on soluble metabolites including carbohydrates, soluble proteins, free amino acids and phenolics in shoots and roots of *Chenopodium quinoa* L. seedlings.

Priming application	ZnO NPs levels (mg L^{-1})	Measured parameters*							
		Soluble carbohydrates (mg g^{-1} FW)		Soluble proteins (mg g^{-1} FW)		Free amino acids (mg g^{-1} FW)		Phenolic content (µg g^{-1} FW)	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
without GA3 priming	0	160.59±0.6^{a}	17.98±0.5^{b}	109.59±0.64^{b}	55.93±1.1^{a}	0.62±0.02^{f}	2.77±0.01^{d}	1.65±0.01^{a}	0.43±0.00^{a}
	50	88.54±0.5^{c}	63.56±2.6^{a}	74.45±3.1^{d}	26.51±0.61^{cd}	0.75±0.03^{e}	1.44±0.00^{f}	0.92±0.04^{b}	0.24±0.00^{d}
	100	41.19±0.4^{e}	20.58±0.2^{b}	107.95±0.9^{b}	32.69±0.32^{b}	1.77±0.01^{b}	2.00±0.01^{e}	0.39±0.01^{f}	0.08±0.00^{e}
with GA3 priming	0	32.18±0.2^{f}	12.51±0.1^{c}	84.77±0.23^{c}	27.64±0.19^{c}	1.11±0.03^{c}	11.3±0.1^{a}	0.62±0.03^{d}	0.25±0.00^{c}
	50	105.55±0.12^{b}	3.1±0.01^{d}	85.27±0.13^{c}	8.56±0.02^{e}	0.92±0.03^{d}	3.76±0.07^{c}	0.76±0.00^{c}	0.08±0.00^{e}
	100	51.27±0.9^{d}	6.42±0.12^{d}	136.76±1.1^{a}	25.97±0.02^{d}	3.44±0.05^{a}	6.87±0.01^{b}	0.49±0.03^{e}	0.26±0.00^{b}

*The data are means of six replicates ± SE. Means followed by different letters are significantly different according to Duncan's Multiple Range Test ($p \leq 0.05$).

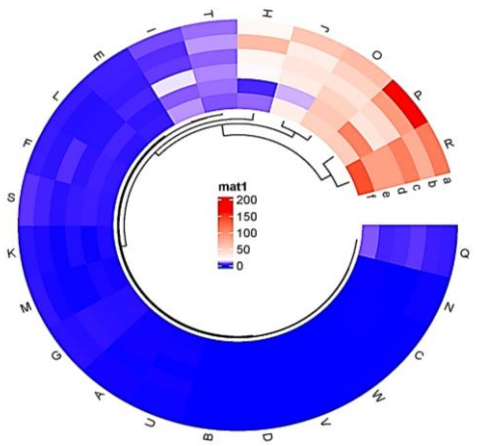

Figure (9): The heatmap provides a graphical representation of the relationships among six investigated treatments and 23 measured growth and physiological traits under varying concentrations of zinc oxide nanoparticles (ZnO NPs) and gibberellic acid (GA3) priming application. The color gradient and intensity in the heatmap were calibrated based on the associations aming treatments and traits. A darker blue scale represents lower values, whereas a darker red scale indicates higher values. Treatments are as follow: a, control; b, 50 mg L^{-1} ZnO NPs; c, 100 mg L^{-1} ZnO NPs; d, 0 mg L^{-1} ZnO NPs +GA3 priming; e, 50 mg L^{-1} ZnO NPs +GA3 priming, f=100 mg L^{-1} ZnO NPs +GA3 priming parameters: A, shoot fresh weight; B, shoot dry weight; C, root fresh weight, D, root dry weight; E, chlorophyll a; F, chlorophyll b; G, carotenoids; H, carbohydrates in root; I, amino acids in root; J, proteins in root; K, phenolics in roots; L, Ascorbate peroxidase (APX) activity in roots; M, Peroxidase (POD) activity in roots; N, Catalase (CAT) activity in roots; O, Superoxide dismutase (SOD) activity in roots; P, Carbohydrates in shoots; Q, Amino acids in shoots; R, Proteins in shoots; S, Phenolic content in shoots; T, SOD activity in shoots; U, APX activity in shoots; V, CAT activity in shoots; W, POD activity in shoots.

DISCUSSION

This study was conducted to detect the strains that might take place in response of quinoa plants to zinc oxide nanoparticles (ZnO NPs) along with gibberellic acid (GA3) as priming agent. Recently, Zinc Oxide Nanoparticles (ZnO NPs) have gained significant popularity in science, industry, and medicine. However, their disposal can lead to environmental contamination of soil and water (Sharma *et al*., 2023). Additionally, ZnO NPs can re-dissolve and release heavy metal ions, further contributing to pollution (Sobhanan *et al*., 2022). As a consequence, the growth and productivity of plants can be adversely affected by the accumulation of these contaminants. Moreover, consumption of plants contaminated with ZnO NPs can pose health and hygiene risks to humans, either directly or indirectly through animals, fish, poultry, and other livestock that feed on such plants (Zafar *et al*., 2016). On the other hand, nanomaterials like ZnO NPs can also compensate for mineral deficiencies in plants, and hence in animal and human diet (Du *et al*., 2019). In this work, growth, metabolic pools and the antioxidant system of quinoa shoots and roots as influenced by two levels of ZnO NPs (50 and 100 mgL^{-1}) were followed.

To mitigate the negative effects of applying ZnO NPs on quinoa plants, the seed priming technique was employed to improve plant growth and enhance resistance to abiotic stress. Seedlings grown from primed seeds exhibited superior overall growth and resilience compared to those grown from unprimed seeds. Seed priming involves a pre-sowing treatment that enhances the physiological and biochemical condition of seeds, leading to more efficient germination (Lutts *et al*., 2016).The application of ZnO NPs significantly impacted the growth of both shoots and roots, as evidenced by the results shown in Figures 1 and 2. These findings align with previous research on barley (*Hordeum vulgare* L.) seedlings exposed to nano-ZnO, where a reduction in shoot and root weight was observed at concentrations ranging from 300 to 2000 mg/L (Azarin *et al*., 2022).

Seed phytopriming with GA3 improved root and shoot fresh matter gain at 50 and 100 mg L^{-1} ZnO NPs levels, respectively. Similarly, Priming of *Avena sativa* seeds with 100 and 150 ppm GA3 for 24 h curtailed the toxic effects of 25, 50, 75 and 100 mM NaCl by enhancing germination percentage, shoot and root lengths, fresh and dry weights, tissue water contents, and seedling vigour indices contents (Chauhan *et al*., 2019).

Significant reduction in Chlorophyll a (Chl.a) was observed due to Zn ONPs application at 50 and 100 mgL^{-1} and enhanced significantly in plants of GA3 primed seeds at 100 mgL^{-1} ZnO NPs (Figure 3a). Meanwhile, chlorophyll b (Chl. b) and carotenoids were inhibited by 50 mgL^{-1} ZnO NPs, but significantly enhanced at 100 mgL^{-1} ZnO NPs (Figure 3b, c). In accordance with these findings a similar reduction in total chlorophyll content was reported in green leaves of *Pisum sativum*, *Salvinia natans*, and *Arabidopsis thaliana* treated with ZnO NPs (Mukherjee *et al*., 2014; Hu *et al*., 2014; Wang *et al*., 2016), which was probably due to impaired photosynthesis and reduced biomass accumulation (Adams *et al*., 2006). Priming of *Avena sativa* seeds with GA3 seedling growth and chlorophyll contents under salinity stress (Chauhan *et al*., 2019).

The exposure of plants to higher concentrations of ZnO NPs resulted in a significant reduction in soluble proteins in both the shoot and root, as shown in Table (1). However, seed priming led to a further increase in soluble proteins specifically in the shoot. This increase indicates an enhancement in the presence of functional proteins. Functional proteins are typically properly folded and soluble (Vihinen, 2020). On the other hand, if a protein fails to adopt its intended three-dimensional structure (protein misfolding), it often forms biologically inactive and insoluble protein aggregates (Skretas and Ventura, 2020). Soluble proteins play a crucial role in osmoregulation under halo/osmotic conditions and in chelating ions released from nanoparticles (Sitohy *et al*., 2020). They contribute to maintaining the balance of water and ions in the plant cells, as well as sequestering ions that are released from nanoparticles (Athar *et al*., 2022).

Free amino acids content increased significantly in shoot but reduced in root of quinoa plant treated with elevated concentrations of ZnO NPs (Table 1). Contrarily were the soluble carbohydrates, Significant

reduction observed in shoot, and enhancement recorded in roots (Table 1). Application of GA3 as priming agent GA3 increased significantly soluble carbohydrates and proteins contents in shoots. In the present study, additional enhancement due to GA3 phytohormonal priming was reported in free amino acids content in shoots and roots under ZnO NPs toxicity. The presence of abundant soluble fractions is a notable characteristic of osmoregulation, which facilitates continuous water uptake and provides protection for enzyme molecules. The high concentration of soluble fractions corresponds to the increased water content drawn into cells through osmotic potential or the absorption of these compounds (Ozturk *et al*., 2021). The observed enhancement of soluble fractions in this study by ZnO NPs may be attributed to the breakdown of their structural polymers (proteins into amino acids, carbohydrates into soluble sugars), or conversely, the inhibition of polymerization of simpler components.

In order to counteract the harmful effects of reactive oxygen species (ROS) and other oxidants, such as reactive sulfur species (RSS), in stressed cells, both enzymatic and non-enzymatic antioxidants are typically induced to mitigate their negative impacts (Hong *et al*., 2024).

In this study, the impact of ZnO NPs exposure and seed priming on enzymatic and non-enzymatic antioxidant systems was evaluated. The applied treatments significantly affected both the enzymatic and non-enzymatic antioxidant systems. The non-enzymatic antioxidants assessed in this study included phenolics, while the enzymatic antioxidants included superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT). These antioxidants were evaluated in the shoots and roots of quinoa plants under the influence of ZnO NPs phytotoxicity and the GA3 priming agent.

Phenolics were higher at shoots and roots of control, received none of ZnO NPs or priming treatments (Table 1). Exposure to ZnO NPs highly significantly reduced the content of phenolics. Contrarily, Phenolics content increased significantly due to GA3 priming at shoot and roots as compared to corresponding treatment with GA3 priming (Table 1). Previous study reported similar results, *Pisum sativum* seeds invigorated with 0.5 mM GA3 for 6 hrs exhibited enhancement in total phenol, proline and flavonoid accumulation facilitating seedlings to thrive under salinity stress (Ahmad *et al*., 2021).

Several studies have demonstrated that the exogenous application of GA3 can enhance the activities of antioxidant enzymes, providing protection to plants against various abiotic stresses (Jiang *et al*., 2020). Consistent with these findings, our study observed that the activity of superoxide dismutase (SOD) did not show a significant change in the shoot under both the ZnO NPs and GA3 priming treatments. While in roots significant reduction in SOD activity observed due to 50 and 100 mg L^{-1} Zn ONPs application, meanwhile GA3 priming improved SOD activity under the two ZnO NPs levels.

The application of 100 mg L^{-1} ZnO NPs increased significantly APX activity in shoot, while GA3 priming induced APX activity at both levels of Zn ONPs (50 and 100 mg L^{-1}). On the other hand, ZnO NPs phytotoxicity induced no change in APX activity in root, meanwhile GA3 application induced APX in root at 50 mg L^{-1} ZnO NPs. CAT activity induced slightly in shoot and significantly in root by the phytotoxicity of Zn ONPS (Fig. 6). GA3 application as priming agents stimulated CAT activity to the highest levels in shoots and roots of quinoa plants under 100 mg L^{-1} ZnO NPs.

The activity of peroxidase (POD) in both the shoots and roots of quinoa plants was significantly reduced due to the phytotoxicity of ZnO NPs. However, when the plants were treated with the GA3 priming agent under 100 mg L^{-1} ZnO NPs, there was a significant increase in POD activity specifically in the roots compared to the corresponding treatment without priming. Prolonged exposure to high concentrations of ZnO NPs triggers the activation of enzymatic antioxidants, which leads to the depletion of plant energy resources and has a negative impact on plant growth and yield. The findings from Azarin *et al*. (2022) provide valuable insights for predicting the risks associated with the continued release of nano-ZnO into the environment. The study revealed that the activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione S-transferase (GST) increased in both the roots and shoots of barley seedlings exposed to bulk- and nano-ZnO for 7 days. Gene expression analysis specifically identified CAT-1 as the main contributor to the increase in catalase activity in treated *Hordeum* vulgare. Generally, the antioxidant defense system in *Hordeum vulgare* is activated in response to the impact of ZnO forms, effectively mitigating oxidative stress during the early stages of plant development (Azarin *et al*., 2022). Pre-soaking treatment of *Isatis indigotica* seeds with 0.2, 0.4, 0.6 g L^{-1} concentrations of GA3 for 12 h accelerated germination and seedling performance by reducing oxidative injury and enhancing activities of antioxidant enzymes under salinity stress (Jiang *et al.*, 2020).

CONCLUSION

Overall, this study demonstrates that seed priming using GA3 showed important beneficial effects on ZnO NPs phytotoxicity stressed quinoa plants. The phytohormonal priming contributed to the maintenance growth and physiological traits of plants under ZnO NPs phytotoxicity including root and shoot fresh matter gain, maintenance of chlorophyll a content, in addition to metabolic pools (soluble carbohydrates and soluble proteins) of shoots. Our results showed that the higher SOD and POD activation in the roots, accompanied with APX and CAT activation in shoots and roots due to GA3 priming under ZnO NPs phytotoxicity conditions. Thus, seed priming approach represents a viable, economically accessible, and sustainable management strategy. However, even though the growth and

physiological indicators show the efficiency of the treatments, further studies are needed on the impacts of treatments on quinoa productivity. This agricultural practice is recommended for ZnO NPs contaminated soils. 550 nm was calculated spectrophotometrically in order to quantify the free aldehydic groups of hexosamine that were freed during chitin digestion, as measured by chitinase using a 3,5-dinitro salicylic acid reagent.

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تحسين تحمل نبات الكينوا (*Chenopodium quinoa L.*) وتوازنها التأكسدي لسمية جسيمات أكسيد الزنك النانوية (ZnO NPs) من خلال تهيئة فيتوهرمونية عن طريق النقع في حمض الجبريليك

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تتعلق هذه الدراسة بمحاولة إثبات التأثير التحسيني لحمض الجبريليك (GA3) كعامل نقع هرموني نباتي (فيتوهورموني) بمعدل 0.1 ملل مول على نمو ونظام مضادات الأكسدة لنبات الكينوا (*Chenopodium quinoa* L.) تحت التأثيرات السلبية لسمية جسيمات أكسيد الزنك النانوية (ZnO NPs) بالتركيزات الاتية (0، 50، و 100 ملل جرام/لتر). حمض الجبريليك GA3 هو أحد الهرمونات النباتية (فيتوهرمون) التي تعززنظام مضاد الأكسدة في الخلية النباتية. أظهرت نتائج الدراسة تثبيط في نمو نبات الكينوا (الكتلة الطازجة والجافة، والكلوروفيل أ) تحت تأثير سمية جسيمات أكسيد الزنك النانوية ZnO NPs. كما أوضحت نتائج الدراسة أن تثبيط النمو في نبات الكينوا كان مصاحبًا لتغييرات في تثبيط مكونات الايض الابتدائي في المجموع الخضري لكل من الكربوهيدرات والبروتينات القابلة للذوبان. وقد بينت الدراسة التأثير السلبي لجسيمات أكسيد الزنك النانوية ZnO NPs حيث أظهرت النتائج التثبيط الملحوظ في نشاط الإنزيمات المضادة للأكسدة بالإضافة إلى المركبات الفينولية كإحدي مضادات الاكسدة اللاأنزيمية بشكل كبير. ومن جهة أخري كشفت نتائج الدراسه عن التأثير التحفيزي لتهيئة البذور عن طريق نقعها في حمض الجبريليك GA3 وذلك من خلال التنشيط الملحوظ في نشاط مضادات الأكسدة الأنزيمية والفينولات. وقد خلصت الدراسة انه يمكن تقليل التأثيرات الخطرة لسمية جسيمات أكسيد الزنك النانوية ZnO NPs، على الأقل جزئيًا، وذلك عبر أستخدام تقنية النقع من خلال تهيئة بذور الكينوا عن طريق نقعها في حمض الجبريليكGA3.