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Zinc Oxide Nanoparticles and Biochar Enhance Wheat Resilience to Salinity through Biochemical and Yield Traits

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ABSTRACT

Salinity stress significantly constrains global wheat (*Triticum aestivum* L.) production, necessitating novel amelioration strategies. This experiment investigated the biochemical and yield responses of 11 diverse wheat genotypes to salinity stress, and the mitigating effects of foliar-applied zinc oxide nanoparticles (ZnONP) and soil-incorporated biochar (BC). A randomized complete block design with three replications was employed. ANOVA revealed highly significant differences ($p \leq 0.001$) among genotypes, treatments, and their interactions for all assessed traits. Salinity significantly reduced thousand grain weight (TGW) from a control average of 24.49 g to 18.51 g, and grain yield per plant (GYP) from 34.96 g to 26.02 g. Conversely, proline content increased from 23.06 µg/g FW under control conditions to 82.28 µg/g FW under salinity stress. Both ZnONP and BC treatments partially restored TGW (22.60 g and 22.70 g, respectively) and GYP (31.96 g and 33.04 g, respectively), with BC demonstrating slightly superior efficacy for GYP. Zn contents in grains was markedly enhanced by ZnONP (212.92 mg/kg) and BC (216.93 mg/kg) compared to control (72.77 mg/kg) and salinity (52.81 mg/kg). Antioxidant enzyme activities, including glutathione reductase (GR) and superoxide dismutase (SOD), generally increased under stress and were further augmented by amendments. GR activity was highest with BC (17.34 EU/mg), while SOD peaked under ZnONP (62.98 EU/mg). Genotypes G10 and G9 consistently exhibited superior performance across most ameliorative treatments. These findings underscore the potential of ZnONPs and biochar as sustainable solutions for enhancing wheat productivity under saline conditions, particularly when integrated with tolerant genotypes.

Keywords: genotypes, proline, GR, SOD, productivity, mitigation.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a key contributor to global food security, accounting for nearly 20% of the caloric intake and protein of the global population. It is cultivated throughout the world in a variety of agro-ecological zones and is one of the most important crops for billions of people. Nonetheless, the productivity of this crucial cereal crop is under increasing threat from abiotic stresses, particularly soil salinity, which is rapidly becoming a global issue as one of the most important environmental hurdles for sustainable agriculture (Akhtar et al., 2015; Francis et al., 2024). Agricultural land is increasingly threatened by soil salinization, and estimates suggest that over 20% of total cropland and nearly half of irrigated land are salt affected globally, and these numbers are anticipated to rise from climate change, unsustainable agricultural

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irrigation, and sea-level rise. This growing problem threatens global food production and will require immediate and innovative approaches to improving crop resilience and minimizing the loss of crop yield stability on marginal lands (Al-Zahrani et al., 2021; Saraugi and Routray, 2024).

Salinity stress, is characterized by an excessive amount of soluble salts (mainly NaCl) in the vicinity of plant roots, causes a multi-faceted assault on the growth and development of the plant. Salinity causes harmful effects at the physiological, biochemical, and molecular levels. One important effect is that high external salt concentrations create osmotic pressure, which reduces the water potential of the soil solution, thereby hindering the ability for plants to take up water (similar to drought). This osmotic imbalance disrupts turgor pressure, cell expansion, and overall plant growth (Saleh et al., 2009; Abel et al., 2013). Second, excessive uptake and accumulation of particular ions, namely Na^+ and Cl^-, lead to ion toxicity. Plants have complex antioxidant defense mechanisms that include enzymatic [e.g., Superoxide Dismutase (SOD), Glutathione Reductase (GR)] and non-enzymatic (e.g., proline, ascorbate, glutathione) ROS scavengers, which, in effect, help to maintain cellular homeostasis. However, in response to prolonged severe salinity stress, ROS production can exceed the detoxifying ability of the plant resulting in oxidative damage and metabolic impairment. The emergence of nanotechnology will provide innovative opportunities for sustainable agriculture management in the coming years (Akhtar et al. 2015; Cîmpeanu et al., 2021; Petcu et al., 2021; Saraugi and Routray, 2024).

Nanoparticles (NPs) have unique physicochemical properties, including a large surface area to volume ratio, increased reactivity, and improved penetration, that may have applications in nutrient delivery, pest management, and abiotic stress mitigation. Among several nanoparticles, zinc oxide nanoparticles (ZnONPs) are one of the most noteworthy nanoparticles due to the role of zinc as a micronutrient for crop development and its involvement in a variety of physiological and biochemical functions (Al-Zahrani et al., 2021; Shah et al., 2024). Zinc is a co-factor for more than 300 enzymes, including those related to carbohydrate metabolism, protein synthesis, nucleic acid metabolism, and stress defense. Zinc, in particular, is a key contributing/constituent enzyme in the superoxide dismutase (SOD) enzyme responsible for scavenging superoxide radicals while coping with different stresses (underlie stress) to keep redox homeostasis (Ali et al., 2019; Shukla et al., 2023). While there is evidence that foliar-applied ZnONPs can increase zinc uptake efficiency, help photosynthetic efficiency, and activate antioxidant defenses/capacity to enhance growth and salt stress even under saline conditions, the nanoscale nature allows the potential for improved absorption and translocation and to be more efficient than conventional zinc fertilizers (Bashir et al., 2021; Shukla et al., 2024).

At the same time, biochar, which is a charcoal-like substance generated from the pyrolysis of biomass in an oxygen-limited environment, has become a strong soil amendment that has good potential for improving soil and crop health, particularly under stress. In many regards, biochar can be characterized by a highly porous structure, a large surface area, and its high carbon content, leading to a range of benefits (El-Sayed et al., 2021; Singh et al., 2022). Biochar can augment soil water retention capacity, help with nutrient availability by increasing cation exchange capacity, buffer soil pH, and provide habitat for beneficial microorganisms in the soil. Furthermore, the sustainable production of biochar from agricultural wastes makes it an environmentally friendly approach to waste management and carbon sequestration. Although the individual roles of ZnONPs and biochar in mitigating abiotic stresses, including salinity, are increasingly recognized, the synergistic potential of their combination needs further exploration (Takagi and Yamada, 2013; Francis et al., 2024). It is suggested that the combined effects of ZnONPs contributing to increased nutrient uptake and improved enzyme activity and biochar ameliorative soil properties and enhanced root environment could represent a more integrated and effective form of ameliorating stress in wheat with salinity compared with either form of amelioration alone. An integrated approach could lead an increase in plant growth, yield, and stress defense (García-Gómez et al., 2023; Ullah et al., 2024).

The current study sought to assess the effects of salinity stress, foliar-applied zinc oxide nanoparticles, and soil incorporated biochar (alone and in combination) on the agronomic performance of 11 wheat genotypes. The aim was to assess how the changes in the aforementioned agronomic parameters (e.g. thousand grain weight, grain yield per plant) and biochemical indicators (e.g. proline content, zinc content, glutathione reductase and superoxide dismutase activity) impact the mechanisms underpinning both salinity resilience and salinity toleration (the two components illustrated in the salinity resilience framework). Ultimately, this study will contribute to identifying the best amelioration practices and genotypes that can provide a measure of resilience or tolerance when food security is threatened.

MATERIAL AND METHODS

The research examines 11 wheat genotypes, which were selected based on their genetic diversity and their ability to grow under different environmental conditions. The experiment is pot-based and designed as a randomized complete block design (RCBD). All wheat genotypes are subjected to four treatments performed across three replications: (i) Normal conditions (Control), (ii) Salinity stress, (iii) Salinity + Zinc Nanoparticles, and (iv) Salinity + Zinc Nanoparticles + Biochar. Application of Treatments T1: Normal Conditions (Control, control treatment, wheat grown without subjected stress): All pots labeled as control received no treatments, and thus, optimal growth conditions were maintained. T2: Salinity Stress: Sodium chloride (NaCl 60 mM NaCl concentration) was applied to develop salinity stress. This concentration was established through current literature protocols or pilot studies along with the concentrations being selected that provided a moderate stress treatment. T3: Salinity (60 mM NaCl concentration) + Zinc Nanoparticles: Zn NPs (50 mg/L) was applied to test for improvements in the responses of salinity stressed plants. Dose of (Zn NPs) was given at a concentration of 50 mg/L by foliar application after four weeks and six weeks of seedling growth. All treatment received a total of 4 liters of the NP solution per rep while the non-NP treated plants received the same amount of distilled water. This NP treatment dose was based on previous observations of NP phytotoxicity (Bashir et al., 2021). T4: Salinity + Zn Nanoparticles + Biochar: Biochar that was generated from organic waste using pyrolysis, was added to the soil (Ali et al., 2019; Singh et al., 2022). This treatment would test the combined effectiveness of ZnO-NPs and biochar for stress mitigation.

Assessment of biochemical and yield traits

Evaluation of different biochemical and yield traits, including 1000 grain weight, grains yield per plant, proline contents (µg/g FW) and Zinc contents, gives indications on the performance of the wheat genotypes under varying treatments. The proline content of fresh leaves was estimated using the protocol with ninhydrine (Bates et al., 1973). The Zn content estimated from the powder of leaves was measured using the protocol described by (Singh et al., 2022). Further, characterization of antioxidant enzyme activities, such as glutathione reductase (GR), superoxide dismutase (SOD) provides valuable information regarding the antioxidative defense mechanism of the plant. GR, SOD activities were measured from the leaves of genotypes studied following (Takagi and Yamada, 2013; Shukla et al., 2023).

Statistical Analysis

The data collected was thoroughly analyzed, utilizing appropriate statistical analysis tools to detect significant differences between treatments and genotypes. Following the methods outlined by (Steel and Torrie, 1980), statistical analysis was used to determine variance from the data collected for the various traits. The means of the 11 genotypes were compared across the four treatments for all traits, using the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

The results of the analysis of variance (ANOVA) presented in Table 1, indicated that highly significant differences (p≤0.001) existed among genotypes, treatments, and genotype × treatment interactions for all biochemical and yield traits studied. These traits included thousand grain weight (TGW), grain yield per plant (GYP), zinc (Zn) content, protein (Pro), germination rate (GR), and superoxide dismutase (SOD) activity. Significant genotypic variation resulted in mainly genetic diversity in the studied traits among the 11 wheat genotypes that were evaluated, which can be of benefit in breeding efforts for improvement of those traits for future generations. The findings of the current study is well established with (Hussain et al., 2019; Rizwan et al., 2023), who developed considerable genetic variation for yield and biochemical parameter between stress and non-stress environments, displaying potential for selection as well as genetic improvement.

The noteworthy treatment effects for all traits, but notably Zn (476.53**), protein content (3623.75**), and SOD activity (1073.96**), illustrate the impact of environmental or management modifications (e.g., fertilization, irrigation regimes, or different stress treatments) on wheat performance. This data support the results reported by (Pratiwi et al., 2022; Kannan et al., 2025) and show that management of nutrients and stress management can significantly affect biochemical responses and yield in wheat. Moreover, the significant genotype × treatment interactions (G × T) for all traits, whilst less prominent, also indicate that genotypes respond differently depending on treatment; thus, indicating differences in adaptability or stress resistance. This interaction is valuable in identifying stable and high-quality genotypes in the face of changing environments. Our results are consistent with those of (Phares et al., 2020; Kumari and Garg, 2025) who also recognized the role of G × T interactions for selecting wheat varieties best suited to an agro-environment, based on their physiological and biochemical plasticity.

The overall low error variance across traits is an indicator of reliable experimental results, suggesting the differences observed are primarily caused by genetic and treatment effects rather than random variability. Collectively, these results demonstrate strong evidence for the effectiveness of combining both genetic and environmental approaches to improve wheat productivity and stress resilience in a climate-affected future, affirming the earlier findings by (Lehmann and Joseph, 2015; Nosratabad et al., 2024) regarding the multifactorial regulation of yield and biochemical traits of cereals.

Table 1. Analysis of variances for biochemical and yield traits for various treatments in 11 wheat genotypes

Sources of Variation	DF	TGW	GYP	Zn	Pro	GR	SOD
Replications	2	6.91	10.34	8.12	6.45	6.82	7.11
Genotypes	10	82.34**	90.21**	112.67**	137.43**	81.92**	92.65**
Treatments	3	64.28**	215.89**	476.53**	3623.75**	11.83**	1073.96**
Genotypes × Treatments	30	15.34*	13.16*	15.21*	11.38*	12.06*	13.84*
Error	86	2.08	1.17	1.26	1.12	1.42	1.93
Total	131						

*** = highly significant (p=0.001), * = significant (p=0.01), ns = non-significant.*

Thousand Grain Weight (TGW)

The treatments significantly affected the thousand grain weight (TGW) where the control treatment reached the highest average value (24.49 g), and the lowest was for the salinity treatment (18.51 g). Standard deviations (SD) ranged from 0.72 (control and salinity) to 1.12 (ZnONP), and the coefficient of variations (CV) were in the ranging from 2.93% (control) and 4.97% (ZnONP) demonstrating similarities of variations showing very moderately low variability (Table 2). Biochar (BC) and zinc oxide nanoparticles (ZnONPs) treatments partially restored TGW where it was observed BL (22.70 g), and ZnONP (22.60 g). Decline for salinity was associated with ionic toxicity and osmotic stress leading to reduced grain filling and impaired assimilate translocation consistent with (Mujeeb-Kazi et al., 2019; El-Sayed et al., 2021).

Across all genotypes, salinity reduced TGW, suggesting that G10 (20.18 g) and G9 (19.15 g) were tolerant genotypes (Figure 1). The reductions were anticipated and consistent with research by (Lehmann and Joseph, 2015; Francis et al., 2024) who concluded that salinity disrupts grain filling by restricting the uptake of water and nutrients. The addition of ZnONPs was shown to increase TGW, notably in G5 (25.07 g) and G9 (24.55 g) perhaps linked to the role of zinc in plant enzymatic activity, metabolic processes, and seed development under stress conditions (Al-Zahrani et al., 2021; Shah et al., 2024). Biochar improved the TGW for most of the genotypes after salinity, specifically G10 (24.37 g) and G8 (23.35 g). This is believed to be due to the effect of biochar on soil structure and nutrient holding capacity of soil.

Treatments had a significant effect on thousand grain weight (TGW) whereby the highest mean value was obtained under control (24.49 g; "a") followed by biochar (22.7 g; "b"); ZnONP (22.6 g; "bc"); and the lowest value was observed under salinity with 18.51 g (d) as showed in Table 3. The decline of TGW observed under salinity stress was largely due to reduced carbohydrate accumulation, assimilate partitioning, and premature senescence, according to the explanation given by (Kannan et al., 2025; Kumari and Garg, 2025). However, the partial recovery of TGW seen with ZnONP and BC demonstrated their ability to mitigate salt-induced injury. It is thought that zinc oxide nanoparticles improve photosynthesis efficiencies and enzymatic activity for more fruitful seed maturation (Abel et al., 2013; Phares et al., 2020; Rizwan et al., 2023).

Table 2. Descriptive statistics (Minimum, Maximum, Average, Standard Deviation, and Coefficient of Variation) of various biochemical and yield traits under different treatments

Traits	Treatments	Minimum	Maximum	Average	Standard Deviation	Coefficient of Variation
TGW (g)	Control	23.61	26.16	24.49	0.72	2.93
	Salinity	17.63	20.18	18.51	0.72	3.87
	ZnONP	21.49	25.07	22.60	1.12	4.97
	BC	21.82	24.37	22.70	0.72	3.16
GYP (g)	Control	32.20	39.37	34.96	2.21	6.32
	Salinity	23.26	30.43	26.02	2.21	8.49
	ZnONP	29.19	36.36	31.96	2.21	6.93
	BC	30.22	37.39	33.04	2.25	6.80
Zn (mg/kg)	Control	71.13	75.11	72.77	1.18	1.62
	Salinity	51.80	55.39	52.81	0.96	1.82
	ZnONP	210.20	220.25	212.92	3.22	1.51
	BC	212.11	224.11	216.93	4.30	1.98
Pro (µg/g FW)	Control	21.24	24.27	23.06	0.86	3.73
	Salinity	80.11	84.15	82.28	1.47	1.78
	ZnONP	30.13	34.18	32.29	1.27	3.92
	BC	36.57	44.30	41.48	2.18	5.25
GR (EU/mg)	Control	14.30	15.33	14.82	0.33	2.21
	Salinity	15.29	16.32	15.83	0.33	2.06
	ZnONP	16.19	17.22	16.70	0.35	2.12
	BC	16.86	17.89	17.34	0.33	1.88
SOD (EU/mg)	Control	40.61	45.09	42.48	1.43	3.36
	Salinity	59.13	65.17	60.93	1.62	2.66
	ZnONP	61.14	67.19	62.98	1.63	2.59
	BC	53.73	59.69	55.40	1.66	3.00

Note: 1000 grain weight (TGW), Grain Yield per plant (YP), Zinc contents (Zn), Proline (Pro), Glutathione reductase (GR), superoxide dismutase (SOD), zinc oxide nanoparticles (ZnONP), biochar (BC).

Grain Yield per Plant (GYP)

The grain yield basis exhibited almost identical patterns characterized by the highest mean yield under control (34.96 g) conditions and the lowest mean yield under salinity conditions (26.02 g). The standard deviation was fairly stable across treatments (~2.21-2.25) while coefficients of variation ranged from 6.32% (control) to 8.49% (salinity) indicating highly variable yields under stress conditions (Table 2). Yield was significantly improved in the BC (33.04 g) and the ZnONP (31.96 g) treatments. Salinity stress has an impact on yield primarily as a result of reductions in photosynthesis, reproductive development, and grain filling, which agrees with prior findings by (García-Gómez et al., 2023; Francis et al., 2024). The reduction in yield loss through the use of ZnONP and BC demonstrates their ability to alleviate yield under stress. The ZnONP may have enhanced plant micronutrient uptake, increased the activity of antioxidant enzymes, improved metabolic efficiency and therefore improved stress tolerance (Saleh et al., 2009; Bashir et al., 2021).

Salinity stress resulted in a marked decrease of GYP across genotypes, with the greatest declines observed in G1 (23.26 g) and G5 (23.36 g), consistent with (García-Gómez et al., 2023) who cited the negative impacts of osmotic stress and ionic toxicity on wheat productivity (Figure 1). However, ZnONP application significantly increased GYP relative to controls; ZnONPs had the largest increase over controls in G10 (36.36 g) and G9 (34.34 g), likely due to the known important role of zinc in metabolic activity and chlorophyll synthesis as shown in Rizwan et al. (2023). In a similar way, all genotypes produced more GYP with the application of biochar relative to controls. Biochar produced the greatest GYP in G10 (37.39 g) and G9 (35.53 g), also consistent with (Saraugi and Routray, 2024; Ullah et al., 2024), who found that biochar can improve water retention and nutrient availability, which contributed to the enhancement of crop performance under salinity.

Grain yield per plant was greatest in the control (34.96 g, "a") and biochar (33.04 g, "a") treatments and was slightly less in ZnONP (31.96 g, "ab") treatment, while salinity reduced grain yield significantly (26.02 g, "c") as mentioned in Table 3. Salinity has been reported to lower yield, because of its adverse effects on photosynthesis, reproductive development and grain filling (Phares et al., 2020; Singh et al., 2022). The ZnONP treatment and BC treatment significantly alleviated grain yield loss from salinity by improving nutrient bioavailability, antioxidative response, and water use efficiency.

Table 3. Mean comparison of biochemical and yield traits under different treatments using alphabetical grouping

Traits	Treatments	Average	Traits	Treatments	Average
1000 Grain Weight (g)	Control	24.49a	Proline (µg/g FW)	Control	23.06d
	Salinity	18.51d		Salinity	82.28a
	ZnONP	22.6bc		ZnONP	32.29c
	BC	22.7b		BC	41.48b
Grain Yield per Plant (g)	Control	34.96a	Glutathione Reductase (EU/mg)	Control	14.82bc
	Salinity	26.02c		Salinity	15.83b
	ZnONP	31.96ab		ZnONP	16.7ab
	BC	33.04a		BC	17.34a
Zinc Content (mg/kg)	Control	72.77a	Superoxide Dismutase (EU/mg)	Control	42.48c
	Salinity	52.81c		Salinity	60.93ab
	ZnONP	212.92b		ZnONP	62.98a
	BC	216.93b		BC	55.4b

Note: followed by different letters within each trait indicate significant differences at p≤0.05 according to LSD test. ZnONP = Zinc Oxide Nanoparticles; BC = Biochar.

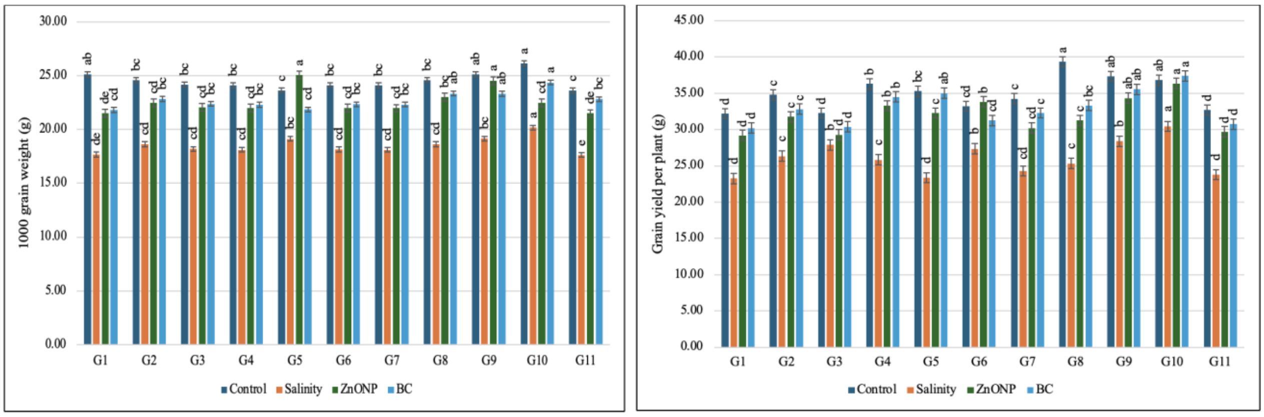

Figure 1. Effect of different treatments (Control, Salinity, ZnONP, and BC) on 1000-grain weight (g) and grain Yield per plant across various wheat genotypes (G1-G11). Bars represent means, and different letters indicate significant differences (p<0.05).

Zinc Content (Zn)

The concentrations of zinc in grains showed good improvements for ZnONP (212.92 mg/kg; SD = 3.22, CV = 1.51%) and BC (216.93 mg/kg; SD = 4.30, CV = 1.98%) treatments relative to control (72.77 mg/kg; SD = 1.18, CV = 1.62%) and salinity (51.81 mg/kg; SD = 0.96, CV = 1.82%) treatment (Table 2). These results demonstrate the potential of ZnONPs and biochar for biofortification purposes. The levels of Zn that we obtained were consistent with (Francis et al., 2024; Kannan et al., 2025) that showed improved translocation and accumulation of Zn in wheat with ZnONPs. Biochar also reduces soil pH, increases nutrient retention properties, which may have benefited Zn availability.

Significant declines in Zn content were documented under salinity stress, between 51.80 mg/kg and 55.39 mg/kg (Figure 2). This drop supports the results in Cakmak's earlier research (2008), which concluded that the competitive uptake of ions (like Na^+ and Cl^-) under saline conditions, in addition to root impairment, reduced the uptake of micronutrients. However, the introduction of ZnONPs enhanced zinc accumulation in all genotypes tested, with the greatest concentrations occurring in G10 (220.25 mg/kg), G2 (215.70 mg/kg), and G8 (216.22 mg/kg). This agrees with the data produced by (Pratiwi et al., 2022; Rizwan et al., 2023), who suggested that nanoparticulate zinc has increased bioavailability, and plant uptake efficiency in contrast to traditional zinc sources.

The highest grain zinc content was observed in the biochar treatment (216.93 mg/kg, "b") and the ZnONP treatment (212.92 mg/kg, "b"), and the control was slightly lower (72.77 mg/kg, "a"), while the salinity treatment had the lowest grain zinc content (52.81 mg/kg, "c") as displayed in Table 3. This marked increase in Zn content in the grain among the ZnONP and BC treatments indicated the capability of these materials to conduct agronomic biofortification. As mentioned previously, zinc oxide nanoparticles supply readily available zinc ions that plant roots absorb quickly and translocate to the grain (El-Sayed et al., 2021; Singh et al., 2022). Furthermore, our findings suggest that BC and ZnONP may offer sustainable management practice options for biofortification and addressing issues associated with zinc malnutrition.

Proline Content (Pro)

The level of proline was significantly increased during salinity stress (82.28 µg/g FW; SD = 1.47, CV = 1.78%), verifying its contribution as a stress indicator and osmoprotectant (Table 2). The values were lower under control (23.06 µg/g FW; SD = 0.86, CV = 3.73%). The treatments involving ZnONP (32.29 µg/g; SD = 1.27, CV = 3.92%) and BC (41.48 µg/g; SD = 2.18, CV = 5.25%) resulted in moderate values, just above the control. This outcome corroborates proline's protective function in osmotic adjustment, free radical scavenging, and protein stabilization during stress (Abel et al., 2013; El-Sayed et al., 2021). These results reinforce proline's role as a stress indicator and biochemical response to the mitigation treatments.

Under salinity stress, all genotypes had significantly elevated proline accumulation, with maximum values for G3 (84.15 µmol/g FW), G8 (84.09 µmol/g FW), and G2 (83.63 µmol/g FW) as mentioned in Figure 2. The increase in proline is a known stress-response strategy, where proline acts as an osmolyte and an antioxidant preventing salt-induced oxidative damage. The current study findings are consistent with those reported in (Phares et al., 2020; Pratiwi et al., 2022) and describe proline accumulation as an important plants response to abiotic stress. Moreover, biochar-treated plants continued to have lower proline values than salinity-stressed control plants but higher values than unstressed control plants. The biochar-treated plants had higher proline values for G3 (44.22 µmol/g FW) and G8 (44.30 µmol/g FW). Found that biochar-exposed plants were still experiencing stress, but the proline values indicated the use of mechanisms to ameliorate the salt stress that was overcome by having a combination of improved soil structure and increased nutrient availability (El-Sayed et al., 2021; Francis et al., 2024; Kannan et al., 2025).

The proline amounts significantly increased under salinity stress (82.28 µg/g FW, "a"), followed by BC (41.48, "b"), ZnONP (32.29, "c"), and the control had the least (23.06, "d"). These results demonstrate proline's role as an osmoprotectant under stress conditions (Table 3). The accumulation of proline under salinity stress helped stabilize membranes, remove ROS (reactive oxygen species), and maintain cellular homeostasis (Ali et al., 2019; Shukla et al., 2023). Although ZnONP and BC reduced proline accumulation in comparison to salinity stress, their values were still higher compared to the control, suggesting a mild alleviation of salt-stress responses. Thus, we believe that both treatments helped mitigate the severity of stress which is reflected by the intermediate amount of proline compared to the control.

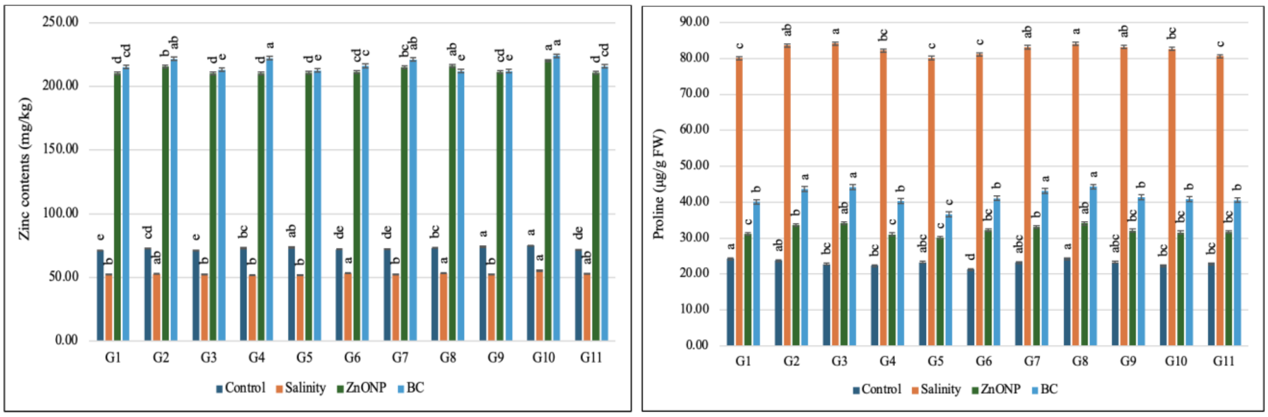

Figure 2. Effect of different treatments (Control, Salinity, ZnONP, and BC) on zinc contents and proline contents across various wheat genotypes (G1-G11). Bars represent means, and different letters indicate significant differences ($p<0.05$).

Glutathione Reductase (GR) Activity

The rate of GR activity significantly increased progressively from control (14.82 EU/mg; SD = 0.33, CV = 2.21%) to salinity (15.83; SD = 0.33, CV = 2.06%), ZnONP (16.70; SD = 0.35, CV = 2.12%), and BC (17.34; SD = 0.33, CV = 1.88%), suggesting enhanced antioxidative defense mechanisms in response to both stress and supplemented treatments (Table 2). GR, which is central to the ascorbate–glutathione cycle, helps maintain redox homeostasis under stress (El-Sayed et al., 2021; García-Gómez et al., 2023). This increase under ZnONP and BC applications, indicates its potential to activate antioxidant machinery and protects the plant from oxidative damage. The greatest GR levels under salinity were recorded for G6 (16.23 U/mg protein), G9 (16.32 U/mg protein), and G3 (16.19 U/mg protein), which suggests that these genotypes possess strong antioxidant enzyme systems (Figure 3). Also, the application of ZnONPs and biochar increased GR in most genotypes, with the largest increase seen for G6 and G9 under each treatment. Prior findings support the enhancement of the antioxidant enzyme activity by ZnONPs, which modulated redox signaling and improved stress tolerance (Lehmann and Joseph, 2015; Nosratabad et al., 2024). Therefore, using ZnONPs and biochar, in addition to G6 and G9 for the tolerant genotypes, could be a good approach to improve wheat adaptation to stress.

Glutathione reductase activity increased steadily in all treatments but was greatest in the biochar treatment (17.34 EU/mg, "a"), followed by the zinc oxide NP treatment (16.7, "ab"), the salinity treatment (15.83, "b"), and the control treatment (14.82, "bc") as indicated in Table 3. The increase in GR activity demonstrates the activation of the antioxidant defense system under stress and can be enhanced by adding to the treatments. Glutathione reductase is an important component of the cellular redox balance that converts oxidized glutathione to its reduced form, which is particularly important when cells are exposed to oxidative stress (Rizwan et al., 2023; Shah et al., 2024; Shukla et al., 2024). The increase in GR further suggests that the ZnONP and BC treatments promote increases in stress tolerance.

Superoxide Dismutase (SOD) Activity

The highest SOD activity was obtained from the ZnONP (62.98 EU/mg; SD = 1.63, CV = 2.59%) followed by the salinity (60.93; SD = 1.62, CV = 2.66%) treatments, indicating that the plant is actively detoxifying reactive oxygen species (ROS) as a response to oxidative stress (Table 2). Conversely, the control treatments had significantly lower SOD activity (42.48; SD = 1.43, CV = 3.36%), but SOD activity increased to moderate amounts in the BC treatments (55.40; SD = 1.66, CV = 3.00%). These results denote that oxidative stress occurred in the salinity treatment and that ZnONP and BC enhanced SOD activity as part of the plant's defensive responses to stress. SOD converts superoxide radicals to hydrogen peroxide, which is the first step of the antioxidative pathway. Nosratabad et al. (2024) and Kannan et al. (2025) support these results by highlighting that Zn acts as a cofactor for SOD enzyme synthesis and activity. Furthermore, BC has also been shown to enhance activities of antioxidative enzymes in some plants exposed to abiotic stress (El-Sayed et al., 2021; Ullah et al., 2024). Thus, the improvement of SOD activity in both the ZnONP and BC treatments suggests that the enzymes improved the mechanisms of stress tolerance as an adaptive response by reducing oxidative damage.

All the genotypes displayed higher SOD activity than control under salinity stress with the highest SOD activity recorded in G10 (65.17 U/mg protein), G9 (62.13), and G5 (61.61), illustrating their effectiveness in scavenging reactive oxygen species (ROS) produced in saline environments (Figure 3). This elevation in activities is consistent with previous studies that indicate SOD is the first line of defense against oxidative stress by detoxifying the superoxide radical to hydrogen peroxide and oxygen (Abel et al., 2013; Ali et al., 2019). Furthermore, the ZnO nanoparticles (ZnONPs) and biochar (BC) significantly stimulated the SOD level across all genotypes with G10 surprisingly exhibiting 67.19 and 59.69 U/mg protein, respectively, followed by G9 and G5 to receive the elevation in SOD activity. These findings are supported by the study from (Lehmann and Joseph, 2015; Saraugi and Routray, 2024; Shah et al., 2024), which indicated ZnONP application enhances antioxidant enzyme systems by serving a catalytic and structural role to proteins in the enzymatic reaction.

ZnONP significantly increased SOD activity (62.98 EU/mg, "a") along with salinity (60.93, "ab"), followed by BC (55.4, "b") and control (42.48, "c") showing the lowest SOD activity (Table 3). The high SOD activity significantly indicated that ROS detoxification is imperative under abiotic stress. SOD catalyzes the reduction of superoxide radicals to hydrogen peroxide, which constitutes the first line of defense against oxidative damage. ZnONP showing the maximum activity relates to (Pratiwi et al., 2022; Nosratabad et al., 2024) findings that zinc acts to increase SOD synthesis. Additionally, studies have shown BC enhances antioxidant responses by improving nutrient uptake to promote growth and decrease oxidative stress.

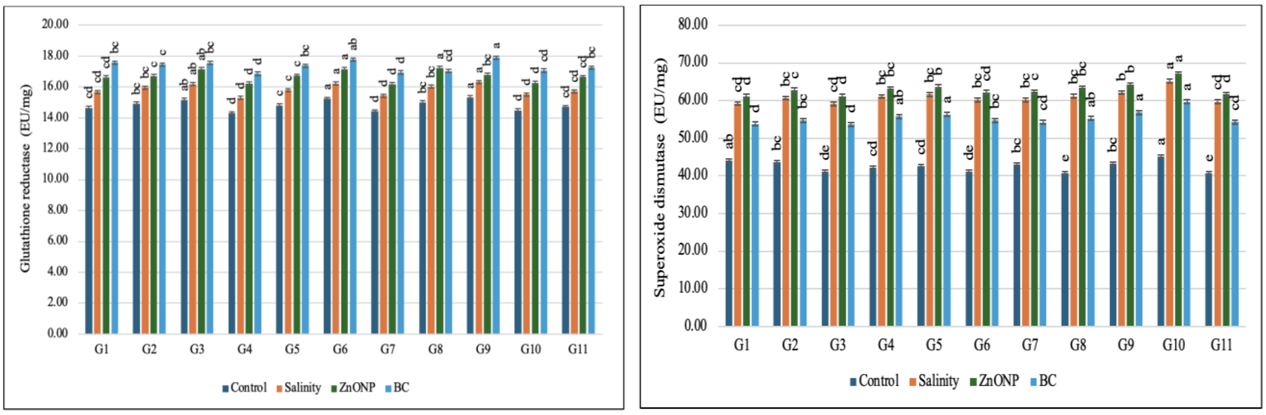

Figure 3. Effect of different treatments (Control, Salinity, ZnONP, and BC) on glutathione reductase and superoxide dismutase across various wheat genotypes (G1-G11). Bars represent means, and different letters indicate significant differences ($p < 0.05$).

CONCLUSIONS

This research unequivocally showed the significant negative effects of salinity stress on wheat growth and yield, as reflected in changes in critical traits and biochemical defense pathways. Salinity reduced thousand grain weight (TGW) by 24.5% (from 24.49 g to 18.51 g), and grain yield per plant (GYP) by 25.7% (from 34.96 g to 26.02 g) relative to control. At the same time, salinity raised proline concentration by a staggering 256.8% (from 23.06 μg/g FW to 82.28 μg/g FW), confirming osmotic and oxidative stress experienced by the plants. In addition, the applied treatments enhanced grain zinc contents significantly; compared to salinity-stressed plants. Thus, ZnONP and BC treatments produced average grain zinc enhanced by 303% and 310% respectively, indicating their capacity for biofortification. The increases in antioxidant enzymes GR (17.34 EU/mg with BC treatment) and SOD (62.98 EU/mg with ZnONP treatment) confirm that these ameliorants stimulate sturdy antioxidative defense mechanisms. Responses among the 11 wheat genotypes to the combination of salt stress and ZnONPs + biochar application differ and highlight the potentially positive role of genetic diversity in the adaptation to stress, with genotypes G10 and G9 outperforming the others ones genotypes in both instances. Moving forward, this research supports the co-application of ZnONPs + biochar with the selection of salt-resistant genotypes as a cost-effective, sustainable and productive approach to improving wheat yield and profitability in salt-affected agro-ecosystems.

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