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Nutrient-coated urea mitigates deleterious impacts of salinity and supports wheat performance by enhancing antioxidant activities, photosynthetic performance and nitrogen use efficiency

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Highlights

- Coated urea alleviated the toxic effect of salinity stress on wheat.
- Coated urea improved leaf water status, and photosynthetic pigments under saline conditions.
- Coated urea improved antioxidant activity and osmolytes in response to salinity.
- Coated urea restricted the uptake of toxic ions including Na and Cl.

Abstract

Soil salinization has increased over recent years and is negatively affecting crop productivity. Nutrient application is an effective strategy to improve abiotic stress tolerance in crops. The application of coated fertilizers has emerged as an excellent approach to mitigate the adverse impacts of soil salinity. Therefore, the present study was conducted to determine the effects of zinc and sulfur coated urea on the performance of wheat growing under saline conditions. The study comprised of diverse salinity stress levels; 0, 6 and 12 dS m^{-1}, cross combined with normal urea (NU), zinc coated urea (ZCU) and sulfur coated urea (SCU). Salinity stress reduced wheat yield by impairing leaf water status, reducing photosynthetic pigments, osmolytes accumulation, potassium (K) and nitrogen (N) uptake while increasing sodium (Na) and chloride (Cl) uptake and hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and electrolyte leakage (EL) accumulation. The application of ZCU increased the wheat yield by enhancing photosynthetic pigments, leaf water status, antioxidant activities, osmolytes accumulation, and reducing H_2O_2, MDA and EL accumulation. Furthermore, the significant increase in growth and yield of wheat with ZCU and SCU was also linked with improved K and N uptake, higher nitrogen use efficiency (NUE) and reduced Na and Cl concentration. Thus, the application of ZCU could be an effective approach to improve wheat productivity under saline conditions.

Introduction

Soil salinization is a serious abiotic stress and socio-economic threat across the globe (Hassani *et al.,* 2021). It is one of the most significant abiotic limitations to agricultural production which is drastically reducing the productivity of agronomic and horticultural crops (Sangiorgio *et al.,* 2023, Dustgeer *et al.,* 2021). Globally, 6% of land area is salt affected which accounts for 20% of the total cultivated area (Bhattarai *et al.,* 2020, Qin *et al.,* 2020, Zhao *et al.,* 2021a). The extent of salinity stress (SS) is increasing due to rapid climate change, the use of salty water in irrigation, poor drainage and intensive agriculture practices (Wu *et al.,* 2023). Salt stress negatively affects plant growth and productivity by impairing plant physiological, molecular and biochemical functioning (Dewi *et al.,* 2023). Firstly, SS limits water absorption through the creation of negative water potential which limits

seed germination; thereby, reducing seedling growth and development (Ahmad *et al*., 2023). Secondly, toxic ions (Na and Cl) enter into the transpiration stream and damages plant cellular structures therefore, impairs nutrient homeostasis, physiological functioning, cell division, and subsequent plant growth and development (Hao *et al.,* 2021; Rawat *et al.,* 2021).

Salt stress also decreases chlorophyll synthesis, induces enzyme denaturation, and alters stomata movement by decreasing K^+ uptake. Therefore, it negatively affects photosynthesis and dry matter production (Pan *et al.,* 2021; Lu *et al.,* 2023). Furthermore, SS also reduces tissue relative water contents (RWC) and increases production of reactive oxygen species (ROS) that damage cellular membranes, proteins and lipids (Kesawat *et al.,* 2023). Salt stress also affects the availability of both nitrogen and other nutrients, resulting in significant reductions in plant growth (Aouz *et al.,* 2023, Haj-Amor *et al.,* 2022). Salt stress also influences nutrient homeostasis and negatively affects plant reproductive growth and final yield (Acosta-Motos *et al.,* 2018). Plants have excellent enzymatic and non-enzymatic antioxidant defense system to quench or scavenge the ROS to mitigate the adverse impacts of salinity (Balasubramaniam *et al.,* 2023). Hydrogen peroxides and their derivatives produced under saline conditions are broken down by ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) leading to improve plant performance (Azeem et al., 2023). Globally, different strategies are used to mitigate the adverse impacts of SS on crops. The application of nutrients (zinc, nitrogen, potassium and sulfur) has emerged as a promising strategy to improve crop production under stress conditions.

Nitrogen (N) is an important nutrient for crops and its deficiency is generally increasing due to intensive agricultural practices (Govindasamy *et al.,* 2023). The use of N not only improves crop productivity, but also enhances plant tolerance against stress conditions (Zörb *et al.,* 2018). Nitrogen is an essential nutrient that plays an important role in chlorophyll, protein and amino acid synthesis (Agami *et al.,* 2018). However, the excessive use of N causes environmental and water pollution and heavy economic losses (Altaf *et al.,* 2021). Urea is an important N source used across the globe to meet crop N requirements; however, a large proportion of applied urea may be lost as a result of volatilization and leaching losses (Zörb *et al.,* 2018). Thus, to reduce N losses under stress conditions and improve crop productivity, it is suggested to consider four different principles in fertilizer application including, right time, amount, source, and place of N application (Ghafoor *et al.,* 2021). In this context, the application of slow-release N fertilizers (SRNF) is an effective practice to improve crop productivity, abiotic stress tolerance and decrease environment and water pollution (Zörb *et al.,* 2018; Gautam *et al.,* 2022).).

Slow release nitrogen fertilizers contain a semi-permeable layer of different oils and nutrients that control the water solubility of fertilizers by slowing hydrolysis processes. Zinc (Zn) and sulfur (S)

coated urea are the most important SRNF that promote plant growth and productivity and reduce environmental losses (Eghbali-Babadi *et al.,* 2019). Zinc is an important micro-nutrient that improves enzymatic activities, protein synthesis, and resistance against abiotic stresses (Altaf *et al.,* 2021).
The application of coated urea reduces leaching losses and substantially improves crop yield under abiotic stresses (Gooding *et al.,* 2002). For instance, the application of coated urea augmented root development, shoot growth, physiological functioning, nitrogen use efficiency (NUE) and productivity of wheat under saline conditions (Nazir *et al.,* 2021) Slow release nitrogen fertilizers contain a layer of nutrients that ensure N availability over a long time, therefore, improving NUE and salinity tolerance (Sikora *et al.,* 2020; Waqar *et al.,* 2022). Furthermore, coated urea also alleviates the toxic effects of salinity by increasing photosynthetic activity, stomata opening, chlorophyll synthesis, and activity of antioxidants including APX, CAT, glutathione peroxidase (GPX), glutathione S-transferase (GST), glutathione reductase (GR), and SOD (Ain *et al.,* 2020). In the literature, limited studies are available about the effect of coated urea on growth, yield, physiological and biochemical activities of wheat growing under saline conditions. Therefore, the present study was conducted to determine the impacts of sulfur and Zn coated urea on growth, yield, nutrient homeostasis, antioxidant activities and NUE of wheat crop growing under saline conditions.

Materials and Methods

Experimental details

The present pot experiment was conducted to determine the impacts of sulfur and zinc coated urea on growth, physiological traits, and NUE of wheat under saline conditions. The wheat variety "Akbar 2019" (salt sensitive) was collected from Ayub Agriculture Research Institute (AARI), Faisalabad. The soil for filling of pots was taken from Agronomic Research area. The collected soil was thoroughly mixed and all the debris removed. Thereafter, pots having a capacity of 5 kg were filled with soil and silt (3:1). The collected soil was a clay loam with pH 7.78, organic matter 8.24 g kg^{-1}, electrical conductivity 0.98 dS m^{-1}, total N 0.035 g kg^{-1}, and available phosphorus 9.4 mg kg^{-1} and available potassium 178 mg kg^{-1} respectively. Twelve wheat seeds were sown in each pot during the last week of November 2021. Urea (46% N) was coated with zinc and sulfur and was applied at a rate of 625 g per pot (=287.5 mg N) in three doses: first at sowing, second at tillering and third at the flag leaf stages.

Experimental treatments

The study consisted of three different SS levels: control, 6 dS m^{-1} and 12 dS m^{-1}; and different urea types normal urea (NU), zinc coated urea (ZCU) and sulfur coated urea (SCU). The study was

performed in a completely randomized design (CRD) with three replicates with a factorial combination of SS levels and urea types. The concentration of NaCl salt for each treatment was calculated using the equation below

$$Salt\ req.\left(\frac{g}{kg}\right) =, Tss\ x\ mol.weight\ x, saturation\ (\%)\ /\ 100\ x, 1000$$

NaCl salt was added at rates of 1.179 and 2.58 g/kg of soil to achieve 6 and 12 dS m^{-1} levels. Moreover, a soil sample was taken and soil paste was made by adding distilled water. Then this paste was allowed to reach equilibrium and filtrate was obtained with filter paper. Thereafter, it was oven dried (105°C), and soil saturation was determined with the equation given below.

$$Saturation\ (\%) = \frac{loss\ in\ soil\ weight\ on\ drying}{weight\ of\ soil\ after\ drying} \times, 100$$

Determination of growth traits

Plant samples were taken to determine the various growth traits at the flag leaf stage. Three plants were selected and the roots were separated from shoots. Then the root and shoots were weighed to determine fresh weight, and then oven dried (65 °C) for 24 hours and weighed to determine dry weights. The number of leaves from three random plants from each pot were counted and their average calculated.

Determination of physiological traits

Portions of fresh leaves taken from multiple leaves were placed together to make up 1 g and soaked in distilled water for 24 hours, after that leaves were removed from the water and weighed to record their turgid weight. Then leaves were packed in paper bags and oven dried (70 °C). Therelative water content (RWC) was determined with the following formula: RWC = (FW-DW) / (TW-DW) × 100 suggested by Diaz-Pérez et al. (1995). To determine of leaf electrolyte leakage (EL); 0.5 g of plant leaf samples were cut into pieces and placed in distilled water for 30 minutes and EC_1 was taken. These samples were then heated in a water bath (90°C) for 50 minutes and the second EC_2 was taken. Finally, electrolyte leakage (EL) was determined with the following equation: EL% = (EC_1 / EC_2) × 100. The leaf concentration of chlorophyll and carotenoid was determined by the method of Lichtenthaler *et al.* (1987). In 80% methanol solution; 0.5 g of leaf samples were homogenized by mortar and pestle and the extract was obtained. The extract was then centrifuged and filtrate was obtained. Later, absorbance was noted at 663, 645 and 480 nm wavelengths by using spectrophotometer (Hitachi U-2001, Tokyo, Japan) to determine chlorophyll and carotenoid concentration.

Determination of oxidative stress markers and antioxidant activities

To determine total soluble protein (TSP); leaf samples were ground in 5 ml of phosphate buffer, and then centrifuged for 15 minutes. The samples were then treated with Bradford regent for 15-20 minutes for the chemical reaction, and absorbance was noted at 595 nm to determine TSP (Bradford, 1976). In the case of free amino acids (FAA), 0.5 g of plant samples were ground in 5 ml phosphate buffer and centrifuged for 15-20 minutes. Then 1 ml extract was taken and mixed with 1 ml pyridine and 1 ml ninhydrin. Then these test tubes were placed for 30 minutes in a water bath (90 °C) and the volume of the mixture was increased to 25 ml and the concentration of FAA was determined at 570 nm (Hamilton and Van Slyke, 1943). To determine hydrogen peroxide (H_2O_2) concentration, 0.5 g leaf samples was ground in 5ml of trichloroacetic acid (TCA) and centrifuged. Afterward, 1 ml extract was placed in tubes containing 1 M potassium iodide (166 mg) and 100 µL potassium phosphate buffer (PPB), and absorbance was noted at 390 nm using the same spectrophotometer. To determine leaf malondialdehyde (MDA) concentration, 0.5 g of frozen plant samples (0.5 g) were ground in TCA solution (5 ml) using mortar and pestle and centrifuged for 15 minutes. The mixture then quickly heated (100 °C) for 30 minutes and cooled rapidly (4 °C). Then absorbance was noted at 532 nm to determine the MDA concentration Rao and Sresty, 2000). For CAT, leaf samples were grind in 2.5 ml of 50 mM K-buffer. After that, supernatant was centrifuged for 15 min at 4 °C and supernatant was collected, then 0.1 ml extract was added to 0.1 ml of H_2O_2 (5.9 mM) and 2.5 ml of 5 % TCA buffer, and absorbance was noted at 240 to determine CAT activity by using spectrophotometer (Aebi, 1984). To assess POD activity; 0.5 g of leaf sample was homogenized in 5 ml of PPB (pH 7.8) and centrifuged (10000 rpm) for 15 minutes and supernatant was taken. Then absorbance was noted at 470 nm with spectrophotometer to determine POD activity (Zhang, 1992). For APX activity, 0.5 g of leaf sample was homogenized in 5 ml of PPB (pH 7.8). Then the extract was centrifuged for 15 minutes, and the supernatant was obtained to measure APX activity by using spectrophotometer (Nakano and Asada, 1981). To determine anthocyanin concentration, 0.5 g of fresh leaves was homogenized in 5ml PPB using a pestle and mortar. The extract was taken and centrifuged for 15 minutes, and absorbance was noted at 535 nm with a spectrophotometer.

Element concentrations

To determine elemental concentration, different plant organs were oven dried (70 °C) and then ground to make powder. After that, 0.5 g of the powdered samples were digested with two acids (HCl and HNO_3: 1:2) using a hot plate (180 °C: Hsu and Kao, 2003). Then distilled water was added to dilute the extract and Cl- concentration was determined with chloride analyzer (model 926, Sherwood Scientific, Cambridge, UK) and Na^+ and K^+ concentration were determined with a flame photometer

(Jenway PFP-7, Burlington, NJ, USA). The N concentration in digested samples from plant tissues was determined by the Kjeldahl procedure (Sáez-Plaza et al., 2013).

Yield traits, agronomic and nitrogen use efficiencies

To determine different yield traits, three plants were taken at physiological maturity and the tillers, spike length, spikelets, and grains per spike were counted. Then these plants were weighted to determine biomass yield and later spikes were separated and threshed to determine grain yield. The nitrogen use efficiency (NUE) was determined using the method of Rehman *et al.* (2021) using following formula:

$$\boldsymbol{NUE = grain\ yield/N\ accumulation\ in\ plant}.$$

The nitrogen productive efficiency (NPE) was determined by the method of Jadon *et al.* (2018) using the following formula: $\boldsymbol{NPE = grain\ yield\ /amount\ of\ N\ applied}$

Statistical and principal component analysis

The experimental data were analyzed by two-way analysis of variance (ANOVA) for SS, and their interaction, while the least significant difference (LSD) test ($p \leq 0.05$) was used to detect the significant levels among ANOVA sources. Moreover, a principal component analysis (PCA) was performed to explore the potential relationship among the studied traits. In principal component analysis (PCA) different growth, yield, photosynthetic traits, physiological traits, antioxidant activity, osmolytes, elements concentration, and NUE were set as quantitative variables, while coated urea and SS were used as supplementary categorical variables.

Results

Growth and morphological traits

The results indicated that SS, had a significant ($P \leq 0.05$) impact on all growth and morphological traits of wheat (*Supplementary Table 1*). The results indicate that maximum shoot fresh weight (SFW) was observed in the control treatment and the lowest was noted at the highest SS level. Likewise, ZCU also significantly increased the SFW as compared to SCU and control (Table 1). On the other hand, root fresh weight (RFW) also decreased by 22.8% at 6 and dS m^{-1} SS.However, ZCU and SCU appreciably increased the RFW (26.5% and 22.1%) at moderate and higher SS levels (Table 1). Different levels of SS and UT showed a significant ($P \leq 0.05$) impact on the leaves per plant (LPP) of wheat (Table 1). Salinity reduced the production of leaves, however, ZCU and SCU decreased the toxic effect of SS resulting in higher leaf production in ZCU and relative to the control treatment (Table 1). The application of ZCU and SCU increased the LPP by 41.5% and 21.6% under 6 dS m^{-1}

while ZCU and SCU increased the LPP by 57.1% and 29.1% under 12 dS m^{-1} as compared to control (Table 1).

Photosynthetic pigments and relative water contents

Salinity, CU, and interactive effect of SS, and CU showed a significant ($P \leq 0.05$) impact on photosynthetic pigments and leaf RWC (*Supplementary Table 1*). The maximum chlorophyll-a concentration (0.570 mg g^{-1} FW) observed in the control treatment and the minimum chlorophyll-a (0.235 mg g^{-1} FW) was recorded at 12 dS m^{-1} SS (Table 2). The minimum chlorophyll-b (0.033 mg g^{-1} FW) was recorded at 12 dS m^{-1} SS without application of CU and the maximum chlorophyll-b concentration was observed in the control with ZCU application treatment (Table 2).The carotenoid concentration showed a reduction of 54.6% and 67.3% respectively at 6 dS m^{-1} and 12 dS m^{-1} SS levels. However, the application of ZCU and SCU increased the carotenoid concentration by 44.9% and 20.2% respectively under 12 dS m^{-1} SS as compared to control (Table 2). Leaf relative water contents also showed a reduction of 41.1% and 70.4% under 6 dS m^{-1} and 12 dS m^{-1} SS. However, ZCU increased the RWC by 83.4% and 173.9% under 6 and 12 dS m^{-1} while SCU increased the RWC by 35.3% and 97.8% under 6 and 12 dS m^{-1} SS as compared to the control (Table 2).

Oxidative stress markers and potential osmolyte

Different levels of SS, CU and the interactive effect of CU and SS showed a significant ($P \leq 0.05$) impact on oxidative stress and potential osmolytes (*Supplementary Table 2*). Maximum EL (51.6%) was observed under stronger SS, followed by moderate SS (31.9%) without coated urea application (Table 3). Whilst, ZCU reduced EL by 40.5% while SCU decreased EL by 21.8% under 12 dS m^{-1} as compared to normal urea application (Table 3). The concentration of MDA and H_2O_2 was significantly increased under saline conditions (Table 3). The application of ZCU was the top performer in mitigating MDA and H_2O_2 concentration compared to SCU and NU under both moderate and higher SS levels (Table 3). The concentrations of TSP and FAA were significantly decreased under saline conditions. A decrease of 43.6% and 72% in TSP was observed at 6 and 12 dS m^{-1} salinity levels, and was 34.6% and 44.9% lower at 6 and 12 dS m^{-1} SS as compared to control (Table 3). However, ZCU increased TSP and FAA by 48.4% and 33.9% while SCU increased TSP and FAA by 30.7% and 17.4% under both moderate and higher levels of SS compared to the normal urea (Table 3).

Antioxidant activities

Different types of urea, SS and the interactive effects showed a significant ($P \leq 0.05$) impact on the activity of APX, and CAT. However, interactive effect of (SS× CU) showed a non-significant impact on POD activity (*Supplementary Table 2*). The CAT activity was increased by 19.9% and 51.6% at 6 dS m^{-1} and 12 dS m^{-1} SS in comparison to the control treatment. Furthermore, ZCU increased CAT activity by 41.9% and SCU increased CAT by 19.9% under 12 dS m^{-1} SS as compared to normal urea (Figure 1). Zinc coated urea also increased APX activities by 46.5% under 12 dS m^{-1} SS while SCU increased APX activity by 20.8% compared to NU (Figure 1). The overall trend of the different types of urea in increasing antioxidant activities under SS was observed as; ZCU>SCU>NU (Figure 1**).** The concentration of anthocyanin was also reduced by 31.9% and 58.3% at medium and higher SS. Application of ZCU and SCU significantly increased anthocyanin concentration by 34.1% and 15.4% under moderate and higher SS levels as compared to NU (Figure 1).

Yield traits

The application of coated urea SS and their interactive effect showed a significant ($P \leq 0.05$) impact on the yield traits of wheat crop with the exception of biological yield per plant (BYPP: *Supplementary Table 3*). Salinity stress curbed the yield and yield traits of wheat (Table 4), however, coated urea mitigated the negative effects of SS and improved the yield, and yield traits of wheat with the following ranking ZCU>SCU>NU (Table 4). Zinc coated urea increased spike length (SL), tillers per plant (TPP) and spikelet's per spike (SLPS) by 44.2%, 43.2% and 41.2% under 12 dS m^{-1} SS compared to NU. On the other hand, SCU increased SL, TPP, and SLPS by 21.4%, 20.7% and 21.5% as compared to NU (Table 4). Furthermore, ZCU also appreciably increased 100-grain weight (GW), and grain yield per plant (GYPP) by 30.4%, and 31.9% while SCU increased 100-GW, and GYPP by 15.1%, and 15.5% at higher salinity stress level compared to NU (Table 4).

Element concentration in plant parts

Salinity stress, different coated urea and their interactive effect determined a significant positive ($P \leq 0.05$) impact on the concentration of different elements in plant parts (*Supplementary Table 4*). Salinity stress resulted in a sizeable increase in Na and chloride Cl concentration in wheat plant parts and the maximum amount of these toxic ions was observed under the highest salinity level (Table 5). On the other hand, a substantial reduction in K was observed in plant parts at higher SS (Table 5). Similarly, a significant reduction in N concentration was also observed under salinity conditions (Table 5). Moreover, application of coated urea particularly ZCU curbed the uptake of toxic ions (Na and Cl) and substantially increased the uptake and concentration K and N in plant parts (Table 5).

Nitrogen use efficiency

Salinity stress, coated urea and their interactive effect showed a significant ($P \leq 0.05$) impact on and nitrogen use efficiency (NUE) and nitrogen productive efficiency (NPE: *Supplementary Table 3*). Salinity stress reduced the NUE and NPE in wheat. However, application of CU increased both NUE and NPE (Figure 2). Minimum NUE and NPE were observed under higher saline stress (12 dS m^{-1}) than moderate SS (6 dS m^{-1}) and normal conditions (Figure 2). The maximum NUE and NPE was recorded with application of ZCU as compared to other treatments under both salinity levels (Figure 2).

Principal component analysis

The collected data set was subjected to PCA to determine the relationships between different treatments and studied traits. The results indicate that two components (PC1 and PC2) showed 97.4% of the total variance in which PC1 had a contribution of 87.7%, and PC2 had a share of 9.7% (Figure 3). The results of the PCA indicated that the studied traits were distributed in the dataset indicating clearly that application of SS and CU had a significant impact on growth, yield, physiological traits, photosynthetic pigments, antioxidant activities, AUE, and NUE of wheat. The results indicate that chlorophyll contents, RWC, root and shoot growth, yield traits, K, N, AUE and NUE were grouped in PCA1 and they showed a significant negative relationship with SS. However, some variables like antioxidant activities, MDA, H_2O_2, EL, Na and Cl accumulation were grouped into PCA 2 and they had a positive relationship with SS (Figure 3). Of the two SS levels, the higher level of SS showed more negative effects on growth, yield, physiological traits, photosynthetic pigments, AUE and NUE of wheat crop. Conversely, ZCU effectively was more effective in decreasing the toxic effects of salinity growth, yield, physiological traits, photosynthetic pigments, AUE and NUE of wheat compared to SCU and NU (Figure 3).

Discussion

It has been reported that soil salinity negatively affects wheat growth and productivity. In the present study, SS curbed the growth and yield of wheat (Table 1 and 5) due to impaired physiological functions, increased oxidative stress markers, disturbed nutrient homeostasis, and reduced photosynthetic pigments (Guo *et al.,* 2019, Yang *et al.,* 2020, Badawy *et al.,* 2021; Homayouni *et al.,* 2024; Stefanov *et al.,* 2024). Salinity stress also increases ROS production which damages DNA, protein and membranes. Furthermore, SS also negatively affects cell division, and cell elongation resulting in a reduction in plant growth (Dabravolski and Isayenkov, 2024; El-maghraby *et al.,* 2024). In the present study K and N uptake was also inhibited under SS (Table 1) which might have disturbed

stomatal opening and impaired the photosynthesis (Kumar *et al.,* 2022), thus led to a significant decrease in wheat growth and yield (Fu *et al.,* 2023). The application of coated urea offset the negative impacts of SS and significantly improved wheat growth and yield (Table 1 and 5). Zinc and sulfur coated urea favored the uptake of N and K and maintained nutrient availability for a longer time thanks to slow N release, resulting in better wheat growth and yield under normal and saline conditions (Yaseen *et al.,* 2017). Coated urea was associated with higher N in plant parts under both normal and saline conditions and the same pattern was observed for K. Therefore, an increase in N uptake favoured K uptake which improved wheat growth (Yaseen *et al.,* 2017; Xu *et al.,* 2020). Furthermore, coated urea application also offset the negative impacts of salinity by increasing photosynthetic pigments, osmolytes accumulation and antioxidant activity which ensured better wheat growth and yield (Ahanger *et al.,* 2019, Giambalvo *et al.,* 2022).

In the present study, photosynthetic pigments were significantly decreased under SS which is in line with the findings of different authors (Farag *et al.,* 2022; Lungoci *et al.,* 2022).). Salinity stress increases the activity of chlorophyll degrading enzymes (chlorophyllase) which decreases synthesis of chlorophyll (Taiz *et al.,* 2015). Furthermore, salinity stress also decreases Mg uptake which also leads to a reduction in chlorophyll content owing to the fact Mg is a building block of chlorophyll synthesis in plants (Lacerda *et al.,* 2020, Metwally *et al.,* 2021, Zhoa *et al.,* 2021b, Elkarmout *et al.,* 2022). Coated urea ensures prolonged availability of nitrogen which maintains better chlorophyll synthesis under stress conditions (Docimo *et al.,* 2020, Wang *et al.,* 2021). Besides this, Zn present on the urea surface also supports the plants by increasing enzymatic activities, protein synthesis and protecting the photosynthetic apparatus which could be an indirect cause of the increase in chlorophyll synthesis under saline soil.

In the present study, we have noted that coated urea application, particularly ZCU, improved RWC. Salinity induced osmotic stress and reduced water uptake which in turn decreased the RWC (Table 2) (Kim *et al.,* 2022, Fatma *et al.,* 2021). Coated urea improved the leaf water status under saline conditions due to improved nutrient uptake, and better root growth, which in turn improved water uptake and leaf hydration (Mumtaz *et al.,* 2018; Docimo *et al.,* 2020). In the present study, EL was significantly increased owing to a substantial increase in two oxidative stress markers (MDA and H_2O_2; Farag *et al.,* 2022. However, the application of coated urea reduced the MDA and H_2O_2 by increasing antioxidant activities (Figure 1) and osmolyte accumulation (Table 3). Malondialdehyde is a product of lipid peroxidation and a reduction in MDA is consistent with improved membrane stability and reduced EL under saline conditions (Table 3: Mumtaz *et al.,* 2018; Cordiano et al., 2023). Saline stress exerted a decrease in FAA and TSP (Table 3), however, coated urea, particularly ZCU, increased TSP and FAA under saline conditions. The decrease in compounds such as TSP and FAA

under salinity stress was directly linked to insufficient N uptake. Free amino acids create a gradient of osmotic potential that facilitates inward water movement to prevent SS effects, while TSP plays a key role in protecting the enzymes thus ensuring better plant growth under saline conditions (El-Saidi 1997; Feng *et al.,* 2023). The study findings indicate that the activities of all the antioxidants were significantly increased under SS which were further increased by the application of coated urea (Figure 1). Antioxidant enzymes play an important role in ROS scavenging, and are essential to mitigate abiotic stresses (Zand and Schnug, 2022; Mansoor *et al.,* 2023). The application of N effectively inhibited the H_2O_2 accumulation by strengthening antioxidant activities (SOD, CAT, and POD), and increasing concentration of FAA and TSP (Borella *et al.,* 2019, Sikder *et al.,* 2020).

Salinity stress significantly increased the accumulation of toxic ions (Na and Cl) and reduced the accumulation of both K and N in wheat plants (Table 5). The increased concentration of toxic ions (Na and Cl) is responsible for disturbed ionic homeostasis and nutrient concentration in plant tissues (Chen *et al.,* 2012, Tarighaleslami *et al.,* 2012). Salinity induces an increase in Na concentration effects on the guard cells of stomata (Munns *et al.,* 2008), and it also decreases the ability of Na/K anti-porters to exclude excessive Na (Munns *et al.,* 2008). As a result salinity leads to a substantial increase in Na accumulation and decrease in K accumulation (Munns *et al.,* 2008). Excessive Na and Cl also reduced N accumulation as Na competes with the cationic form (NH_4^+) and Cl competes with anionic forms (NO_3^-) of N (Carpici *et al.,* 2010, Khan *et al.,* 2023). However, the application of coated urea decreased Na and Cl accumulation and increased K and N accumulation. Zinc coated urea and SCU favored K and N uptake, which is consistent with less damaged to the root system (Table 1) alongside lower release of N, and decreased Na competition at the soil-root interface (Altaf *et al.,* 2021)

Conclusions

Salinity stress reduced the growth and yield of wheat owing to salinity-induced oxidative damage, ionic and osmotic stress. However, application of zinc coated urea, and to a lesser extent sulfur coated urea, significantly increased the growth and yield (~ 40%) of wheat crop under saline conditions. The zinc and sulfur coated urea appreciably increased antioxidant activities, photosynthetic pigments, relative water content, osmolyte accumulation, and nitrogen efficiency which induced a substantial increase in wheat yield under both normal and saline conditions. The experimental results indicate that zinc coated urea is beneficial in improving wheat productivity under saline soils. However, more field studies are needed under saline conditions before making recommendations for farming communities.

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Table 1. Effect of coated urea on growth traits of wheat under saline conditions.

SS	CA	SFW (g)	SDW (g)	RFW (g)	DRW (g)	LPP
Control	NU	5.10±0.073	3.04c±0.093	3.80c±0.042	1.82±0.014	4.58d±0.16
	ZCU	6.98±0.031	4.23a±0.086	4.32a±0.050	2.08±0.016	6.83a±0.083
	SCU	6.32±0.052	3.54b±0.029	4.08b±0.017	1.90±0.029	5.92b±0.088
6 dS m^{-1}	NU	4.49±0.026	2.41d±0.084	3.13e±0.022	1.48±0.032	3.47f±0.074
	ZCU	6.21±0.104	3.54b±0.046	3.52d±0.017	1.67±0.026	4.91c±0.083
	SCU	5.35±0.147	3.083c±0.023	3.23e±0.025	1.52±0.024	4.22e±0.024
12 dS m^{-1}	NU	2.60±0.061	0.95g±0.058	2.72fg±0.042	1.22±0.020	2.23h±0.029
	ZCU	4.07±0.082	1.84e±0.079	2.95f±0.021	1.39±0.018	3.56f±0.080
	SCU	3.37±0.078	1.37f±0.051	2.80f±0.011	1.26±0.016	2.88g±0.011

SS: salinity stress, UA: urea application, NU: normal urea, ZCU: zinc coated urea, SC: sulfur coated urea. The letters with mean values indicating significance at $P \leq 0.05$ with ±SE using two-way ANOVA. SFW and SDW are shoot fresh and dry weights and RFW and RDW are root fresh and dry weights while LPP indicating leaves per plant.

Table 2. Effect of coated urea on photosynthetic pigments and RWC of wheat under saline conditions.

SS	CA	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Carotenoids (mg/g FW)	RWC (%)
Control	NU	0.353d±0.016	0.186d±0.008	1.56c±0.025	57.27c±1.14
	ZCU	0.570a±0.011	0.414a±0.016	2.40a±0.005	93.74a±0.59
	SCU	0.437b±0.009	0.308b±0.002	2.02b±0.016	72.09b±1.19
6 dS m^{-1}	NU	0.300e±0.0018	0.073d±0.005	0.56g±0.037	31.46e±1.24
	ZCU	0.457b±0.005	0.314b±0.004	1.19d±0.029	57.70c±0.57
	SCU	0.387c±0.008	0.228c±0.001	0.971e±0.013	42.56d±0.82
12 dS m^{-1}	NU	0.235f±0.007	0.033g±0.003	0.373h±0.011	11.58g±0.89
	ZCU	0.373cd±0.002	0.135e±0.012	0.955e±0.011	31.72e±0.29
	SCU	0.295e±0.004	0.097f±0.004	0.636g±0.007	22.91f±0.32

SS: salinity stress, UA: urea application, NU: normal urea, ZCU: zinc coated urea, SC: sulfur coated urea. The letters with mean values indicating significance at $P \leq 0.05$ with ±SE using two-way ANOVA. RWC indicating relative water contents.

Table 3. Effect of coated urea on oxidative stress markers and osmolytes concentration of wheat under saline conditions

SS	CA	EL (%)	MDA (µmol g^{-1} FA)	H_2O_2 (µmol g^{-1} FA)	TSP (mg/g FW)	FAA (mg/g FW)
Control	NU	56.8f±1.32	3.23g±0.22	2.59f±0.012	17.25c±0.57	11.95d±0.10
	ZCU	29.45i±1.29	2.89f±0.29	2.32h±0.19	29.00a±0.66	17.90a±0.17
	SCU	41.99h±0.78	3.10g±0.17	2.46g±0.20	21.66b±0.43	15.34b±0.26
6 dS m^{-1}	NU	74.64c±0.82	5.10d±0.32	3.92c±0.15	8.70e±0.20	7.47g±0.42
	ZCU	45.19g±0.13	4.58e±0.40	3.52e±0.10	18.15c±0.28	12.73c±0.72
	SCU	60.75e±0.29	4.98e±0.12	3.72d±0.14	11.50d±0.24	9.40e±0.24
12 dS m^{-1}	NU	88.50a±0.99	6.78a±0.50	4.72a±0.22	3.33g±0.30	7.08g±0.38
	ZCU	78.20d±1.12	5.77b±0.40	4.44b±0.10	9.58e±0.08	9.43e±0.52
	SCU	85.97b±1.19	6.12c±0.20	4.52b±0.17	6.16f±0.43	8.36f±0.58

SS: salinity stress, UA: urea application, NU: normal urea, ZCU: zinc coated urea, SC: sulfur coated urea. The letters with mean values indicating significance at $P \leq 0.05$ with ±SE using two-way ANOVA. EL indicating electrolyte leakage, MDA is malondialdehyde and H_2O_2 is hydrogen peroxide while TSP and FAA are total soluble proteins and free amino acids.

Table 4. Effect of coated urea on yield traits of wheat under saline conditions.

SS	CA	SL (cm)	TPP	SLPS	GPS	100-GW	GYPP (g)	BYPP (g)
Control	NU	8.16c±0.13	4.08d±0.29	25.58c±0.28	44.50c±0.88	4.34d±0.023	5.75d±0.072	17.83d±0.66
	ZCU	11.75a±0.08	7.25a±0.32	36.00a±0.43	58.66a±1.12	5.93a±0.029	8.12a±0.041	25.33a±0.72
	SCU	9.92b±0.17	5.50b±0.43	31.50b±0.78	50.33b±0.89	5.15b±0.13	7.01b±0.144	23.00b±0.60
6 dS m^{-1}	NU	4.35f±0.10	2.66f±0.18	11.66g±0.42	21.66f±0.78	3.38f±0.017	4.26f±0.0188	13.50f±0.36
	ZCU	9.99b±0.09	4.50c±0.23	24.50d±0.98	35.50d±1.2	4.81c±0.05	6.32c±0.017	19.42c±0.42
	SCU	7.76d±0.03	3.58e±0.14	17.66e±0.72	29.16e±0.89	4.10e±0.06	5.33e±0.144	16.25e±0.38
12 dS m^{-1}	NU	3.07g±0.04	1.20h±0.10	8.50h±0.42	6.16i±0.44	1.58i±0.04	2.72h±0.026	7.99h±0.30
	ZCU	6.19e±0.11	2.23g±0.18	15.83f±0.62	19.66g±0.58	2.62g±0.018	4.22f±0.017	13.50f±0.14
	SCU	4.28f±0.15	2.01g±0.22	11.16g±0.70	15.98h±0.82	2.11h±0.049	3.44g±0.020	10.75g±0.20

SS: salinity stress, UA: urea application, NU: normal urea, ZCU: zinc coated urea, SC: sulfur coated urea. The letters with mean values indicating significance at $P \leq 0.05$ with ±SE using two-way ANOVA. SL and TPP are spike length and tillers per plant, SLPS and GPS indicating spikelets/spike and grains/spike while 100 GW, GYPP and BYPP are indicating 100 grains weight, grain and biological yield per plant respectively.

Table 5. Effect of coated urea on yield traits of wheat under saline conditions.

SS	CA	Root Na	Shoot Na	Root K	Shoot K	Root Cl	Shoot Cl	Root N	Shoot N
mg g^{-1} DW									
Control	NU	2.05g±0.12	2.72f±0.012	22.87c±0.54	29.81b±1.22	2.22f±0.14	1.54f±0.087	10.32c±0.22	16.21c±0.45
	ZCU	1.52i±0.022	2.00g±0.042	24.98a±0.29	33.42a±1.50	1.78f±0.032	1.38f±0.050	11.89a±0.52	17.89a±0.75
	SCU	1.78h±0.13	2.23g±0.028	23.89b±0.62	32.33b±1.33	2.00f±0.010	1.42f±0.018	11.20b±042	17.20b±0.50
6 dS m^{-1}	NU	15.16d±0.14	16.5c±0.76	18.4f±0.49	23.55f±1.16	24.45d±0.78	18.52c±1.23	7.88e±0.45	12.20f±0.44
	ZCU	12.14f±0.25	15.10e±0.32	20.2d±0.81	27.70d±1.20	22.20e±0.43	15.89e±0.78	9.20d±0.22	14.56d±0.31
	SCU	14.12e±0.20	15.89d±0.42	19.5e±0.41	25.42e±1.45	23.45d±0.54	17.23d±0.72	8.88d±0.78	13.76e±0.50
12 dS m^{-1}	NU	18.22a±0.18	18.82a±0.50	13.4i±0.40	17.89i±1.40	29.30a±0.87	22.42a±0.99	6.48g±0.42	8.89i±0.62
	ZCU	17.29b±0.22	17.82bc±0.62	16.5f±0.42	19.82g±1.89	26.56c±0.65	18.99c±0.52	7.10f±0.22	11.20g±0.78
	SCU	17.85c±0.29	18.02b±0.42	14.5g±0.52	18.89h±1.52	27.89b±0.50	20.87b±1.20	6.78g±0.29	10.10h±0.62

SS: salinity stress, UA: urea application, NU: normal urea, ZCU: zinc coated urea, SC: sulfur coated urea. The letters with mean values indicating significance at $P \leq 0.05$ with ±SE using two-way ANOVA.

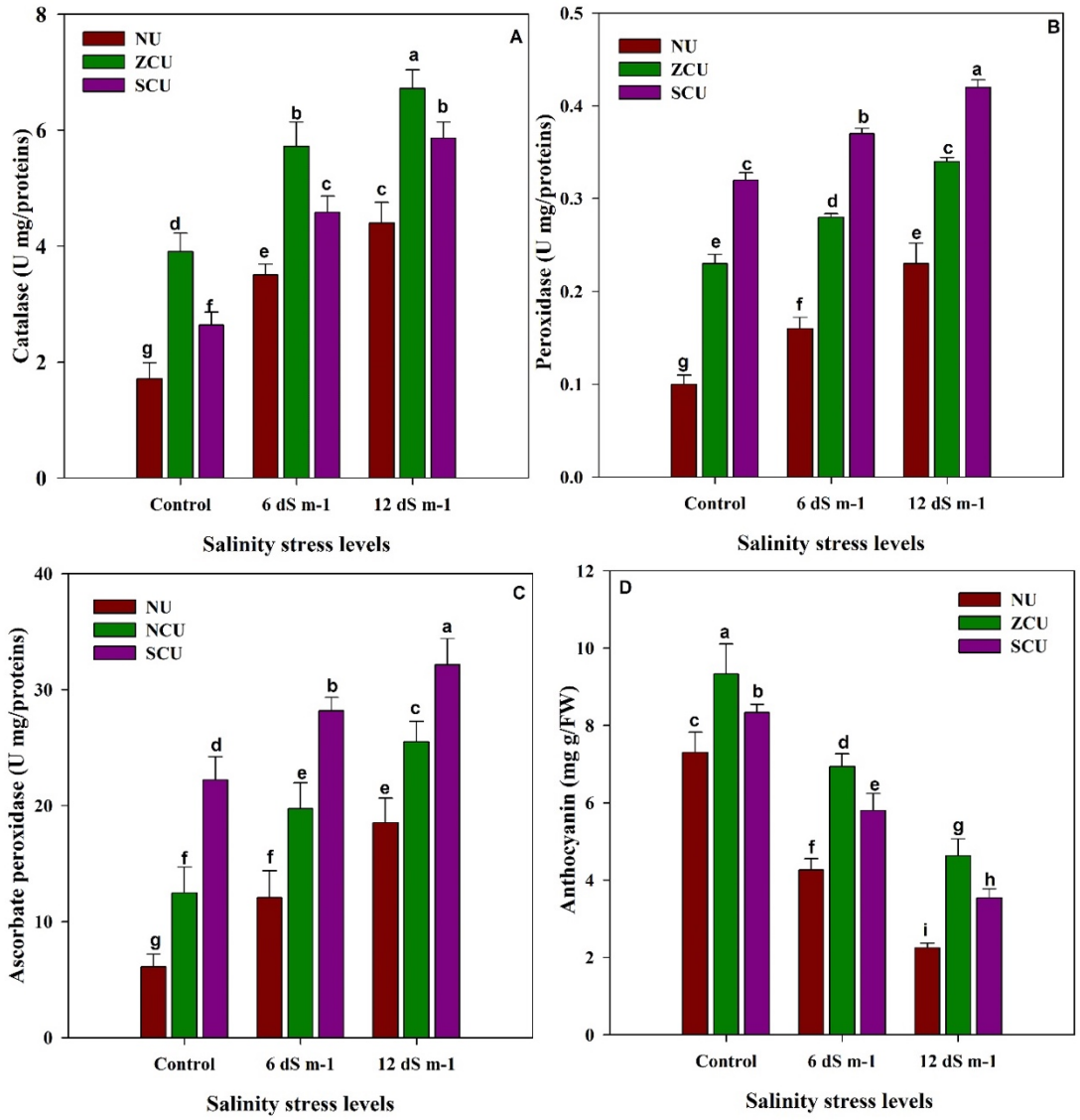

Figure 1. Effect of coated urea on antioxidant activities of wheat under saline conditions. The bars represent the mean values of three replicates and different letters indicate significance (LSD) at P≤0.05 with ±SE.

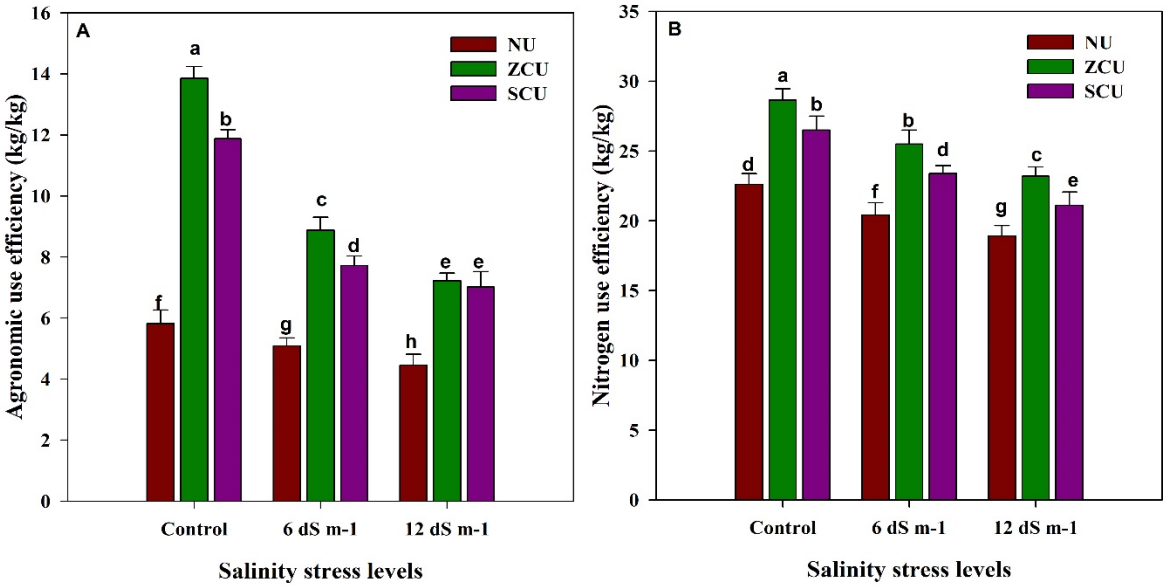

Figure 2. Effect of coated urea on agronomic use efficiency (A) and nitrogen use efficiency (B) of wheat under saline conditions. The bars represent the mean values of three replicates and different letters indicate significance (LSD) at P ≤ 0.05 with ±SE.

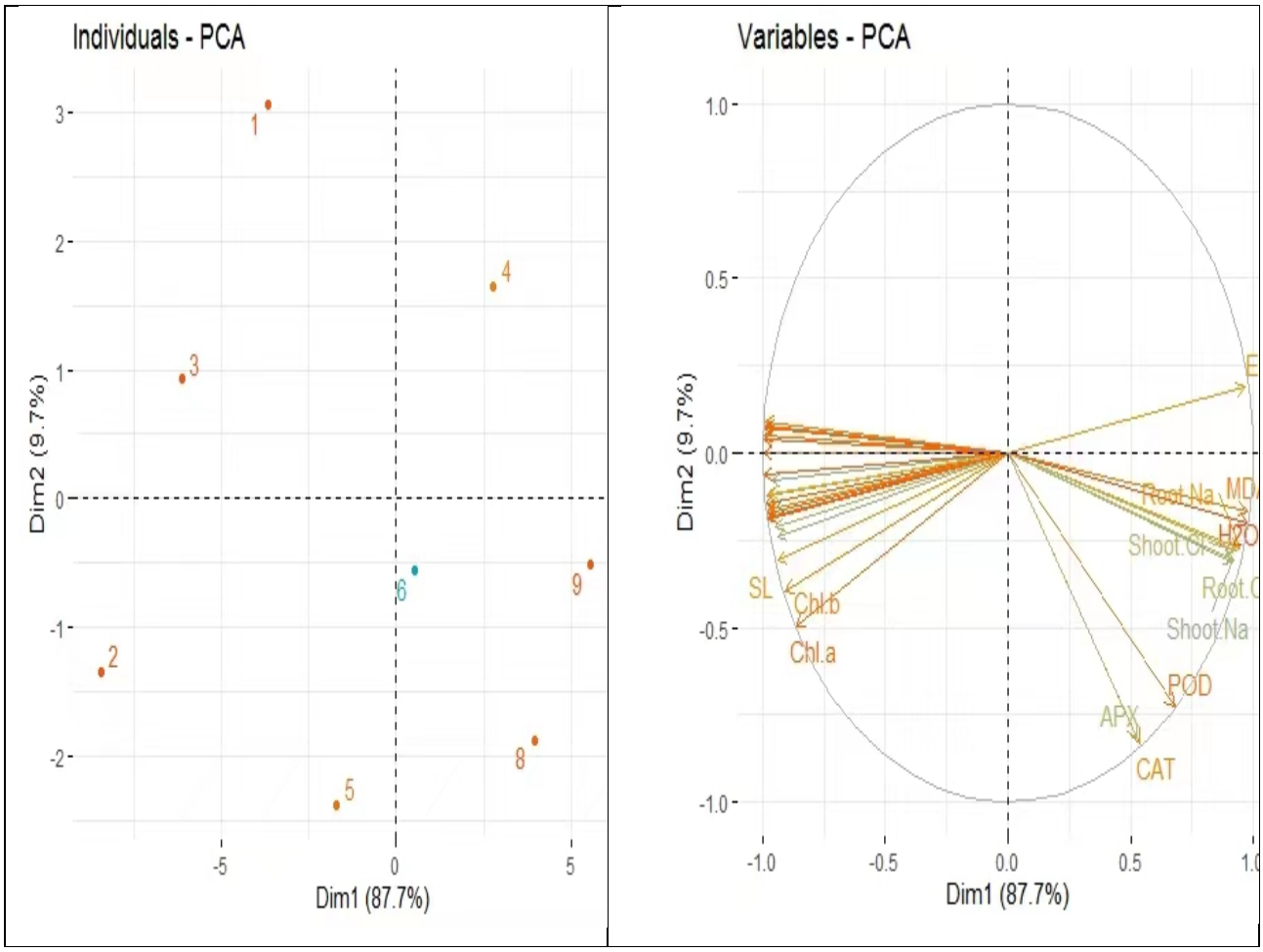

Figure 3. The scores on left and loading plots on right of principal component analysis (PCA) showing the effect of diverse treatments on examined traits. SL: shoot lenght, Chl-a: chlorophyll a, Chl-b: chlorophyll b, EL: electrolyte leakage, MDA: malondialdehyde, H_2O_2: hydrogen peroxide, Na, sodium Cl: chloride, POD: peroxidase, CAT: catalase, APX: ascorbate peroxidase. SFW: shoot fresh, weight, RFW: root fresh weight, LPP: leaves per plant, TSP: total soluble poteins, FAA: free amino acids, K: potassium, TPP: tillers per plant, SLPS: spikelets/spike, GPS: grains/spike, 100 GW: grain weight, GYPP: grain yield per plant, BYPP: biological yield per plant.

Supplementary material online:

Table S1. ANOVA sources, F-values and significance in plant growth, photosynthetic pigments, and leaf water status.

Table S2. ANOVA sources, F-values, and significance in plant oxidative stress markers, osmolytes and antioxidant activities.

Table S3. ANOVA sources, F-values, and significance in yield traits and nitrogen use efficiency.

Table S4. ANOVA sources, F-values, and significance in element (Na, K, Cl and N) concentrations in plant roots and shoots.