




Sulphur coated urea increases drought tolerance in rice by improving photosynthetic efficiency, antioxidant performance, osmolyte synthesis, and nitrogen use efficiency

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ABSTRACT

Drought stress (DS) significantly threatens crop productivity and global food security. The intensity of DS is continuously soaring due to climate change, which is causing a substantial reduction in plant productivity. Coated fertilizers emerged as an excellent strategy to improve crop productivity and reduce the toxic effects of abiotic stresses. Thus, this study determined the role of coated urea fertilizers in mitigating DS in rice (*Oryza sativa* L.) plants. The experiment comprised different DS levels: Control (100% field capacity: FC), 70% FC, and 50% FC; and different types of coated urea: Control, normal urea (NU), Zn-coated urea (ZCU), S-coated urea (SCU). Drought stress decreased grain yield by 43.7%, enhanced electrolyte leakage, hydrogen peroxide and malondialdehyde (+151%, +105% and +217%, respectively), and reduced chlorophyll-*a*, total soluble proteins and free amino acids by -84%, -85% and -69%, respectively. However, SCU application in DS augmented grain yield by ~46%, boosted ascorbic acid activity (+119%) and agronomic N use efficiency (67.12%). Therefore, applying SCU could be an effective strategy for mitigating the adverse impacts of drought and enhancing rice productivity.

Key words: Antioxidant, coated urea, field capacity, nitrogen use efficiency, *Oryza sativa*, yield.

INTRODUCTION

Global climate change is leading to the depletion of natural resources and degradation of agroecosystem and crop productivity. Agriculture is a significant water user. Water deficiency is becoming a severe problem due to the lack of rain and the increasing intensity of frequent droughts (Corral-Bermudez et al., 2019). Drought stress has reduced the recharge of water bodies and aquifers owing to the overexploitation of water resources, leading to the availability of low-quality and quantity water for different purposes (Rasheed et al., 2022). This imbalance is leading to the diminishing of water resources and causing social, economic, and environmental impacts as studies documented that drought stress reduced the crop yield from 30%-90% depending on crop type (Dietz et al., 2021).

Drought stress (DS) negatively affects nutrient uptake by disturbing membrane permeability, active transport, and reducing transpiration rate, leading to a profound decrease in plant growth (Wahab et al., 2022). The severity of DS also depends on the stage of plant growth; for instance, DS decreases germination, seedling

growth, and vigor, which are the most susceptible stages in plant life (Lu et al., 2022). Water deficiency also damages photosynthetic apparatus and causes oxidation of membranes, pigments involved in the photosynthetic process, DNA, lipids, and proteins (Wahab et al., 2022). However, plants use different physiological and molecular strategies to counter the toxicity of DS. For instance, they increase the synthesis of different soluble compounds and also increase antioxidant activities to counter the toxicity of DS (Saha et al., 2022).

Different approaches are used to mitigate adverse impacts of DS to ensure sustainable crop production. Among these strategies, the nutrient application is tailored to improve plant growth under stressful conditions. Nitrogen (N) is an essential nutrient used globally to improve crop production (Ma et al., 2022). The application of N increases leaf area, photosynthetic efficiency, crop growth, productivity, and N accumulation. The optimized N application increases crop yield, water use efficiency (WUE), and crop productivity, while excessive N decreases crop productivity and both water and N use efficiencies (Du et al., 2021). Besides this, N application also modulates the toxicity of abiotic stresses in plants. For instance, Agami et al. (2018) noted N reduced the severity of DS in wheat by maintaining plant metabolic activities while N application promoted growth and improved the N utilization efficiency and mitigated toxic impacts of drought, improving photosynthesis and mitigating stress-induced oxidative damage.

Urea is an important source of N fertilizers, and most N applied through urea is lost to the environment. This reduces N use efficiency (NUE) and negatively impacts the climate, soil, and water quality (Mustafa et al., 2022). This calls for developing appropriate measures to improve NUE and crop production while reducing N losses. Therefore, coated fertilizers are an excellent approach to enhance plant growth stress tolerance and reduce environmental consequences (Aslam et al., 2026). Coated urea fertilizers can reduce N losses and improve NUE efficiency, crop productivity, and stress tolerance (Dimkpa et al., 2022). The coated urea improves NUE and N recovery, which are reasons for better plant growth under stress conditions (Mustafa et al., 2022).

Rice (*Oryza sativa* L.) is a staple food for > 50% of the world's population, and there is a dire need to increase its yield to meet rising food needs (Niu et al., 2022). However, abiotic stresses are a severe threat to rice productivity. Therefore, it is essential to develop critical measures to ensure better rice productivity. The coated urea fertilizers showed appreciable results against different stress conditions (Altaf et al., 2021). Despite these beneficial impacts, no study is available in the literature regarding the role of coated urea fertilizers in mitigating the toxicity of DS. Therefore, this study was done to fill the knowledge gap concerning applying coated N fertilizers on the rice facing the DS. The objectives of the current study were to investigate the effect of Zn- and S-coated urea fertilizers in modulating plant growth, physiological and biochemical activities, yield, and NUE under DS.

MATERIALS AND METHODS

Experiment site

The pot study was performed at the University of Agriculture, Faisalabad, Pakistan, during the summer of 2021. The rice (*Oryza sativa* L.) 'Pak Basmati 1121' (*indica*) was used for the experiment. Plastic pots with a capacity of 10 kg were filled with 9 kg soil, and 30-d-old 3 to 4 seedlings were transplanted into each pot.

Experimental treatments

The experiment was performed in completely randomized design (CRD) with a two-factor factorial along with three replicates. The experiment comprised different drought stress (DS) levels: Control (100% field capacity: FC), 70% FC, and 50% FC, and different types of coated urea: Control, normal urea (NU), Zn-coated urea (ZCU), S-coated urea (SCU). To maintain various levels of FC; the soil-filled pot was weighed initially, then covered with water and left in that state for 48 h to allow the gravimetric water to drain. The pots were reweighed, and the moisture content at FC estimated as variance between the initial (before saturation) and final (48 h after saturation) weights. Throughout the experiment, pots were weighed daily, and water was provided to each pot by the quantity necessary to maintain the required water levels (100%, 70%, and 50% FC). All doses of single super phosphate (21% P) and potassium sulfate (50% K) were applied at the rate of 0.483 and 2 g K₂O pot⁻¹, respectively as basal dose. The dose of normal urea and coated urea (S and Zn coated urea) fertilizers was 0.1222 g urea pot⁻¹ which was applied in three equal splits at basal, tillering, and panicle initiation stage.

Physiological parameters

The samples for physiological and biochemical parameters were taken at the flag leaf emergence stage. The rice leaf samples (0.5 g) were homogenized using 80% methanol and centrifuged, and absorbance was read at 663, 645, and 480 nm to determine the chlorophyll *a*, *b*, and carotenoids contents (Lichtenthaler, 1987). For relative water content (RWC), leaves were collected and weighed (FW), and after that, they were placed in water for 24 h. Then, leaves were collected and weighed (TW), after that the leaf sample was dried in an oven at 65 °C and weighed again (DW). Finally, RWC was calculated using the following formula: $RWC (\%) = (FW - DW) / (TW - DW) \times 100$. In case of electrolyte leakage (EL), fresh leaf samples were harvested and washed with distilled water to avoid contamination. Leaf samples were incubated at 25 °C on a rotary shaker. Initially, electrical conductivity (EC_1) was recorded after 24 h. Then, samples were autoclaved at 120 °C for 20 min, and the final EC (EC_2) was recorded upon equilibrium at 25 °C. The EL was determined with the following formula: $EC_1 / EC_2 \times 100$.

Biochemical parameters

The concentration of hydrogen peroxide (H_2O_2) was determined by Velikova et al. (2000) approach. The leaf sample (0.5 g) after being ground in 5 mL trichloroacetic acid (TCA) and supernatant was taken. Then supernatant was mixed with potassium phosphate buffer (PPB) and 1 M potassium iodide and absorbance (390 nm) was noted. For malondialdehyde (MDA), plant fresh samples (0.5 g) were extracted by using polypropylene centrifuge tubes (PCT) at 12 000 rpm. Thereafter, supernatant was mixed with TCA solution (5 mL) and heated for 20 min in water-bath (100 °C) and absorbance (532 nm) was read to determine MDA contents.

For ascorbate peroxidase (APX), plants were homogenized while using PPB in mortar and pestle and centrifuged (10 000 rpm) for 15 min and absorbance was read at 290 nm (Aebi, 1984). In case of catalase, 0.5 g was blended with 5 mL PPB and centrifuged (10 000 rpm) for 15 min and absorbance was noted at 240 nm (Chance, 1955). For peroxidase (POD); fresh samples (0.5 g) were blended with PPB (5 mL) and centrifuged (10 000 rpm) and absorbance (470 nm) was read. For ascorbic acid, fresh samples (0.5 g) were blended with 5 mL TCA (10%) solution and centrifuged (8000 rpm) for 10 min. Then 2 mL supernatant and 0.5 mL chromogenic reagent (i.e., 2% 2,4-dinitrophenylhydrazine, 0.4% thiourea, and 0.5% copper sulfate in 9 N sulfuric acid, DTC) solution was mixed and incubated (37 °C) for 3 h. Afterward, it was cooled rapidly with the help of cold ice for about 10 min, and then added dropwise 2 mL sulfuric acid solution in it and shaken slightly and absorbance was taken at 520 nm (Mukherjee and Choudhuri, 1983). For total soluble protein (TSP): Fresh leaves were blended with PPB (5 mL) and centrifuged (14 000 rpm) for 15 min. Then samples were treated with 2 mL Bradford reagent and left to sit for 15 to 20 min and absorbance was measured at 595 nm (Bradford, 1976). The total free amino acid was determined by the method of Hamilton and Van-Slyke (1943). Moreover, 0.5 g leaves were taken and homogenized with PPB solution and anthocyanin contents were estimated with methods of Kubo et al. (1999).

Growth and yield traits

Five plants were randomly selected from each pot and their roots were separated from shoots to determine their lengths and biomass. Ten rice plants were selected at random from each experimental unit, to determine plant height, panicle length, branches per panicle, tillers, and grains per panicle. The pots were hand-harvested with a sickle to determine grain and biomass yields and the harvest index was worked as the ratio of both grain and biomass yield.

Nitrogen use efficiency (%)

Agronomic N use efficiency (NUE) was calculated as the ratio of difference between yield of fertilized pots (g) and yield of unfertilized pots (g) to the total N applied by using the following formula:

$$NUE (\%) = (Yield\ of\ fertilized\ pots - Yield\ of\ control) / Applied\ N.$$

Statistical analysis

The data of different traits was analyzed with ANOVA by Statistix 8.1 (Analytical Software, Tallahassee, Florida, USA) and difference amid means were sorted out by least significant difference test at a 5% probability level (Steel et al., 1997).

RESULTS

Growth traits

The results indicate that different types of coated urea had a substantiated impact on the growth traits of rice under DS (Table 1). The maximum root fresh weight (RFW) and root dry weight (RDW) were obtained with SCU under 100% FC, and the lowest RFW and RDW was observed in control conditions without the application of any urea (Table 1). Different urea types and FC levels also significantly affect the root biomass. The SCU significantly increased root biomass compared to other types of coated materials. The maximum root biomass (101 m³) was SCU under 100% FC, followed by ZCU under 100% FC, and the lowest root biomass (54.83 m³) was observed in control without the application of any urea (Table 1).

Table 1. Effect of different types of coated urea on growth traits of rice under different levels of drought stress. Data indicates mean ($n = 3$) and different letters indicate significance among means ($P \leq 0.05$). FC: Field capacity; NU: normal urea; ZCU: Zn-coated urea; SCU: S-coated urea.

| Treatments | | Root fresh weight | Root dry weight | Root biomass |
|-----------------------------|---------|---------------------|---------------------|---------------------|
| | | g | g | m ³ |
| Control | 100% FC | 61.580 ^k | 10.420 ^l | 54.83 ^K |
| Control | 70% FC | 68.547 ^j | 15.547 ^k | 58.93 ^J |
| Control | 50% FC | 73.767 ^h | 32.687 ^e | 86.00 ^D |
| NU | 100% FC | 69.120 ⁱ | 18.450 ^j | 63.00 ^I |
| NU | 70% FC | 75.377 ^g | 23.487 ⁱ | 72.67 ^G |
| NU | 50% FC | 80.803 ^f | 36.700 ^c | 91.00 ^C |
| ZCU | 100% FC | 75.227 ^g | 25.727 ^h | 65.00 ^I |
| ZCU | 70% FC | 83.607 ^d | 29.457 ^g | 76.00 ^F |
| ZCU | 50% FC | 87.757 ^c | 40.440 ^b | 97.00 ^B |
| SCU | 100% FC | 81.840 ^e | 30.783 ^f | 68.00 ^H |
| SCU | 70% FC | 89.440 ^b | 34.863 ^d | 81.33 ^E |
| SCU | 50% FC | 95.797 ^a | 45.420 ^b | 101.00 ^A |
| LSD value ($P \leq 0.05$) | | 0.39 | 0.40 | 2.15 |

Physiological traits

The interaction between field capacity and slow release/coated urea shows that maximum RWC (87.07%) was recorded at 50% FC with SCU. In comparison, the minimum RWC (40.73) was observed at 100% FC without using urea (Table 2). On the contrary, the maximum EL (40.06%) was observed at 50% FC without the application of any urea, and the lowest EL (15.965%) was obtained at 100% drought stress with SCU (Table 2). The results indicated urea fertilizers significantly enhanced the carotenoids, chlorophyll-*a* and chlorophyll-*b* contents under both 100% FC and drought stress conditions. The results showed that SCU under 100% FC significantly enhanced the chlorophyll-*a* and chlorophyll-*b* contents by 57.44% and 48.64% than the control 100% FC. Furthermore, SCU under 50% increased both chlorophyll-*a* and chlorophyll-*b* contents by 100% and 158.74% than the control 50% FC (Table 2). The application of coated fertilizers also significantly enhanced carotenoids and anthocyanin contents of rice. Notable under 50% FC, the application of SCU significantly enhanced carotenoids and anthocyanin contents by 145.79% and 51.10% respectively, than alone 50% FC (Table 2).

Table 2. Effect of different types of coated urea on leaf water status, electrolyte and photosynthetic pigments of rice under different levels of drought stress. RWC: Relative water contents; EL: electrolyte leakage; Cart: carotenoid; Chl: chlorophyll; Anth: anthocyanin; NU: normal urea; ZCU: Zn-coated urea; SCU: S-coated urea; FC: field capacity.

| Treatments | | RWC | EL | Cart. | Chl. <i>a</i> | Chl. <i>b</i> | Anth. |
|------------------------------|---------|--------------------|--------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| | | % | % | mg g ⁻¹ FW | mg g ⁻¹ FW | mg g ⁻¹ FW | mg g ⁻¹ FW |
| Control | 100% FC | 68.80 ^f | 24.70 ^h | 2.43 ^g | 0.470 ^d | 0.370 ^g | 3.50 ⁱ |
| Control | 70% FC | 59.57 ^h | 32.47 ^d | 2.38 ^g | 0.250 ^h | 0.287 ⁱ | 8.20 ^d |
| Control | 50% FC | 40.73 ^k | 40.07 ^a | 1.07 ⁱ | 0.120 ^k | 0.143 ^k | 5.43 ^h |
| NU | 100% FC | 80.40 ^c | 21.93 ⁱ | 4.70 ^c | 0.550 ^c | 0.480 ^c | 5.10 ⁱ |
| NU | 70% FC | 68.40 ^f | 30.40 ^e | 3.57 ^e | 0.360 ^g | 0.400 ^f | 9.10 ^c |
| NU | 50% FC | 50.60 ^j | 37.97 ^b | 1.42 ^h | 0.180 ^j | 0.270 ^j | 7.20 ^f |
| ZCU | 100% FC | 83.60 ^b | 20.20 ^j | 5.34 ^b | 0.660 ^b | 0.520 ^b | 5.40 ^h |
| ZCU | 70% FC | 71.40 ^e | 28.27 ^f | 4.43 ^d | 0.390 ^f | 0.430 ^e | 9.60 ^b |
| ZCU | 50% FC | 57.80 ⁱ | 36.80 ^c | 2.29 ^g | 0.213 ⁱ | 0.350 ^h | 7.50 ^e |
| SCU | 100% FC | 87.07 ^a | 15.97 ^k | 5.66 ^a | 0.740 ^a | 0.550 ^a | 6.10 ^g |
| SCU | 70% FC | 79.20 ^d | 25.40 ^g | 4.38 ^d | 0.440 ^e | 0.450 ^d | 10.10 ^a |
| SCU | 50% FC | 60.83 ^g | 32.53 ^d | 2.63 ^f | 0.240 ^h | 0.370 ^g | 8.20 ^d |
| LSD value (<i>P</i> ≤ 0.05) | | 0.44 | 0.73 | 0.16 | 0.016 | 0.016 | 0.18 |

Biochemical traits

The concentration of both oxidative stress markers significantly increased with the decrease in FC. However, applying coated urea fertilizers abated this increase and substantially reduced H₂O₂ and MDA under stress conditions (Figure 1). The maximum H₂O₂ and MDA were observed in control at 50% FC, and the lowest H₂O₂ and MDA were recorded at 100% FC with the application of SCU (Figure 1). Notably under 50% FC, the application of SCU significantly decreased the H₂O₂ and MDA by 20.33% and 28.02% respectively compared to alone 50% FC (Figure 1). The maximum concentration of TSP and free amino acid (FAA) was recorded at 100% FC with SCU followed by ZCU at 100% FC and lowest TSP and FAA was observed at 50% without applying any coated urea fertilizer (Figure 1). Overall, SCU performed relatively well and it significantly enhanced TSP and FAA followed with ZCU. Notably SCU under 50% significantly enhanced the TSP and FAA by 132.46% and 52.63%, respectively, than the control 100% FC.

The antioxidant activity significantly increased with increasing DS levels. This indicates that rice plants increased their antioxidant activities to counter the toxicity of drought (Figure 2). Further, coated urea fertilizers also significantly increased the activity of all the antioxidants under normal and DS conditions. The maximum ascorbic acid activity (31.13 mg g⁻¹ FW) was observed at 50% FC with SCU, and the lowest using ascorbic acid activity (14.23 mg g⁻¹ FW) was recorded at 100% FC without any use of urea (Figure 2). Likewise, maximum APX activity (40.20 mg g⁻¹ FW) was observed at 70% FC with SCU, and the lowest activity (9.33 mg g⁻¹ FW) was recorded at 100% FC without any use of urea (Figure 2). Moreover, maximum catalase (CAT) activity (8.20 mg g⁻¹ FW) was observed at 50% FC with SCU and lowest (1.83 mg g⁻¹ FW) at 100% FC without any use of urea (Figure 2). Additionally, maximum POD contents (0.51 mg g⁻¹ FW) were observed at 50% FC with SCU and the lowest was observed at 100% FC without coated urea application (Figure 2). Overall, SCU under 100% FC caused a mark increase of 48.23%, 58.55%, 77.10% and 21.42% respectively in ascorbic acid, APX, CAT and POD activities than the control 50% FC (Figure 2).

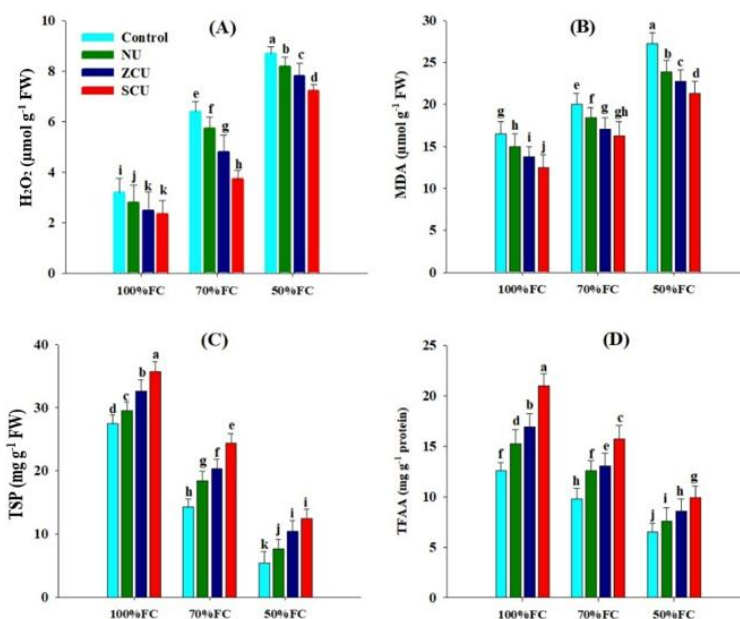


Figure 1. Effect of different types of coated urea on oxidative markers and osmolyte synthesis of rice under different levels of drought stress. FC: Field capacity; NU: normal urea; ZCU: Zn-coated urea; SCU: S-coated urea; H₂O₂: hydrogen peroxide; MDA: malondialdehyde; TSP: total soluble proteins; FAA: free amino acids. Data indicates mean (*n* = 3) with SE and different letters indicate significance among means.

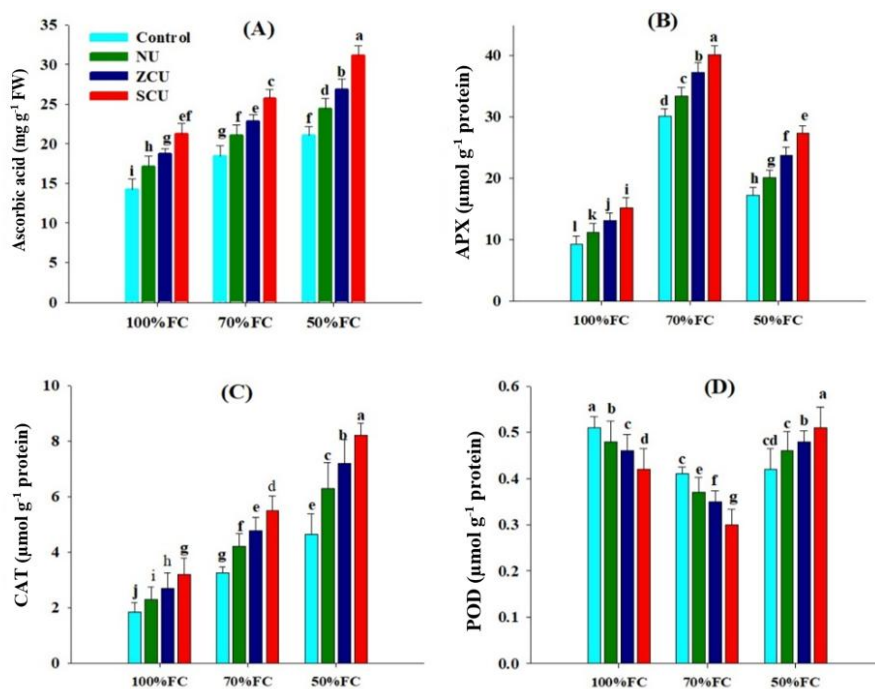


Figure 2. Effect of different types of coated urea on antioxidant activities rice under different levels of drought stress. FC: Field capacity; NU: normal urea; ZCU: Zn-coated urea; SCU: S-coated urea; APX: ascorbate peroxidase, CAT: catalase, POD: peroxidase. Data indicates mean (*n* = 3) with SE and different letters indicate significance among means.

Yield traits

The results indicated a substantiated impact of DS on the yield traits of rice plants (Table 3). The taller plants (118 cm) with longer panicles (24.67 cm) were observed in 100% FC with the application of SCU, followed by ZCU and shorted plants (77 cm) with lowest panicles (8.26 cm) were observed at 50% without applying any urea fertilizer (Table 3). The maximum productive tillers (9.07), nodes plant⁻¹ (1.33), tillers plant⁻¹ (18.67) and grains panicle⁻¹ (54.23) were recorded with SCU at 100% FC. The lowest tillers, nodes plant⁻¹, and grains panicle⁻¹ were noted at 50% FC without applying any urea (Table 3). Overall, SCU performed relative well and it increased the plant height, panicle length, productivity tillers, nodes per plant and tillers per plant by 19.48%, 84.38%, 58.57%, 150.37% and 34.82% than the control 50% FC (Table 3). Further, the maximum 1000-grain weight (45.44 g), grain yield (22.36 g pot⁻¹), biological yield (50.48 g pot⁻¹), and harvest index (45.30%) were obtained with 100% FC by using SCU. The lowest 1000 GW (30.26 g), grain yield (12.59 g pot⁻¹), biological yield (26.87 g pot⁻¹), and harvest index (25.16%) were recorded at 50% drought stress without any use of urea (Table 3). The results displayed SCU under 50% FC significantly enhanced the grains per panicle, 1000-grain weight, grain yield, biological yield and harvest index by 19.97%, 13.24%, 45.90%, 30.62% and 35.20% than the control 50% FC (Table 3).

Table 3. Effect of different types of coated urea on yield and yield traits of rice under different levels of drought stress. PH: Plant height; PL: panicle length; PTP: productive tillers per pot; NP: nodes per plant; TP: total soluble proteins; GPP: grains per panicle; TGW: thousand grains weight; GYP: grain yield/pot; BYP: biological yield per pot; HI: harvest index; NU: normal urea; ZCU: Zn-coated urea; SCU: S-coated urea; FC: Field capacity. Data indicates mean ($n = 3$) and different letters indicate significance among means $P \leq 0.05$).

| Treatments | | PH | PL | PTP | NP | TSP | GPP | TGW | GYP | BYP | HI |
|-----------------------------|---------|------------------|---------------------|-------------------|--------------------|---------------------|---------------------|--------------------|--------------------|--------------------|--------------------|
| | | cm | cm | | | | | g | g | g | % |
| Control | 100% FC | 104 ^d | 17.65 ^e | 6.41 ^e | 4.33 ^a | 15.40 ^d | 45.53 ^e | 40.20 ^d | 17.32 ^e | 40.22 ^f | 35.57 ^e |
| Control | 70% FC | 89 ⁱ | 11.24 ⁱ | 5.28 ^f | 3.67 ^{ab} | 11.34 ^e | 40.53 ^f | 35.17 ^g | 14.36 ^g | 32.20 ⁱ | 30.20 ^h |
| Control | 50% FC | 77 ^l | 8.26 ^j | 4.60 ^g | 3.33 ^{bc} | 8.73 ⁱ | 33.90 ⁱ | 30.27 ^k | 12.59 ^h | 26.87 ^k | 25.17 ^j |
| NU | 100% FC | 109 ^c | 21.67 ^c | 7.60 ^c | 3.67 ^{ab} | 16.43 ^c | 48.68 ^c | 43.30 ^c | 19.51 ^c | 45.08 ^d | 40.20 ^c |
| NU | 70% FC | 95 ^g | 14.58 ^{fg} | 6.34 ^e | 2.67 ^{cd} | 12.37 ^f | 44.56 ^e | 38.23 ^f | 16.48 ^f | 37.29 ^g | 34.47 ^f |
| NU | 50% FC | 81 ^k | 13.28 ^h | 5.40 ^f | 2.67 ^{cd} | 9.37 ⁱ | 36.63 ^h | 32.30 ^j | 14.59 ^g | 30.07 ^j | 9.10 ⁱ |
| ZCU | 100% FC | 113 ^b | 22.92 ^b | 8.50 ^b | 3.33 ^{bc} | 17.17 ^b | 51.37 ^b | 44.49 ^b | 20.51 ^b | 47.43 ^b | 43.43 ^b |
| ZCU | 70% FC | 99 ^f | 17.11 ^e | 7.18 ^d | 2.67 ^{cd} | 13.89 ^e | 47.52 ^d | 39.10 ^e | 18.53 ^d | 42.20 ^e | 36.10 ^e |
| ZCU | 50% FC | 86 ^j | 14.52 ^g | 6.34 ^e | 2.33 ^d | 10.31 ^h | 38.77 ^g | 33.43 ⁱ | 16.74 ^f | 32.43 ⁱ | 31.30 ^g |
| SCU | 100% FC | 118 ^a | 24.67 ^a | 9.07 ^a | 2.33 ^d | 18.62 ^a | 54.23 ^a | 45.47 ^a | 22.36 ^a | 50.48 ^a | 45.30 ^a |
| SCU | 70% FC | 102 ^e | 18.83 ^d | 8.52 ^b | 2.33 ^d | 14.70 ^d | 48.20 ^{cd} | 40.28 ^d | 20.59 ^b | 46.13 ^c | 38.30 ^d |
| SCU | 50% FC | 92 ^h | 15.23 ^f | 7.29 ^d | 1.33 ^e | 11.77 ^{fg} | 40.67 ^f | 34.28 ^h | 18.37 ^d | 35.10 ^h | 34.03 ^f |
| LSD value ($P \leq 0.05$) | | 1.69 | 0.72 | 0.27 | 0.97 | 0.70 | 10.04 | 0.31 | 0.46 | 0.80 | 0.95 |

Nitrogen use efficiency

Both coated urea improved agronomic NUE in all field capacity levels compared to normal urea application. The SCU application at 100% FC significantly increased agronomic NUE by 67.12% than the control. Furthermore, no urea application (control) at all FC levels recorded with minimum agronomic NUE (Figure 3).

Correlation matrix

The data were subjected to correlation matrix to determine the relationship between different studied traits (Figure 4). There was a positive and a negative relationship between root fresh weight (RWF), RDW, RWC, carotenoid, chlorophyll, antioxidants, yield and yield traits, while there was a negative relationship between EL, MDA, H₂O₂, antioxidant activities, osmolyte synthesis, growth and yield traits (Figure 4).

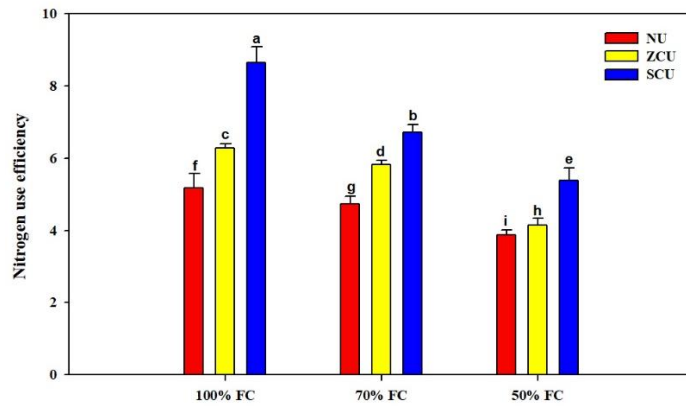


Figure 3. Effect of different types of coated urea on nitrogen use efficiency of rice under different levels of drought stress. FC: Field capacity; NU: normal urea; ZCU: Zn-coated urea; SCU: S-coated urea. Data indicates mean ($n = 3$) with SE and different letters indicate significance among means.

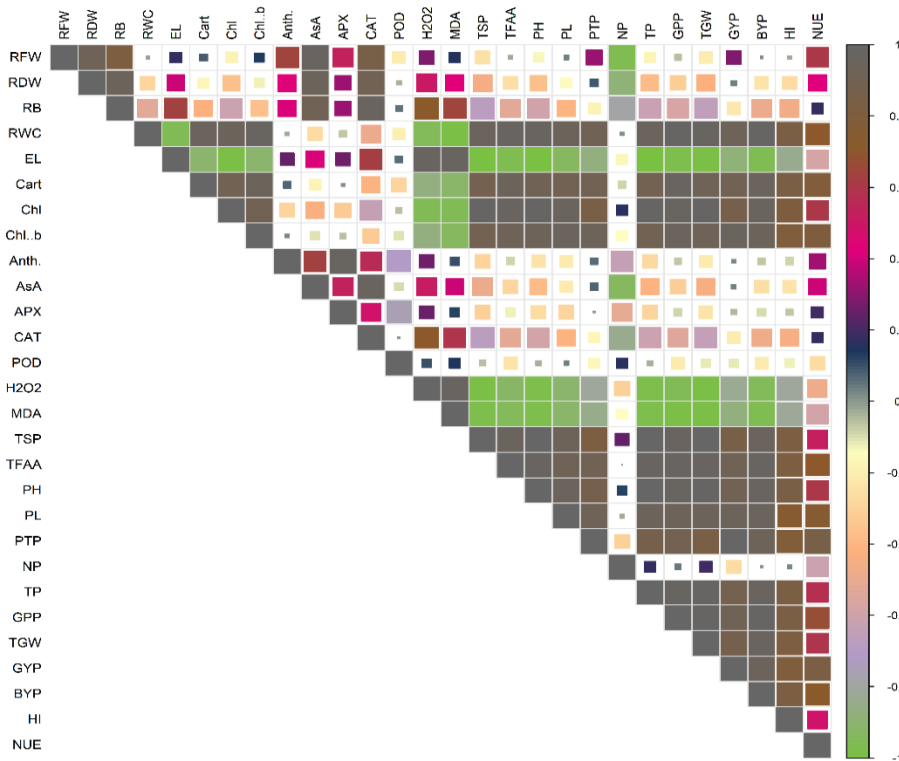


Figure 4. Correlation matrix for the different types of coated urea on growth, physiology, biochemical functioning and NUE (agronomic). The light green, purple, orange and yellow color indicating the negative impact $p \leq 0.05$, while dark blue, pink, grey and brown color indicate the positive impact. RFW: Root fresh weight, RDW: root dry weight; RB: root biomass; RWC: relative water content; EL: electrolyte leakage; Cart: carotenoid; Chl: chlorophyll; Anth: anthocyanin; AsA: ascorbic acid; APX: ascorbate peroxidase, CAT: catalase, POD: peroxidase; H_2O_2 : hydrogen peroxide; MDA: malondialdehyde; TSP: total soluble proteins; TFAA: total free amino acids; PH: plant height; PL: panicle length; PTP: productive tillers per pot; NP: nodes per plant; GPP: grains per panicle; TGW: thousand grains weight; GYP: grain yield/pot; BYP: biological yield per pot; HI: harvest index; NUE: N use efficiency (agronomic).

DISCUSSION

Drought stress (DS) negatively affects crop productivity. However, it largely depends on plant species, drought intensity and growth duration. Drought stress caused a marked decrease in rice productivity (Table 1). Drought stress severely affects cell division and differentiation, leading to a marked reduction in plant growth (Chaves et al., 2009). Besides this, drought also causes oxidative damage, as in the present study, DS increases the production of malondialdehyde (MDA) and H_2O_2 , thereby leading to a marked reduction in plant growth (Wahab et al., 2022). We also observed that electrolyte leakage (EL) was significantly increased under DS due to an increase in MDA and H_2O_2 . This might damage the cellular membranes and could be an essential reason for poor plant growth under DS. Nevertheless, the application of coated urea significantly increased the growth of rice plants. Coated urea effectively reduced the MDA and H_2O_2 , which maintain membrane stability and, in turn, reduced the EL thereby protecting the plants from abiotic stresses (Mustafa et al., 2022).

Drought also reduced the chlorophyll and carotenoid synthesis, which could be ascribed to drought-induced increase in oxidative damages as witnessed by increased synthesis of both MDA and H_2O_2 (Table 1). Previous studies also documented that DS induces stomata closing and decreases chlorophyll synthesis, which inhibits biomass production (Shaaban et al., 2023). Drought-induced stomata closing restricts gas changes and results in a shortage of CO_2 supply to the chloroplast. Consequently, excess electrons are converted into reactive oxygen species (ROS), negatively affecting plants' chlorophyll synthesis and overall photosynthetic efficiency (Aslam et al., 2026).

The results also indicate that DS increased the production of both MDA and H_2O_2 that might increase the damage to the chloroplast, thereby leading to a marked reduction in chlorophyll synthesis (Dumanović et al., 2021). We observed that applying coated urea (S-coated urea [SCU] and Zn-coated urea [ZCU]) effectively increased the chlorophyll and carotenoid synthesis. Coated urea application effectively improved the antioxidant activities, which reduced ROS production, leading to a marked improvement in chlorophyll synthesis. Plants synthesize different osmoregulating compounds which maintain cellular turgor within the cells (Manavalan et al., 2009). Plants accumulate soluble sugars, proteins and proline in response to stress conditions, which enhance plant growth by increasing photosynthetic efficiency and decreasing cell water potential (Dubrovna et al., 2022). The results indicate that DS negatively impacted osmolyte synthesis and significantly reduced the synthesis of both total soluble proteins (TSP) and free amino acid (FAA). This decrease in TSP and FAA is linked with poor uptake of N because it plays an essential role in the metabolism of both TSP and FAA.

Coated urea enhanced agronomic NUE, which counteracts the drought-induced reduction in TSP and FAA. Both these compounds play an essential role against stress conditions because total soluble sugar (TSS) is C source for different organic solutions, and it is essential in protecting enzymes (Feng et al., 2023). The results also showed that SCU more effectively increased the production of osmo-regulating compounds, which preserved the plant hydration and positively impacted plant growth.

Using SCU significantly increased the activity of all the antioxidants, which mitigated both MDA and H_2O_2 production. Nitrogen is the first defense against stress conditions as it increases antioxidant activities (Singh et al., 2019). This suggested that applying coated urea under DS can help alleviate the toxic impacts of drought by increasing antioxidant activities. The study findings demonstrate that the ZCU and SCU increased antioxidant activities and were also linked to increased N uptake. Applying coated urea ensures a slow and continuous supply of N, which is conducive to improving plant growth (Aslam et al., 2026).

Drought stress decreases enzyme activity in N metabolism, leading to a reduction in the production of N compounds, thereby reducing plant productivity (Siddiqui et al., 2021). The results indicate the increasing intensity of DS markedly decreased the rice biomass and grain yield. This suggests that DS caused a marked increase in oxidative damages, and it caused photo-inhibition, thus leading to a reduction in final yield. Photosynthesis is a vital process in plants. However, DS negatively affect photosynthesis by decreasing chlorophyll and carotenoid synthesis, leading to significant losses in plant production (Wahab et al., 2022). In the present study, applying both types of coated urea significantly increased rice productivity. Applying both ZCU and SCU increased the availability of both Zn and S, leading to improved plant productivity. The increase in Zn availability increases enzymes (carbonic anhydrase and ribulose 1,5-biphosphate carboxylase) involved in photosynthesis and synthesis of chlorophyll contents (Ashraf et al., 2019). Besides this, Zn also plays a significant role in sugars, starch, and carbohydrate metabolism, which improves membrane stability by increasing

antioxidant activity. Sulfur is an essential nutrient that protects plants against stress by increasing antioxidant activities. Therefore, increased S following SCU application might increase antioxidant activities and lead to a significant increase in yield and biomass production. The increase in N availability also mitigates the toxicity of drought through an increase in antioxidant activities, photosynthetic efficiency and osmolyte accumulation (Mustafa et al., 2022). The DS decreased agronomic NUE, and oppositely, the application of coated fertilizers significantly increased it (Table 1). The increase in agronomic NUE reduces N losses and provides the benefits of improved crop productivity, environmental quality, and reduced production cost (Langholtz et al., 2021). Both coated urea significantly increased agronomic NUE, which was linked with slower N release and synchronization between N supply and plant demands and reduced N losses into the environment. The results showed that coated urea fertilizers significantly enhanced the N uptake by rice plants. Coated fertilizers have slow-release properties, which increase nutrient retention in soil, thereby enhancing N uptake and subsequent crop yield (Zhang et al., 2024). Coated urea fertilizers also increase soil pH, soil aeration, and water-holding capacity, thereby improving N uptake and resulting in higher NUE (Yue et al., 2021). Moreover, coated urea fertilizers also improve the root growth and vigor, which is beneficial for nutrient uptake; therefore, improved root growth with coated fertilizers may have enhanced the N uptake (Aslam et al., 2026). Additionally, coated fertilizers also reduced volatilization losses and denitrification. This controlled release also decreases environmental losses and keeps the N available in the root zone for a longer time for efficient plant absorption and higher fertilizer use efficiency.

CONCLUSIONS

Drought stress significantly reduced rice production, impairing leaf water status and chlorophyll synthesis and inducing oxidative damage. Sulfur-coated urea mitigated the adverse impacts of drought and improved rice productivity by regulating photosynthetic pigments, osmolyte synthesis, antioxidant activities, leaf water contents, and N use efficiency. Thus, S-coated urea could be an essential practice to improve rice productivity and mitigate the toxic impacts of drought stress. Therefore, additional fields and in-depth studies are desired to authenticate these outcomes for the promising future of S-coated to mitigate adversities of drought stress.

Author contribution

Conceptualization: M.U.C., I.K. Investigation, Writing-original draft preparation: M.U.C., I.K., M.T.A. Data collection: B.B. Writing-review and editing: M.B.C., J.M.A., O.A., B.A., M.I.Aldaej, M.I.Almaghasla. All authors have read and agreed to the published version of the manuscript.

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