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Enhancement of antioxidant defense and alleviation of oxidative stress in proso millet (*Panicum miliaceum* L.) using rice bran-coated urea under heat conditions

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Abstract

Objective: Heat stress triggers overproduction of reactive oxygen species, leading to oxidative damage and crop yield loss. This study investigated the efficacy of rice bran-coated urea in modulating the antioxidant defense system and mitigating oxidative membrane damage in proso millet (*Panicum miliaceum* L.) under field heat stress.

Methods: A two-year field experiment evaluated uncoated urea, rice bran-coated urea, gypsum-coated urea, and cement-coated urea at four nitrogen rates (0, 60, 80, and 120 kg urea ha^{-1}) under optimal (spring) and heat stress (summer) conditions. The experiment was a split-plot factorial design based on a randomized complete block design with three replications. The main plot factor was the planting season (spring and summer seasons). Sub-plot factors were the factorial combination of coating type and urea-N rate. The coating factor included uncoated urea (UCU, control), rice bran-coated urea (RBCU), gypsum-coated urea (GCU), and cement-coated urea (CCU). The urea fertilizers were 0, 60, 80, or 120 kg urea ha^{-1}. At the flowering stage, the catalase (CAT) and peroxidase (POD) activity, total soluble phenol content, total soluble protein content, and malondialdehyde (MDA) concentration were measured on the youngest fully expanded leaves. Grain yield was measured at physiological maturity from a 2-m^2 area in the central two rows of each plot.

Results: Across years, RBCU at 80 kg urea ha^{-1} provided the most consistent balance between antioxidant protection and yield performance under summer heat stress. Under summer heat, RBCU significantly enhanced the enzymatic antioxidant shield, increasing CAT and POD activity, compared to the stressed uncoated urea control. It also elevated non-enzymatic defense by increasing total soluble phenols under summer heat compared with uncoated urea. This coordinated upregulation was associated with a significant containment of oxidative damage. While heat stress increased malondialdehyde content, RBCU at 80 kg urea ha^{-1} resulted in lower MDA compared with the excessive 120 kg urea ha^{-1} treatment. Concurrently, RBCU improved the total soluble protein content under stress. This physiological resilience was associated with improved agronomic performance; RBCU at 80 kg urea ha^{-1} increased grain yield compared with the unfertilized control and outperformed uncoated urea under summer conditions.

Conclusion: RBCU-mediated nitrogen synchronization improved redox balance and limited lipid peroxidation under field heat stress, supporting both physiological stability and yield maintenance in proso millet.

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Introduction

Proso millet (*Panicum miliaceum* L.) is a climate-resilient C_4 cereal, crucial for food security in semi-arid and heat-prone regions. It is a crop with high potential for cultivation under marginal environments; however, its physiological response to combined heat stress and nitrogen management strategies remains poorly understood. The increasing frequency and intensity of heatwaves present a severe threat, often exceeding the crop's innate tolerance limits. Heat stress is increasingly recognized as a major environmental constraint limiting crop productivity, particularly in arid and semi-arid regions. Elevated temperatures during the reproductive stage disrupt cellular homeostasis, impair photosynthetic efficiency, and accelerate senescence, ultimately reducing grain yield (Reynolds *et al.* 2010; Hasanuzzaman *et al.* 2013). High temperatures disrupt chloroplast integrity and photosynthetic electron transport, leading to an overproduction of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide (H_2O_2). One of the primary physiological consequences of heat stress is the excessive generation of ROS, which leads to oxidative damage of membranes, proteins, and nucleic acids if not efficiently scavenged (Mittler 2002). This imbalance between ROS generation and scavenging capacity results in oxidative stress, a primary cause of heat-induced physiological decline (Gill and Tuteja 2010), and biochemically manifests as increased membrane lipid peroxidation, a key marker of oxidative damage (Zanganeh and Jamei 2020). The ensuing oxidation damages membrane integrity and function, leading to cellular leakage, metabolic dysfunction, and ultimately, significant yield loss (Mittler 2017).

A plant's survival under heat stress hinges on the efficiency of its integrated antioxidant defense system, including enzymatic components such as catalase (CAT) and peroxidase (POD), as well as non-enzymatic compounds, such as phenols, to maintain redox balance under stress conditions (Apel and Hirt 2004). CAT and POD directly detoxify H_2O_2 and non-enzymatic compounds like phenolic metabolites, which act as direct ROS scavengers and membrane stabilizers (Sharma *et al.* 2019). The critical role of this system in conferring tolerance has been demonstrated in various crops under abiotic stress, such as drought-stressed sunflower (Sarvari *et al.* 2017) and tomato (Farzane *et al.* 2020). The synthesis and maintenance of this defensive arsenal are heavily dependent on nutrient availability, particularly nitrogen (N) availability, which plays a central role in enzyme synthesis, protein metabolism, and stress signaling pathways (Xu *et al.* 2012). Studies have shown that exogenous application of nitrogenous compounds like arginine can enhance antioxidant capacity and reduce oxidative damage under stress (Kabiri *et al.* 2016; Nasibi *et al.* 2020), highlighting the intricate link between N metabolism and oxidative stress resilience.

Conventional urea fertilization is inefficient due to rapid hydrolysis, volatilization, and leaching, creating a temporal mismatch between N supply and plant demand (Raun and Johnson 1999). Conventional urea fertilizers often suffer from rapid nitrogen release and low nitrogen use efficiency, especially under high-temperature conditions where volatilization and leaching losses are intensified. Under heat stress, this mismatch is catastrophic: an initial ammonium flush can exacerbate ROS production, while subsequent N deficiency cripples the synthesis of the antioxidants needed for repair (Fahad *et al.* 2017). The importance of optimizing N management under stress conditions is evident, as demonstrated in the study on quinoa, where different N levels significantly modulated physiological and yield responses to drought (VaziriMehr *et al.* 2024). Controlled-release fertilizers, especially those coated with organic and biodegradable materials like rice bran, offer a promising solution by synchronizing N release with plant uptake (Shaviv and Mikkelsen 1993). The use of controlled-release nitrogen fertilizers, such as resin-based coated urea (RBCU), has been proposed as an effective strategy to synchronize nitrogen availability with crop demand, thereby improving nitrogen use efficiency and mitigating stress-induced metabolic disturbances (Trenkel 2010). However, in particular, limited information is available on how controlled-release nitrogen fertilizers modulate antioxidant defense mechanisms and membrane stability in proso millet under contrasting planting seasons. While the agronomic benefits of coated urea are documented, their specific role in reprogramming the antioxidant defense cascade and the subsequent containment of oxidative membrane damage under realistic field heat stress remains inadequately explored for millet.

We hypothesized that RBCU, by providing a sustained and balanced nitrogen supply, would specifically and more effectively upregulate key components of the antioxidant system (CAT, POD, and phenols) in heat-stressed millet compared to mineral coatings or uncoated urea. Unlike mineral-coated urea (gypsum or cement), RBCU uses an organic rice bran coating that synchronizes N release with plant uptake, potentially enhancing enzymatic and non-enzymatic antioxidant defenses under heat stress. This enhanced defense, in turn, would lead to a measurable reduction in oxidative membrane damage (lipid peroxidation) and preserve metabolic integrity (protein content), culminating in improved grain yield stability. Therefore, the objectives of this study were to quantify the response of enzymatic (CAT, POD) and non-enzymatic (phenols) antioxidant systems to different urea coatings under field heat stress, assess the resultant effect on oxidative membrane damage via malondialdehyde (MDA), evaluate the associated changes in total soluble protein content, and determine the ultimate impact on grain yield.

Materials and Methods

Plant materials and growth conditions

A two-year field experiment was conducted during the 2024 and 2025 growing seasons in Al-Muthanna Governorate, Iraq (31.5°N, 45.3°E, and 120 m altitude). The region features a semi-arid climate classified as BSh according to the Köppen–Geiger climate classification (Peel *et al.* 2007). To create contrasting thermal regimes, proso millet (cv. Mahoor) was sown in mid-March (optimal spring season with mean maximum temperature of 32.7 ± 2.5 °C) and mid-June (heat stress summer season with mean maximum temperature of 42.5 ± 3.1 °C). The soil was a sandy loam with low organic carbon (0.45%) and an alkaline pH of 8.2.

Experimental design and treatments

The experiment was conducted as a split-plot factorial design based on a randomized complete block design with three replications. The main plot factor was planting season (spring and summer). The subplot factor was a factorial combination of coating type and urea-N rate. Coating factor included: RBCU, gypsum-coated urea (GCU), cement-coated urea (CCU), and uncoated urea (UCU, control). Urea fertilizers (uncoated and coated forms) were applied to the soil at four rates: 0, 60, 80, and 120 kg urea ha^{-1}. Nitrogen was incorporated into the soil surface. Nitrogen fertilizers were split-applied, with 50% applied at tillering (30 DAS) and 50% at stem elongation (55 DAS). All plots received uniform basal applications of phosphorus and potassium fertilizers based on soil test recommendations. Each subplot acquired a 3 m × 4 m (12 m^2) area.

Preparation of coated urea

Coated urea granules were prepared on-site following a physical coating procedure. Commercial urea prills (46% N) were coated in a rotary drum using a 5% (w/v) Arabic gum solution as an adhesive and sieved coating materials (rice bran, gypsum, and cement). The coated granules were dried at 40–

50 °C for 12 hours to ensure stability. Coated granules were inspected visually and sieved to ensure uniform coverage. An in-vitro dissolution test in distilled water confirmed the slow-release property; over 80% of N from coated urea was released over 28 days, compared to over 95% from uncoated urea within 7 days.

Measurements of physiological and biochemical characteristics

At the flowering stage (70 DAS), the youngest fully expanded leaves were sampled from the central rows of each plot between 9:00 and 10:00 AM, immediately frozen in liquid N_2 , and stored at –80 °C for subsequent biochemical analysis through a standard sampling protocol for assessing oxidative stress markers (Nasibi *et al.* 2020; Zanganeh and Jamei 2020).

Antioxidant enzymes**:** Catalase (CAT, EC 1.11.1.6) activity was determined by monitoring the decomposition of H_2O_2 at 240 nm (Beers and Sizer 1952). Peroxidase (POD, EC 1.11.1.7) activity was assayed using guaiacol as the substrate, measuring the increase in absorbance at 470 nm (Chance and Maehly 1955; Velikova *et al.* 2000). Enzyme activities were expressed in units per mg of soluble protein (U mg^{-1} protein).

Non-enzymatic antioxidants: Total soluble phenolic content was estimated using the Folin-Ciocalteu reagent method (Singleton and Rossi 1965). Absorbance was read at 765 nm, and results were expressed as milligrams of gallic acid equivalent per gram fresh weight (mg GAE g^{-1} FW).

Oxidative stress marker: The extent of lipid peroxidation was estimated by measuring the concentration of MDA, a thiobarbituric acid reactive substance (TBARS), according to Heath and Packer (1968). MDA content was calculated and expressed as nmol g^{-1} fresh weight (nmol g^{-1} FW), a common unit for reporting oxidative damage in plant tissues under stress (Kabiri *et al.* 2016; Farzane *et al.* 2020).

Total soluble proteins: Protein concentration in the enzyme extract was determined using the Bradford (1976) method with bovine serum albumin as the standard. Results were expressed as mg protein g^{-1} dry weight (mg g^{-1} DW).

Grain yield measurement

At physiological maturity, plants from a 2-m^2 area in the central two rows of each plot were manually harvested to avoid border effects. The harvested plants were threshed, and the grains were cleaned and sun-dried to a constant weight. Grain yield was recorded and expressed as tons per hectare at a standard moisture content of 12%.

Statistical analysis

All data were subjected to a four-way analysis of variance (ANOVA) appropriate for a split-plot factorial design, using SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA). The significance of fixed effects (season, coating, and N rate) and random effects (year and replication) was tested, using the expected mean squares. The assumptions of ANOVA (normality and homogeneity of variances) were verified. All statistical analyses were performed on raw, untransformed data. Treatment means were compared, using the honestly significant difference (HSD) test of Tukey at a significance level of $p \leq 0.05$.

Results

Antioxidant enzyme activities

Analysis of variance indicated that the interaction of year × planting season × urea coating type × nitrogen rate significantly affected CAT and POD activity (Table 1). Although the main effect of planting season was not consistently significant for all traits (Table 1), the significant Y × S and Y × S × C × N interactions indicate that the magnitude and direction of heat stress responses varied between 2024 and 2025. Therefore, seasonal effects should be interpreted in the context of annual environmental variability rather than as isolated main effects. Under summer conditions, CAT activity declined under control conditions (no urea application) in both years (Figure 1). However, RBCU partially alleviated this decline, highlighting its protective role. Under summer planting conditions, CAT activity declined in plants receiving uncoated urea, whereas application of RBCU tended to enhance CAT activity; even higher values were observed at 80 and 120 kg urea in 2024 as compared to the spring season. In general, RBCU showed higher values than UCU and other coating treatments at 80 and 120 kg urea in all seasons and years (Figure 1).

POD activity generally showed a stress-induced decrease in 2024 but an increase in 2025 under control conditions. However, with RBCU-treated plants, the POD activity under heat stress was higher than that of conventional urea treatments (Figure 2). In summer seasons (Figures 2c and 2d), POD activity under RBCU at 80 kg urea ha^{-1} tended to be higher than under UCU, particularly in 2025, reflecting a supportive effect of RBCU on enzymatic antioxidant activity (Figure 2).

Non-enzymatic antioxidants and oxidative damage

Total soluble phenolic content and MDA were significantly influenced by the interaction of year × planting season × urea coating type × nitrogen rate (Table 1). Heat stress under summer conditions increased phenolic accumulation in uncoated urea treatments; however, the increase was higher under

RBCU application (Figures 3c and 3d). Under summer conditions, total soluble phenolic content tended to be higher under RBCU at 80 kg urea ha^{-1} than UCU, highlighting the enhancement of non-enzymatic antioxidant defenses (Figures 3c and 3d).

MDA content, as an indicator of membrane lipid peroxidation, generally increased significantly under summer heat stress in the controls (Figure 4). RBCU at 80 kg urea ha^{-1} tended to have a lower MDA accumulation relative to UCU in summer 2024, although the difference was not significant, indicating improved membrane stability (Figure 4). Under summer conditions, MDA content under RBCU at 80 kg urea ha^{-1} was lower than under 120 kg urea ha^{-1} (Figures 4c and 4d).

Total soluble proteins and grain yield

Total soluble protein content and grain yield were significantly affected by the interaction of year × planting season × urea coating type × nitrogen rate (Table 1). Heat stress in the summer planting generally reduced total soluble protein content in both years. However, RBCU preserved metabolic integrity under heat stress, as evidenced by higher nitrogen assimilation. Under summer planting, RBCU treatments resulted in higher protein accumulation than uncoated urea, with the highest values observed at 120 and 80 kg urea ha^{-1} (Figure 5). RBCU tended to maintain higher total soluble protein compared with UCU under heat stress, with the effect more pronounced in 2025 (Figure 5).

Grain yield generally declined in the uncoated treatments under summer planting compared to spring conditions (Figure 6). However, the RBCU application mitigated yield loss under heat stress, resulting in higher grain yield compared to uncoated urea treatments, particularly in 2024 (Figure 6c). This cascade of physiological benefits, including enhanced antioxidant defense and preserved metabolism, was translated into improved agronomic performance. In summer 2024, grain yield with RBCU at 80 and 120 kg urea ha^{-1} was higher than UCU, while in summer 2025, the differences among RBCU rates and UCU were smaller (Figure 6), establishing a link between cellular resilience and crop productivity. In 2024, the grain yield under RBCU at 80 and 120 kg urea ha^{-1} was much higher than the unfertilized control (Figure 6c).

In general, RBCU performed better than UCU, GCU, and CCU in both spring and summer seasons and both years, concerning all characteristics studied in this experiment.

Discussion

The significant Y × S × C × N interaction indicates that the effectiveness of RBCU was affected by inter-annual climatic variability rather than by heat stress alone. While summer planting imposed

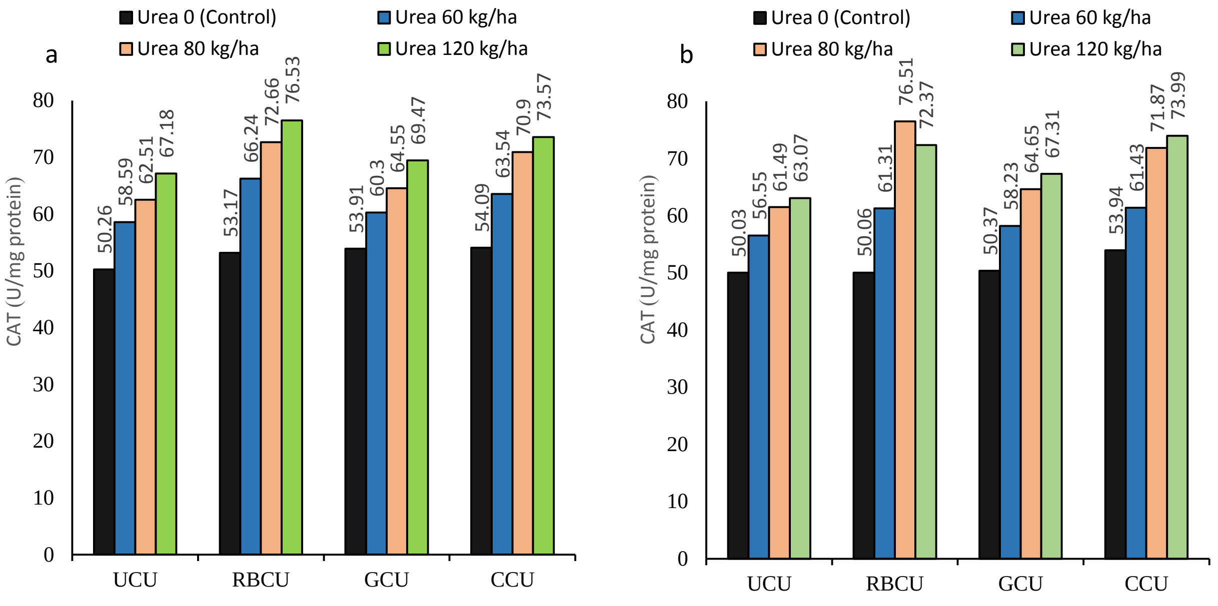
Table 1. The influence of year, planting season, urea coating type, and nitrogen rate on antioxidant defense, oxidative stress, and grain yield traits in proso millet.

Source of variation	**df**	**Mean squares**					
		CAT	**POD**	**TPC**	**MDA**	**TSP**	**GY**
Year (Y)	1	1029.6**	26665.7**	417.5**	113.35**	188.9**	12.63**
Season (S)	1	983.1	3064.9	181.8	1.81	185.1	1.63
Y × S	1	464.9**	1708.4**	39.63**	1.61**	15.62**	0.031**
Rep (Y×S)	8	2.97**	3.12**	0.025	0.00364	0.00465	0.0014
Coating (C)	3	862.6**	139.5	85.52	2.27	43.53	1.17
Nitrogen (N)	3	3204.9**	1698.9*	304.0*	15.26	198.0	4.89**
Y × C	3	7.39**	15.98**	14.86**	1.18**	11.80**	0.68**
S × C	3	32.76	40.53	3.69	0.23	1.81*	0.067
Y × N	3	62.05**	127.8**	15.72**	2.86**	57.14**	0.14**
S × N	3	2.83	99.93	8.37	0.31	1.00	0.012
C × N	9	94.91**	30.60	11.51	0.42	4.39	0.086
Y × S × C	3	14.13**	14.65**	6.11**	0.41**	0.081	0.037**
Y × S × N	3	56.57**	18.02**	12.58**	0.049**	1.39**	0.16**
Y × C × N	9	11.67**	11.62**	6.16**	0.21**	1.44**	0.090**
S × C × N	9	13.71	10.23	5.76	0.099	0.95*	0.046
Y × S × C × N	9	6.69**	10.50**	3.56**	0.073**	0.21**	0.031**
Error	120	0.92	1.12	0.032	0.0031	0.0029	0.0052
Total	191						
CV (%)		1.6	2.0	0.5	2.2	0.5	5.7

Rep: Replication, df: Degrees of freedom, CAT: Catalase activity, POD: Peroxidase activity, TPC: Total phenolic content, MDA: Malondialdehyde; TSP: Total soluble proteins; GY: Grain yield, CV: Coefficient of variation; *, **: Significance at $p \leq 0.05$ and $p \leq 0.01$, respectively; Year and replication were regarded as random factors and planting season, urea coating type, and nitrogen rate were considered as fixed factors, and F-test was conducted according to expected mean squares.

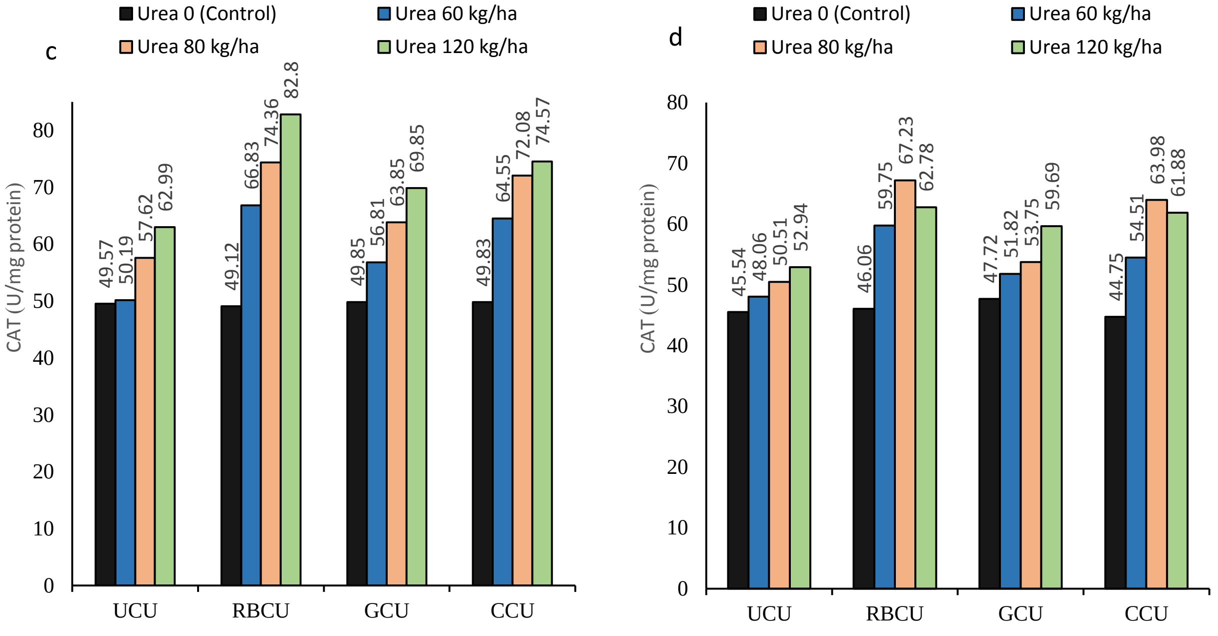
oxidative pressure in both years, the physiological and agronomic responses differed in magnitude between 2024 and 2025. In summer 2024, RBCU at 120 kg urea ha^{-1} maximized grain yield, suggesting that higher nitrogen availability supported productivity under that season's thermal regime. In contrast, during summer 2025, the 80 kg urea ha^{-1} rate provided a more balanced response, maintaining antioxidant activation and limiting lipid peroxidation without the oxidative cost associated with higher nitrogen input. These findings indicate that the optimal nitrogen rate under heat stress should be interpreted as a dynamic balance between productivity and oxidative stability rather than as a fixed maximum-yield value.

RBCU tended to enhance CAT activity, with higher values observed at 80 kg urea ha^{-1} in 2025 and 120 kg urea ha^{-1} in 2024. POD activity generally increased in the summer season as compared



Type of Urea Coverage in Spring cultivation in 2024

Type of Urea Coverage in Spring cultivation in 2025



Type of Urea Coverage in Summer cultivation in 2024

Type of Urea Coverage in Summer cultivation in 2025

Figure 1. Interactive effect of year, planting season, coating type, and urea level on catalase (CAT) activity; (a): Spring planting 2024, (b): Spring planting 2025, (c): Summer planting 2024, (d): Summer planting 2025. Uncoated urea (UCU, control), rice bran-coated urea (RBCU), gypsum-coated urea (GCU), and cement-coated urea (CCU); HSD5% = 3.489.

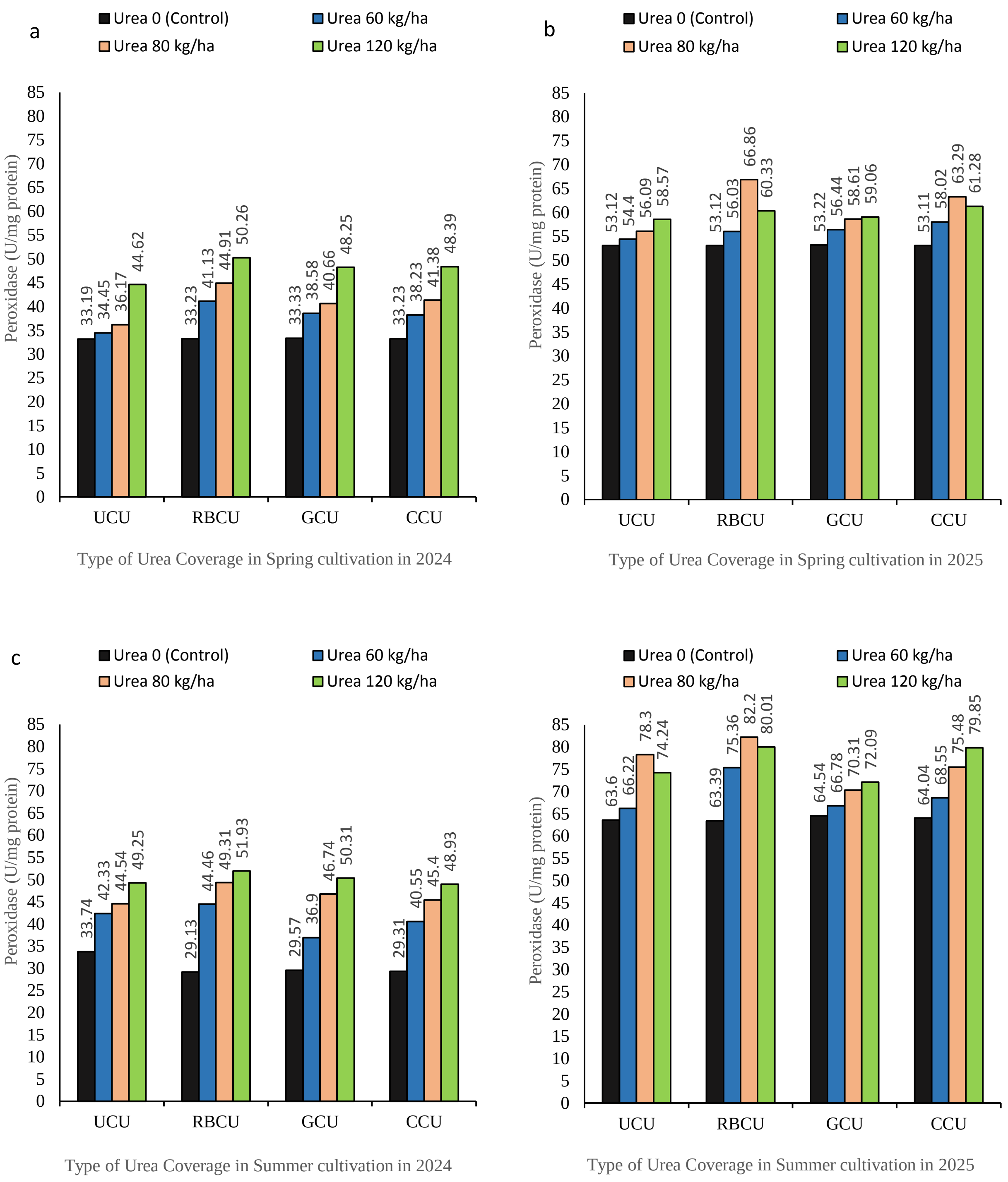


Figure 2. Interactive effect of year, planting season, coating type, and urea level on peroxidase (POD) activity; (a): Spring planting 2024, (b): Spring planting 2025, (c): Summer planting 2024, (d): Summer planting 2025. Uncoated urea (UCU, control), rice bran-coated urea (RBCU), gypsum-coated urea (GCU), and cement-coated urea (CCU); HSD5% = 3.849.

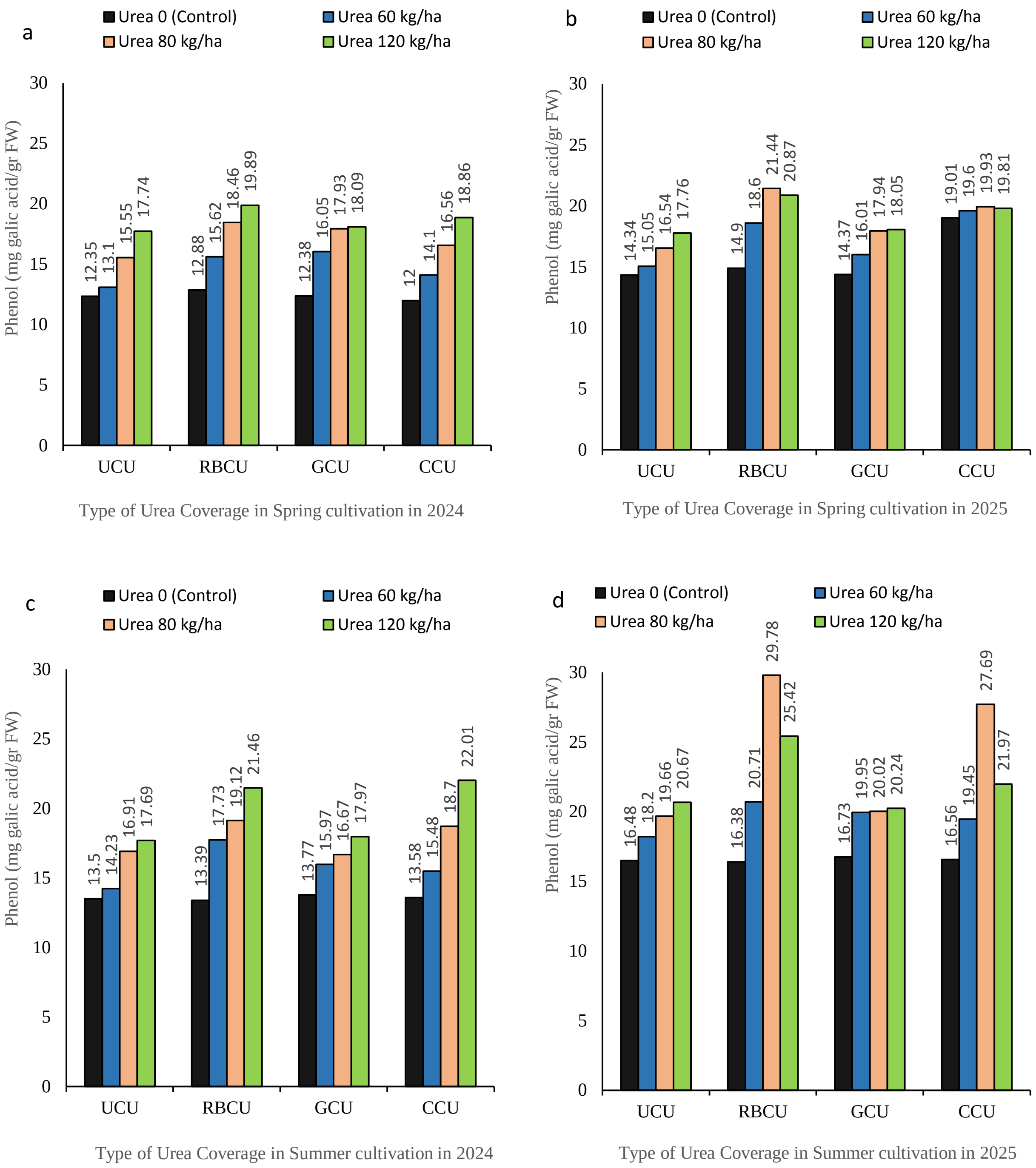


Figure 3. Interactive effect of year, planting season, coating type, and urea level on total soluble phenolic content; (a): Spring planting 2024, (b): Spring planting 2025, (c): Summer planting 2024, (d): Summer planting 2025. Uncoated urea (UCU, control), rice bran-coated urea (RBCU), gypsum-coated urea (GCU), and cement-coated urea (CCU); HSD5% = 0.651.

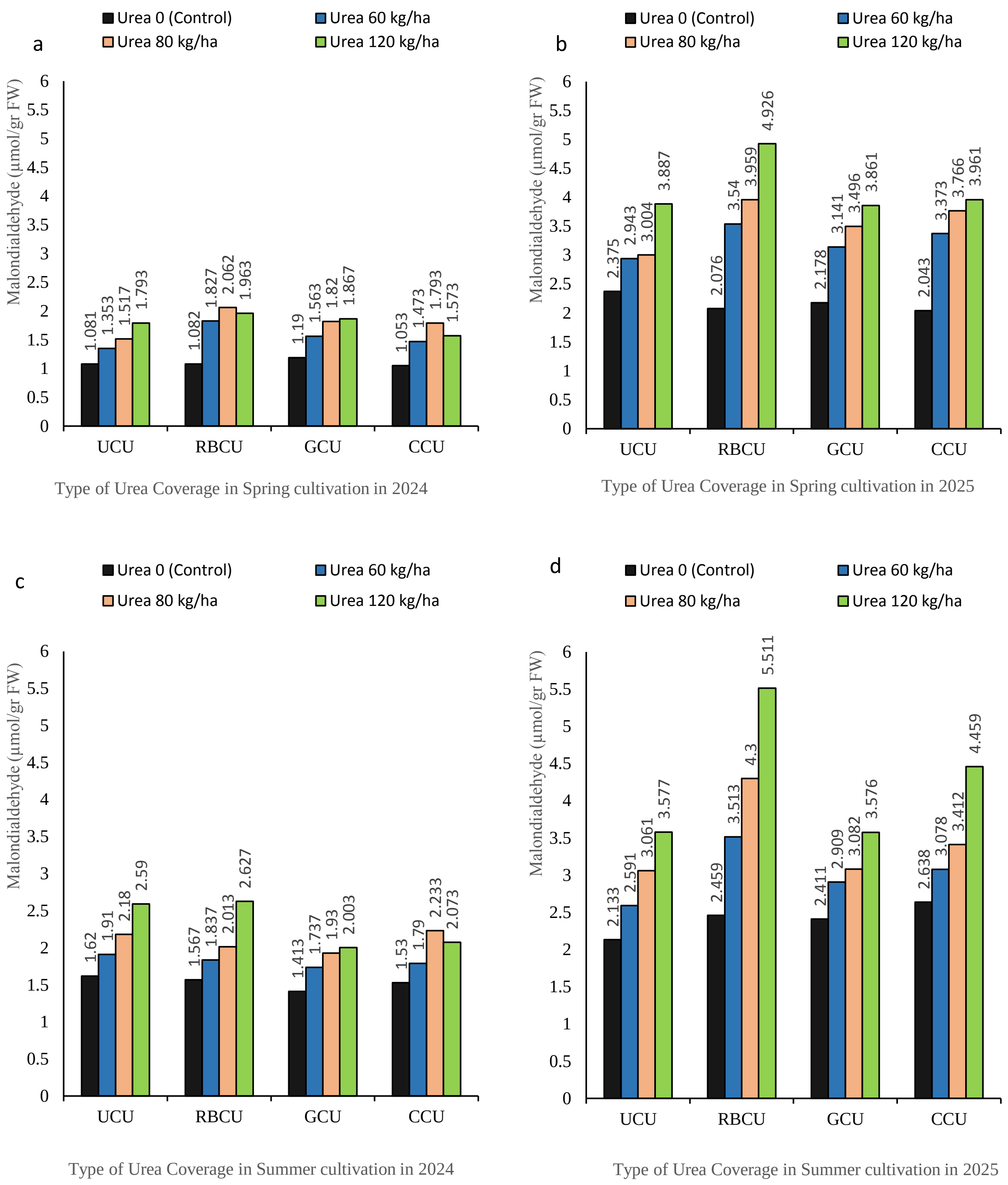


Figure 4. Interactive effect of year, planting season, coating type, and urea level on malondialdehyde content; (a): Spring planting 2024, (b): Spring planting 2025, (c): Summer planting 2024, (d): Summer planting 2025. Uncoated urea (UCU, control), rice bran-coated urea (RBCU), gypsum-coated urea (GCU), and cement-coated urea (CCU); HSD5% = 0.203.

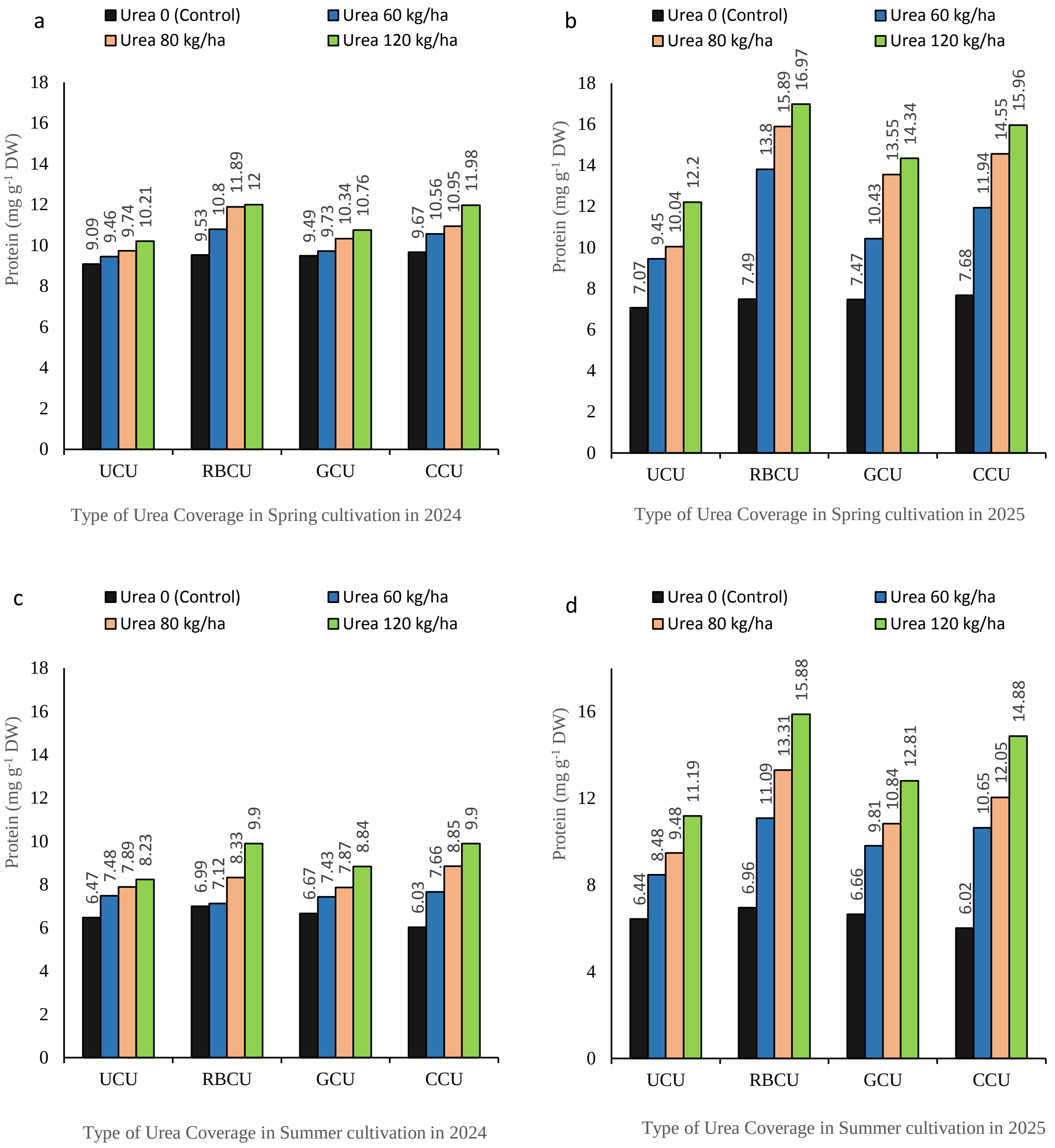


Figure 5. Interactive effect of year, planting season, coating type, and urea level on total soluble protein content; (a): Spring planting 2024, (b): Spring planting 2025, (c): Summer planting 2024, (d): Summer planting 2025. Uncoated urea (UCU, control), rice bran-coated urea (RBCU), gypsum-coated urea (GCU), and cement-coated urea (CCU); HSD5% = 0.196.

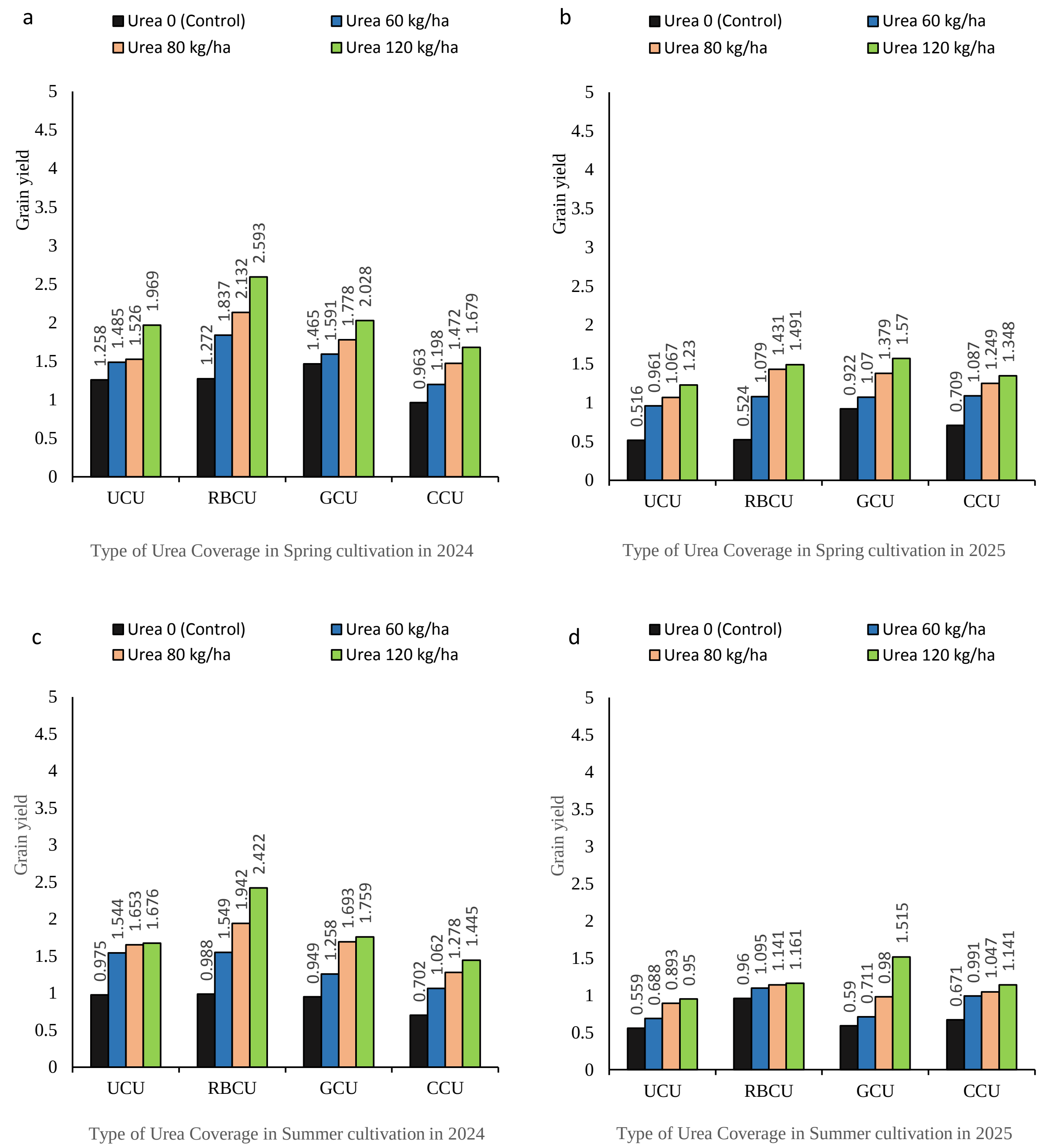


Figure 6. Interactive effect of year, planting season, coating type, and urea level on grain yield; (a): Spring planting 2024, (b): Spring planting 2025, (c): Summer planting 2024, (d): Summer planting 2025. Uncoated urea (UCU, control), rice bran-coated urea (RBCU), gypsum-coated urea (GCU), and cement-coated urea (CCU); HSD5% = 0.262.

to spring. Also, RBCU at 80 kg urea ha^{-1} tended to show higher POD activity than UCU, particularly in 2025. CAT plays a central role in H_2O_2 detoxification, preventing the formation of highly reactive hydroxyl radicals (Mittler 2017). CAT and POD activities generally declined in uncoated urea treatments during summer planting, indicating reduced ROS-scavenging capacity under heat stress, consistent with previous reports in cereals (Apel and Hirt 2004; Hasanuzzaman *et al.* 2013). In contrast, the sustained enzymatic activity observed under RBCU suggests that synchronized nitrogen release maintained enzyme synthesis and turnover during reproductive stages, thereby stabilizing redox homeostasis under fluctuating thermal conditions.

The accumulation of soluble phenolics tended to be higher under RBCU in summer, reinforcing the non-enzymatic antioxidant shield. Enhanced phenolic accumulation under RBCU further contributed to redox homeostasis by providing additional non-enzymatic antioxidant capacity. Phenolic compounds are known to play a key role in scavenging free radicals and protecting cellular membranes under abiotic stress conditions by stabilizing lipid bilayers and neutralizing reactive intermediates (Michalak 2006). This aligns with findings in other crops where enhanced phenolic metabolism is linked to improved stress tolerance (Sharma *et al.* 2019), and underscores the success of RBCU in fueling this N-dependent secondary metabolic pathway (Kabiri *et al.* 2016).

The key finding of this work is the functional link between the RBCU-induced antioxidant upregulation and the mitigation of oxidative membrane damage. RBCU-treated plants tended to show comparable MDA content to UCU in 2014, despite their outperformance in CAT, POD, total phenols, total soluble proteins, and grain yield, suggesting a protective effect against membrane lipid peroxidation. Although the 60 kg urea ha^{-1} rate occasionally exhibited lower absolute MDA values, it did not sustain comparable grain yield or protein accumulation under heat stress. The 120 kg urea ha^{-1} rate enhanced yield but showed elevated lipid peroxidation relative to 80 kg. Therefore, the 80 kg rate represents a functional compromise, simultaneously supporting productive metabolism while avoiding excessive oxidative burden. The observed MDA levels reflect a dynamic balance between ROS production and scavenging. The higher metabolic activity and growth supported by optimal RBCU (evidenced by increased protein content) may inherently generate more ROS substrate, a phenomenon noted in other studies under stress (Mittler 2017, Zanganeh and Jamei 2020). Thus, the relative containment of MDA, rather than its absolute reduction to control levels, indicates that the primed antioxidant system was functionally effective in protecting membranes under intense field heat, a conclusion supported by similar assessments of oxidative damage in applied physiological studies (Nasibi *et al.* 2020).

Improved protein accumulation under RBCU indicates more efficient nitrogen assimilation and reduced stress-induced protein degradation. Nitrogen availability has been directly linked to the synthesis of stress-responsive proteins and enzymes, which are essential for maintaining metabolic stability under adverse environmental conditions (Xu *et al.* 2012). Ultimately, this integrated physiological resilience (encompassing enhanced enzymatic and non-enzymatic antioxidants, reduced membrane damage, and preserved nitrogen metabolism) translated into a yield advantage under heat stress. This yield advantage can be attributed to the combined effects of enhanced antioxidant defense, reduced oxidative damage, and improved nitrogen use efficiency.

While RBCU at 120 kg urea ha^{-1} resulted in the highest grain yield in summer 2024, MDA levels were significantly higher than with 80 kg urea ha^{-1}, suggesting a trade-off between yield and oxidative balance. Differences among treatments in summer 2025 for grain yield were smaller. The 80 kg urea ha^{-1} rate tended to provide a balanced combination of yield, antioxidant activity, and lipid peroxidation control across both years, representing a physiologically stable option rather than a strictly "optimal" rate (Raun and Johnson 1999; Xu *et al.* 2012). This finding resonates with research on other crops, such as quinoa, where optimal N levels were pivotal for maintaining yield under drought stress (VaziriMehr *et al.* 2024).

Our results suggest that RBCU generally performed better than the control and other coating treatments in both seasons, suggesting its role as a slow-releasing fertilizer. However, it may also function as a physiological conditioner that enhances the intrinsic stress tolerance of millet. These findings highlight the importance of integrating nutrient management strategies with physiological stress mitigation approaches to sustain crop productivity under rising temperature regimes.

Conclusion

Across two experimental years, RBCU-mediated nitrogen synchronization in proso millet:

- Moderated oxidative stress under summer heat, tending to maintain higher CAT and POD activities, enhancing total soluble phenols, reducing MDA in the summer of 2024, and supporting protein content compared with UCU.
- Yield was generally improved under heat stress relative to the unfertilized control, although differences among RBCU rates were smaller in some cases, indicating that synchronizing nitrogen availability can support both physiological stability and agronomic performance.
- A physiologically balanced nitrogen supply, rather than maximal input, tended to sustain performance under recurrent summer heat, with 80 kg urea ha^{-1} providing relatively stable responses across years.
- Supported growth and yield under optimal spring conditions, suggesting that RBCU provides a steady nutrient supply even under non-stress conditions.

Overall, these results highlight the potential of controlled-release nitrogen fertilizers as adaptive management tools to stabilize crop productivity across variable temperature conditions.

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Author Contribution

Shrooq Abbas conducted the experiment and wrote the manuscript with support from Sirous Hassannejad. Sirous Hassannejad supervised the project. Soheila Poorheidar Ghafarbi helped in supervising the project. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no known competing interests with any individuals or organizations concerning the subject of this article.

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