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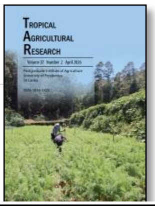

RESEARCH

Effect of single application of rice husk biochar and seasonal application of Urea on Grain Nutritional and Morphometric Traits of Rice: four seasonal field experiment

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ABSTRACT

A four-season field experiment was conducted for rice variety At 362 from the 2023 *Yala* season to the 2024/25 *Maha* season in the low-country dry zone of Sri Lanka, to examine the effects of single application of rice husk biochar and seasonal urea applications on grain nutritional and morphometric traits of rice. Grain amylose, iron (Fe) and ash percentages, antioxidant capacity, grain width, grain length, length-to-width ratio, grain thickness, equivalent diameter, grain volume, grain surface area and grain sphericity were measured. Four biochar rates (0, 1, 2, and 3 t/ha) and the recommended rate of partially burnt paddy husk (625 kg/ha), along with four urea levels (0, 67.5, 157.5 and 225 kg/ha), were arranged in a split-plot design with three replicates. The analysis of variance showed that rice husk biochar had a significant effect on all measured parameters across the four seasons. The application of urea also had a strong impact on some parameters such as antioxidant capacity, Fe content, grain volume, grain length and grain length-to-width ratio, although it did not always significantly affect on other measured parameters. Seasonal variation significantly influenced all measured parameters. Regarding interactions, the biochar × urea and urea × season effects were not significant, while the biochar × season interaction was significant for ash and Fe, indicating that the effect of biochar varied across seasons. The three-way interaction (biochar × urea × season) was not significant for any parameter. Dunnett's test results indicated that most traits were significantly improved at higher levels of rice husk biochar compared to the partially burnt paddy husk treatment, except for antioxidant capacity. Overall, 3 t/ha of rice husk biochar combined with recommended urea level performed better than recommended rate of partially burnt paddy husk in enhancing and maintaining grain nutritional and morphometric traits over four seasons under low-country dry zone conditions in Sri Lanka.

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INTRODUCTION

Rice (*Oryza sativa* L.) serves as a source of essential nutrients and energy for billions of people in the world. With the improvement of living standards among people, there is a notable surge in the demand for high-quality rice. Rice grain quality is primarily measured based on four main characteristics, namely appearance, milling, cooking and nutritional quality. Modern rice farming often relies too heavily on chemical fertilizers to maximize production (Iqbal *et al.,* 2022). However, the heavy use of synthetic N fertilizers such as urea leads to low nitrogen-use efficiency, and causes serious environmental problems, including surface and groundwater pollution, soil acidification, and increased greenhouse gas emissions (Ali *et al.,* 2020a; Ali *et al.,* 2022). Moreover, the improper use of N fertilizer can severely degrade soil health (Francis, 2022) and negatively affect crop growth and grain quality (Ali *et al.,* 2022; Iqbal *et al.,* 2022).

The application of biochar as a soil amendment is globally recognized as an effective approach to addressing these issues (Alburquerque *et al.,* 2013; Haque *et al.,* 2022). Biochar is a stable and efficient soil amendment, produced from various biomass sources using a process called pyrolysis (Sharma *et al.,* 2025; He *et al.,* 2024). Producing biochar using rice husk is a sustainable waste-recycling practice in rice cultivation (Asadi *et al.,* 2021). Researchers have recognized rice husk biochar as an efficient soil amendment due to its high pH (8.67) (Gowthami *et al.,* 2022), carbon content (34.91% to 43.93%) (Karam *et al.,* 2022), and silicon concentration (15-20%) (Gowthami *et al.,* 2022). Therefore, adding biochar can improve soil pH, organic carbon, and cation exchange capacity (Ali *et al.,* 2022; Haque *et al.,* 2022). Therefore, the application of biochar alone can create a better environment for plant growth (Ali *et al.,* 2021). The benefits of applying biochar with N fertilizer go beyond just yield; it also improves the quality of the harvested rice grain (Ali *et al.,* 2022; Gong *et al.,* 2020). However, the biochar effect on grain quality is a complex phenomenon and experimental findings are inconsistent, depending on soil characteristics, biochar type and application rates (He *et al.,* 2024). For instance, He *et al*. (2024) suggested in a meta-analysis that the application of biochar can increase grain milling and appearance qualities. They also reported that the biochar derived from husk and shell has been linked to better processing and appearance qualities. However, according to them there was a statistically insignificant impact on amylose content (AC), protein content (PC) and gel consistency of grains. In contrast to these results Iqbal *et al.* (2022) showed improvements in grain starch content (SC), AC, and PC with the application of biochar. Ali *et al.* (2020) have also reported a positive impact on starch-related enzyme activities. According to Chen *et al.* (2023), higher biochar rates and the combination of biochar and N fertilizer significantly affected starch viscosity characteristics.

The quality of rice grain is vital for consumer satisfaction and impacts its market value. It includes physical appearance, nutritional value, cooking, and eating qualities (Ali *et al.,* 2020b; Ali *et al.,* 2022). However, even though many studies have documented how biochar affects soil health and yield, there is a lack of research that specifically looks at long-term field responses regarding grain quality traits. Given these points, we hypothesized that combining rice husk biochar with chemical N fertilizer would improve the nutritional and morphometric traits of rice grain by optimizing soil conditions and boosting nutrient use. Therefore, the objective of this study was to analyze the effect of a single application of different levels of rice husk biochar and seasonal applications of urea on selected grain nutritional and morphometric traits of rice for four consecutive seasons under low-country dry zone conditions in Sri Lanka.

METHODOLOGY

A field experiment was conducted at the Regional Rice Research Station in Ambalantota, Sri Lanka over four seasons: 2023 *Yala* (S1), 2023/24 *Maha* (S2), 2024 *Yala* (S3), and 2024/25 *Maha* (S4) seasons for the rice variety At 362. Pre-germinated seeds were broadcasted at the rate of 100 kg/ha. The treatments included four levels of biochar applications: 0, 1, 2, and 3 t/ha designated as BC0, BC1, BC2 and BC3, respectively. These levels were chosen to include a control and a range of agronomically feasible, literature-supported application levels, enabling evaluation of dose–response effects and identification of an optimum rate for rice production. As a separate treatment the Department of Agriculture (DOA), Sri Lanka, recommended partially burnt paddy husk rate (625 kg/ha) (Yaya programme, 2019) was also included to compare the difference between rice husk biochar and partially burnt paddy husk effects. There were also four levels of urea (0, 30, 70, and 100%; which means 0, 67.5, 157.5, and 225 kg/ha; U0, U30, U70 and U100 respectively). All fertilizers, except urea, were applied in uniform amounts according to DOA recommendations. The treatments were arranged in a split-plot design with three replicates. The main plot factor was biochar levels, and the subplot factor was urea levels. Biochar was produced by pyrolyzing rice husk at approximately 500 °C for 8 hours, while partially burnt paddy husk was made through open burning. Rice husk biochar was applied once in S1, and urea was applied each season. Rice husk biochar and partially burnt paddy husk were broadcasted after land preparation and mixed with soil in S1. Manual land preparation was done up to 9 inches in other three seasons separately in each plot. Grain quality assessments were done ten weeks after harvesting rice.

Grain morphometric traits

The equivalent diameter (ED) (Equation 1), sphericity (GSP) (Equation 2), surface area (GSA) (Equation 4 and 5) and grain volume (GV) (Equation 3) were determined using mathematical expressions (Varnamkhasti et al., 2008). Grain length (GL), width (GW), length-to-width ratio (L/W R), grain thickness (GT), were measured using a vernier caliper (minimum reading 0.01 mm) after dehusking grains. Thousand grains weight (TGW) was measured using randomly selected grains.

Grain nutritional traits

Five grams of oven-dried rice grain powder from each treatment was added into a clean crucible, which was placed in a muffle furnace and heated up to 550 ℃ and kept for 12 hours, and ash content (ASH) was measured following the procedure described by Park (1996). The amylose (AMY) and iron (Fe) percentages of the grains were assessed through standard procedures. AMY was determined by using spectrophotometric method by measuring UV absorption at 620 nm and analyzing the color development of the starch solution, following the method described by Juliano (1971). Fe was measured through a spectrophotometric method by evaluating the reduction reaction and color development under UV light at 480 nm, as defined by Paul (1995). Standard curves of FeSO4 and potato amylose were utilized to determine the precise values. Ferric molecule reducing antioxidant power (FRAP) of rice grain extracts was used to measure the antioxidant capacity (AO) through the procedure described by Benzie and Szeto (1999). FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM FeCl3.6H2O in the ratio of 10:1:1. Rice extracts in 80% methanol were mixed with working FRAP reagent and incubated at room temperature. Absorbance was read at 593 nm under UV radiation against Fe+3 as a standard calibration, and the results were expressed as ferric reducing antioxidant capacity (μM/mL).

$$\mathrm{ED} = \left(\mathrm{L}\frac{(\mathrm{W}+\mathrm{T})^2}{4}\right)^{1/3} \ldots\ldots\ldots.\ldots \text{(Equation 1)}$$

$$\mathrm{GSP} = \frac{(\mathrm{LWT})^{1/3}}{\mathrm{L}} \ldots\ldots\ldots.\ldots\ldots\ldots.. \text{(Equation 2)}$$

$$\mathrm{GV} = 0.25\left[\left(\frac{\pi}{6}\right)\mathrm{L}(\mathrm{W}+\mathrm{T})^2\right] \ldots\ldots \text{(Equation 3)}$$

$$\mathrm{GSA} = \frac{\pi \mathrm{BL}}{(2\mathrm{L}-\mathrm{B})} \ldots\ldots\ldots\ldots\ldots\ldots \text{(Equation 4)}$$

$$\mathrm{B} = \sqrt{\mathrm{WT}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(Equation 5)}$$

Where,
ED - Equivalent diameter
L - Grain Length
W - Grain width
T - Grain thickness
GV - Grain volume
GSP – Grain sphericity
GSA - Grain surface area
π - 22/7

Statistical analysis

Data was analyzed using ANOVA according to the split-plot design including all treatments. Mean separation among rice husk biochar and urea treatment combinations was performed using the LSD test. In addition, Dunnett's test was applied as a planned comparison to evaluate each rice husk biochar treatment against the partially burnt paddy husk control. All statistical analysis and graphical presentations were conducted using Minitab 21.

RESULTS AND DISCUSSION

Single applications of rice husk biochar and seasonal applications of urea showed significant effects on the nutritional and morphometric traits of rice grains across four consecutive seasons. The experimental site was exposed to a tropical monsoon climate with an average annual rainfall of 1250 mm.

Effect of rice husk biochar and urea on grain nutritional traits across four consecutive seasons

AMY, Fe, AO and ASH were measured as nutritional traits in the current study. Biochar application and season (S) significantly ($p < 0.05$) improved all these nutritional traits indicating fresh and residual effects of biochar and the influence of environmental conditions in different seasons on the nutrient biosynthesis pathways of rice (Figure 1 and Table 1). Although N is one of the key elements of plant biological processes, urea alone had a significant effect only on AO and Fe (Table 1). The interaction effect of BC×S was significant for all traits measured except AO (Figure 1 and Table 1). All other interactions were not significant for any nutritional traits measured.

Amylose percentage is a critical factor determining the texture and quality of cooking rice; for instance, starch with a low amylose concentration produces soft sticky rice, is generally easier to digest and absorb (Zhang *et al.,* 2023). Therefore, high amylose rice has a lower glycemic index and produces firm, separate grains after cooking. In the current study, the highest AMY was observed in the BC3U100 treatment in S2 (29.4%). The variation of AMY ranged from 26.6% to 29.4% (Figure 1) among treatments. Treatments with rice husk biochar showed significant increase in AMY, and the effects were more pronounced in S1 and S2, indicating that fresh biochar had a greater effect compared to residual biochar in S3 and S4.

According to the literature, the effect of applying biochar and urea on AMY content has shown mixed results, with both increases and decreases reported. Studies on noodle rice found significant increase in amylose content compared to the no cassava straw biochar treatment (Ali *et al.,* 2020a). As they described, the increase in amylose content was due to improvements of physiological traits such as net photosynthetic rate, total root length, total average root diameter, root surface area, and root volume during the heading and maturity stages.

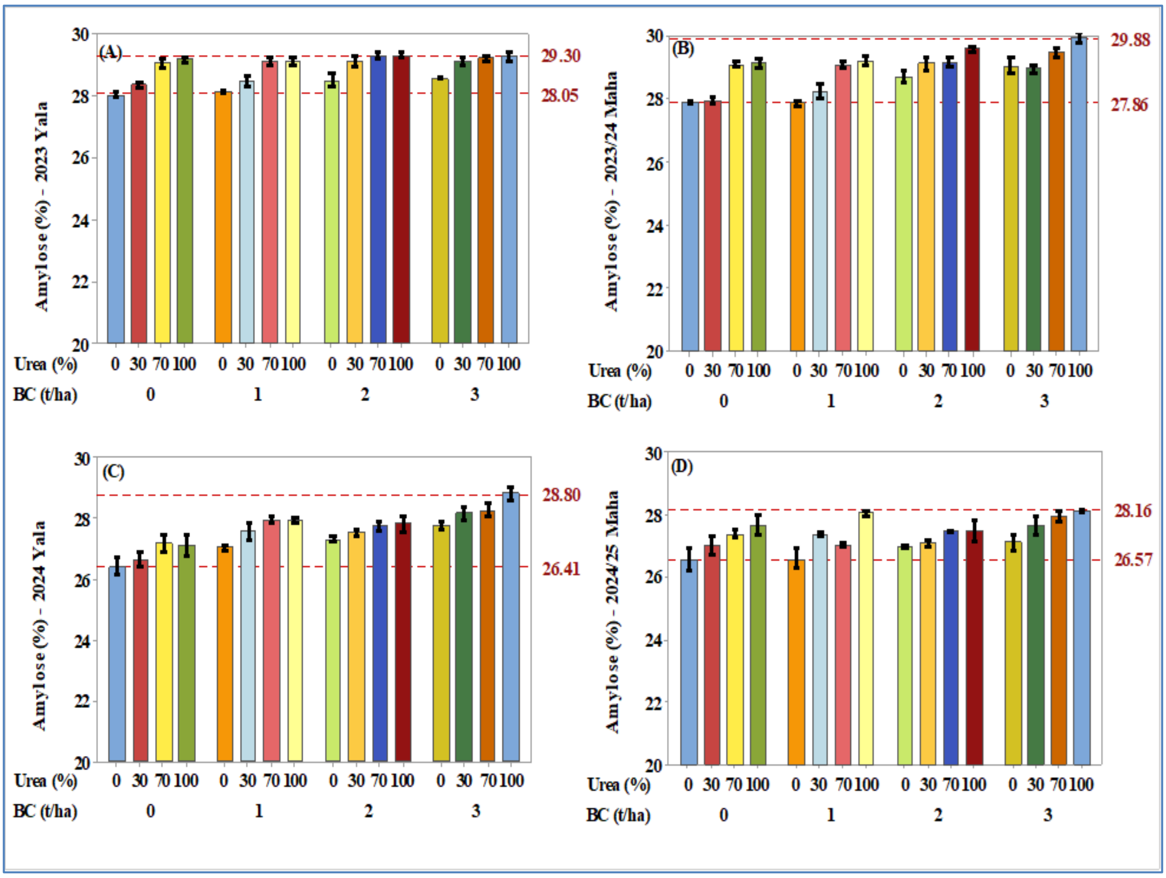

Figure 1: Effect of rice husk biochar and urea levels on grain amylose percentage in four consecutive seasons. Four graphs represent four seasons. Error bars represent the standard error of the mean. Numbers from 0-3 and 0, 30, 70 and 100 in x-axis represent the four biochar and urea levels added respectively. BC, rice husk biochar; U, urea; S, season. Main effects of BC, S and interaction BC×S were significant (P<0.05), whereas main effect U and interactions BC×U, U×S, BC×U×S were not significant.

In contrast, Gong *et al*. (2020) reported a decrease in amylose content with the application of rice husk biochar. It is evident that the influence of biochar and nitrogen on AMY is linked to nutrient availability and starch synthesis enzymes. As reported by Gong *et al*. (2020), biochar treatments decreased the activity of granule-bound starch synthase (GBSS) and increased the activities of soluble starch synthase (SSS), starch branching enzyme (SBE), and starch debranching enzyme (DBE). However, in the absence of biochar, the activities of ADP-glucose pyro phosphorylase (AGPase), SBE, SSS, and DBE were higher in the high-nitrogen treatment, indicating that nitrogen alone also influences these pathways (Ali *et al.,* 2020a). Zhang *et al.* (2023) reported that the application of biochar enhances the effect of N fertilizer, potentially increasing the activities of SBE and soluble SSS, which are responsible for branched-chain starch synthesis and thus decreasing amylose content. According to Ali *et al.* (2020a) the increase in grain quality traits, including amylose content, following biochar application may be due to the enhanced nitrogen uptake, improved soil physicochemical properties, increased nutrient availability, and increased water content in rice kernels through superior water-use efficiency. Furthermore, Ali *et al.* (2022) reported that biochar application combined with urea improved rice starch metabolism enzymes and related gene expressions, thereby influencing grain quality traits such as amylose content. A meta-analysis authored by He *et al.* (2024)

concluded that the overall impact of biochar application on amylose content in rice was statistically in significant. For example, Du *et al.* (2024) reported no significant effect on amylose content in rice among treatments with and without biochar application However, in a two-year experiment, Chen *et al.* (2023) reported a decrease in amylose content in first year and an increase in the second year under low biochar treatments, while a further reduction was observed at high biochar rates. An experiment conducted under drought stress conditions resulted in higher amylose content (Chakraborty *et al.,* 2021). Nitrogen fertilizer alone also affects amylose content (Iqbal *et al.,* 2022). Overuse of synthetic N fertilizer can lead to a continuous decrease in apparent amylose content (Iqbal *et al.,* 2022), but this decrease is less pronounced with the combined application of urea and biochar (Zhang *et al.,* 2023). In contrast, the fitted and forecasted responses of AMY (Figure 2D) to increased levels of biochar showed a nonlinear response. Increasing biochar beyond 3 t ha^{-1} showed slight decline, and this decline started slightly before the 3 t /ha level. These results indicate that rice husk biochar at around 3 t/ha represent the most effective rate for improving AMY of rice under the low-country dry zone conditions in Sri Lanka.

Antioxidant capacity (AO) reflects the grain's ability to neutralize free radicals, which is linked to the prevention of chronic diseases such as cancer and diabetes. Many studies in the literature focus on antioxidant enzyme activities such as superoxide dismutase, peroxidase, and catalase, as well as the concentration of the lipid peroxidation marker malondialdehyde (Du *et al.,* 2024), rather than the total AO of grains. Biochar application notably increases these enzyme activities (Du *et al.,* 2024). In the current study, significantly higher AO was observed in the BC3U100 treatment in each season. The increments compared with BC0U100 were 2.02%, 2.16%, 1.47% and 0.62% in each season, respectively. These results indicate that rice husk biochar applications can increase AO in rice, with fresh biochar showing a greater effect than residual biochar. In S3 and S4, the increase in grain AO was lower compared to S1 and S2; however, it is important to note that the effect of rice husk biochar persisted through S4 (Table 1).

Rice is the staple crop of half of the world's population. Therefore, higher Fe content in rice grains can contribute to nutritional security and biofortification goals. However, there is no explicit information in the literature regarding how the rice husk biochar combined with urea affects ash and Fe content in harvested rice grains. Existing studies mainly discuss Fe in the context of soil dynamics and remediation, and ash content in relation to the biochar material itself. Studies have shown that the ferrous sulfate concentrations in soil increase with biochar application (Chen *et al.,* 2023), whereas Yin *et al*. (2021) reported reduced Fe availability due to the increased soil pH following biochar application. According to these studies, combining biochar and urea can encourage rice root growth and increase organic acid secretion, helping the release of fixed divalent cations such as Fe, suggesting it may influence Fe availability for plants. Therefore, it is reasonable to assume that grain Fe content may rise with biochar application. However, Butnan and Vityakon (2023) studied Fe concentration in rice shoots after rice husk biochar application and reported a reduction. This reduction was linked to the antagonistic effects of Si, abundant in rice husk biochar (Butnan and Vityakon, 2023). Specifically, rice husk biochar reduced the accumulation of Fe, Cd, Zn, Pb, and ash in rice seedlings by affecting Fe plaque formation on roots and the subsequent accumulation of these elements (Karam *et al.,* 2022). Ash content represents the total mineral content, including Ca, Mg, K, P, Zn, and Fe, in rice grains. Although mineral bioavailability depends on many factors, higher ash content generally indicates improved nutritional value of rice grains. While previous studies reported ash content of rice husk biochar, but no studies have evaluated ash content in rice grains following biochar application.

Table 1: Antioxidant capacity, Fe content and ash percentage as influenced by different rice husk biochar and urea fertilization levels over 2023 *Yala* to 2024/25 *Maha*.

Treat.	AO× 10^{-2} µM/mL 2023 *Yala*	AO × 10^{-2} µM/mL 2023/24*Maha*	AO × 10^{2} µM/mL 2024 *Yala*	AO × 10^{-2} µM/mL 2024/25 *Maha*	Fe µg/g 2023 *Yala*	Fe µg/g 2023/24 *Maha*	Fe µg/g 2024 *Yala*	Fe µg/g 2024/25 *Maha*	ASH % 2023 *Yala*	ASH % 2023/24 *Maha*	ASH % 2024 *Yala*	ASH % 2024/25 *Maha*
BC0U0	8.43^{f}	7.16^{g}	9.16^{e}	7.61^{g}	18.77^{i}	18.79^{f}	16.84^{j}	17.64^{f}	45.33^{g}	45.51^{j}	52.00^{h}	37.33^{g}
BC0U30	8.82^{de}	7.30^{ef}	9.41^{d}	7.89^{ef}	19.57^{h}	19.28^{e}	17.69^{ghi}	18.28^{e}	50.66^{f}	47.03^{j}	54.33^{gh}	38.00^{g}
BC0U70	8.81^{de}	7.38^{cde}	9.48^{c}	8.10^{bcd}	19.54^{h}	19.32^{e}	18.07^{def}	18.36^{e}	55.33^{e}	49.52^{i}	57.00^{defg}	42.00^{fg}
BC0U100	8.93^{c}	7.40^{c}	9.54^{b}	8.10^{bcd}	20.14^{f}	19.89^{d}	18.36^{cd}	18.61^{de}	55.66^{e}	52.00^{h}	61.00^{c}	43.66^{f}
BC1U0	8.82^{cd}	7.26^{f}	9.37^{d}	7.78^{f}	19.85^{g}	19.77^{d}	17.80^{fgh}	18.18^{e}	57.00^{e}	51.51^{hi}	55.33^{fg}	39.00^{fg}
BC1U30	8.82^{cd}	7.00^{e}	9.41^{d}	7.85^{f}	20.13^{g}	20.18^{cd}	17.92^{efg}	18.58^{de}	60.66^{d}	55.00^{g}	56.00^{efg}	41.33^{fg}
BC1U70	8.93^{c}	7.32^{def}	9.50^{bc}	8.00^{de}	21.05^{d}	20.52^{bc}	18.27^{cd}	18.66^{de}	61.66^{d}	59.05^{f}	58.00^{de}	50.33^{de}
BC1U100	8.92^{c}	7.30^{ef}	9.51^{bc}	8.11^{bcd}	21.48^{bc}	20.84^{b}	18.37^{cd}	18.98^{d}	61.33^{d}	61.01^{ef}	59.00^{cd}	51.60^{cde}
BC2U0	8.81^{de}	7.40^{c}	9.42^{d}	7.84^{f}	20.14^{f}	19.90^{d}	17.41^{i}	18.59^{de}	60.66^{d}	60.50^{ef}	57.33^{def}	49.00^{e}
BC2U30	8.95^{c}	7.40^{c}	9.52^{bc}	8.10^{bcd}	20.93^{d}	20.68^{bc}	18.17^{cde}	19.67^{c}	62.00^{d}	62.52^{de}	58.33^{de}	51.33^{cde}
BC2U70	9.01^{b}	7.42^{bc}	9.51^{b}	8.00^{cde}	21.38^{c}	20.97^{b}	18.46^{bc}	19.94^{bc}	66.33^{c}	64.01^{cd}	64.33^{b}	53.00^{bcde}
BC2U100	9.02^{b}	7.42^{bc}	9.52^{b}	8.00^{cde}	21.52^{bc}	20.88^{b}	18.70^{b}	20.09^{b}	65.33^{c}	65.91^{b}	64.33^{b}	55.66^{bc}
BC3U0	8.83^{cd}	7.39^{cd}	9.49^{bc}	8.02^{cde}	19.86^{g}	19.87^{d}	17.58^{hi}	18.92^{d}	60.66^{d}	60.52^{ef}	56.00^{efg}	53.33^{bcde}
BC3U30	8.92^{c}	7.40^{c}	9.51^{b}	8.00^{cde}	20.65^{e}	20.56^{bc}	17.90^{efg}	19.62^{c}	62.33^{d}	64.03^{cd}	61.00^{c}	55.00^{bcd}
BC3U70	9.00^{b}	7.53^{a}	9.64^{a}	8.12^{bcd}	21.70^{ab}	20.83^{b}	18.41^{bc}	20.07^{b}	70.33^{b}	66.01^{bc}	64.00^{b}	57.33^{ab}
BC3U100	9.11^{a}	7.56^{a}	9.68^{a}	8.15^{ab}	21.84^{a}	21.56^{a}	18.86^{a}	20.63^{a}	73.33^{a}	68.50^{a}	67.00^{a}	61.00^{a}
BC		s**					s**				s*	
U		s**					s**				ns	
S		s**					s**				s**	
BC×U		ns					ns				ns	
U×S		ns					ns				ns	
BC×S		ns					s*				s*	
BC×U×S		ns					ns				ns	

Note: AO, antioxidant capacity; Fe, content; ASH, ash content; BC, biochar; U, urea; S, season; BC0, BC1, BC2 and BC3, represent 0, 1, 2 and 3t/ha biochar levels respectively while U0, U30, U70 and U100 represent 0, 30, 70 and 100% urea levels based on the recommended rates respectively. S, season; Values followed by the same letters, within the columns, are not significantly different at P<0.05. * Indicate the significant difference at P<0.05 and ** indicate P<0.01

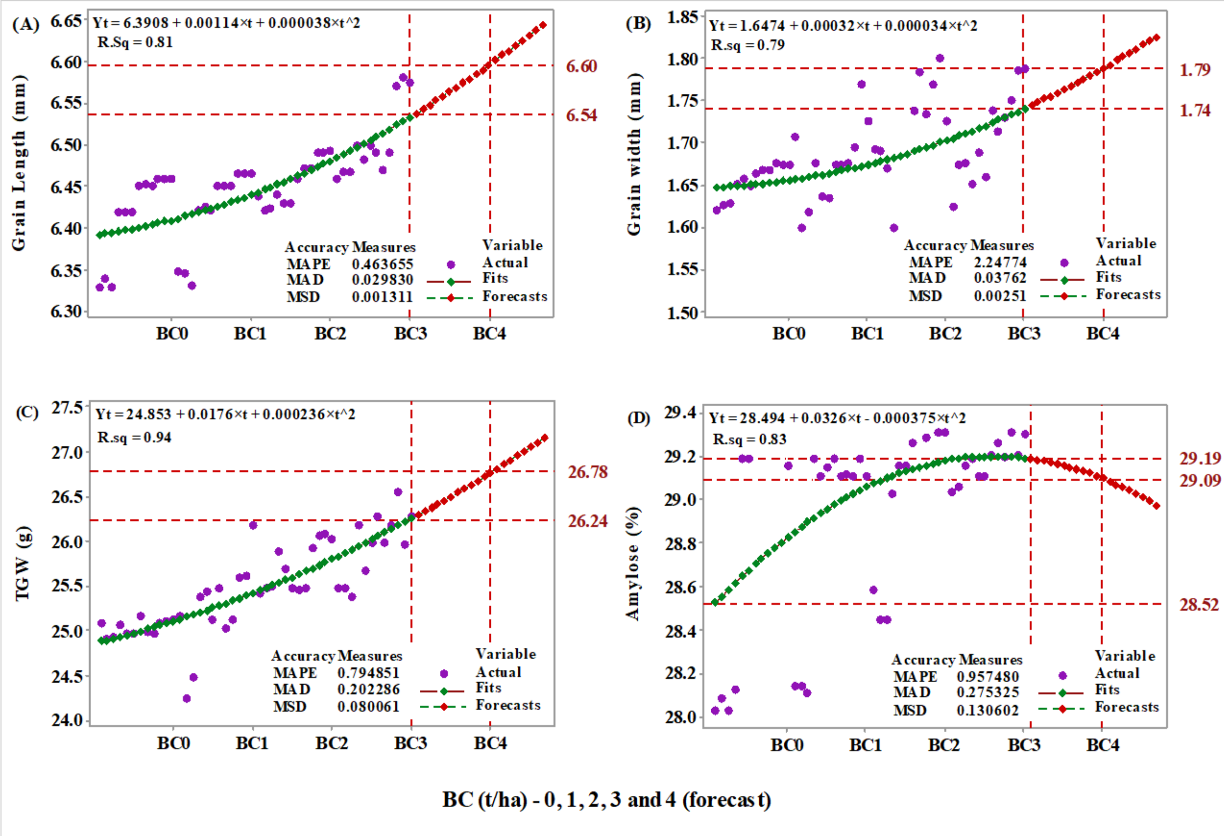

Figure 2: Forecasted effect of increasing rice husk biochar on (A) grain length (B) grain width, (C) 1000-grain weight (TGW) and (D) amylose percentage. The results received for urea and rice husk biochar levels of the current study were used for the forecast analysis. Dots represent the measured values from each treatment and the green lines indicate regression models fitted to the measured data, while the red lines represent the forecasted trends with increasing biochar application up to 4 t ha^{-1}. Model performance is described by quadratic regression equation (a fitted model) along with its R^2 value.

The BC3U100 treatment showed the significantly highest Fe and ASH across all seasons. Compared with BC0U100, Fe increased by 8.44%, 8.4%, 2.72% and 10.85%, while ASH increased by 31.75%, 31.73%, 9.83% and 39.72% in each season, respectively. We can assume that the fresh biochar appears to be more active and can increase mineral availability. The lower increase in Fe and ASH in S3 may be due to depletion of labile nutrients, with remaining minerals in a more stable, slower-releasing form. By S4, microbial activity, soil processes, and biochar weathering may have enhanced the release of these residual minerals. These findings indicate that residual biochar exerts a long-term effect on grain Fe and ASH content. The Pearson correlation matrix (Figure 03) among rice grain nutritional traits, exhibited strong positive correlations between Fe and AMY (r = 0.78) and ASH (r = 0.54), suggesting that enhanced Fe availability may improve grain starch quality. Similarly, ASH showed moderate correlation with TGW (r = 0.52) and GSP (r = 0.72), suggesting that heavier and rounder grains tend to accumulate more minerals.

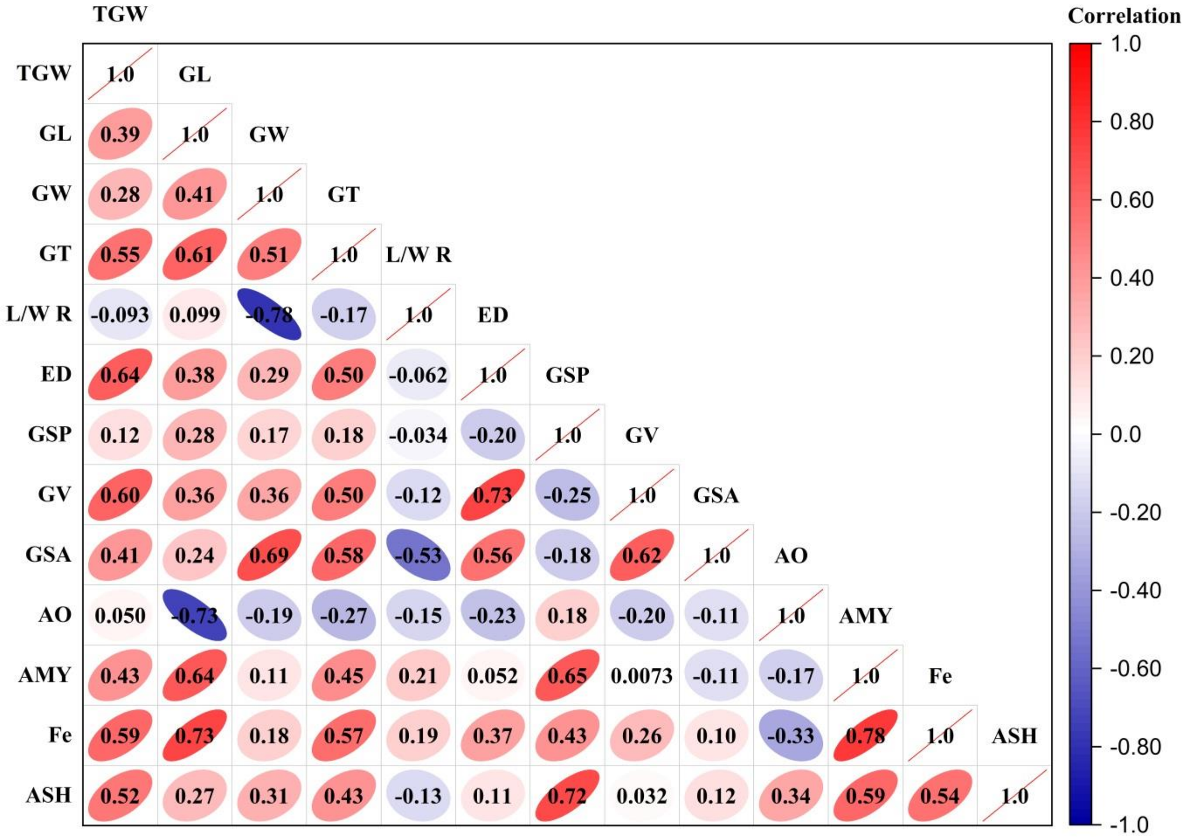

Figure 3: Heat map of the Pearson correlation matrix based on each quality parameter. TGW - thousand grains weight, GL - grain length, GW - grain width, GT - grain thickness, L/W R - grain length-to-width ratio, ED - equivalent diameter, GSP - grain sphericity, GV - grain volume, GSA - grain surface area, AO - antioxidant capacity, AMY - amylose content, Fe-ferrous content, ASH - ash content. Origin Pro software for WOS was used to produce the heat map.

Effect of rice husk biochar and urea on grain morphometric traits across four consecutive seasons

Grain morphometric traits such as thousand grain weight (TGW) (Figure 4), grain thickness (GT), equivalent diameter (ED) (Table 2), sphericity (GSP), grain volume (GV) and specific area (GSA) (Table 3) showed significant differences following biochar application. However, urea levels did not show any significant difference for these parameters except for GV (Table 3). Seasonal variations significantly affected all these traits, indicating the importance of climatic conditions. The significant interaction effect of U×S, on ED and BC×S, on GT indicate that urea and biochar effectiveness depends on environmental conditions. The significantly highest values for these parameters were observed in BC3U70 and BC3U100 treatments across all seasons, indicating the positive influence of higher amount of rice husk biochar and urea rates. Compared with BC0U100, TGW in BC3U100 increased by 4.35% in S1.

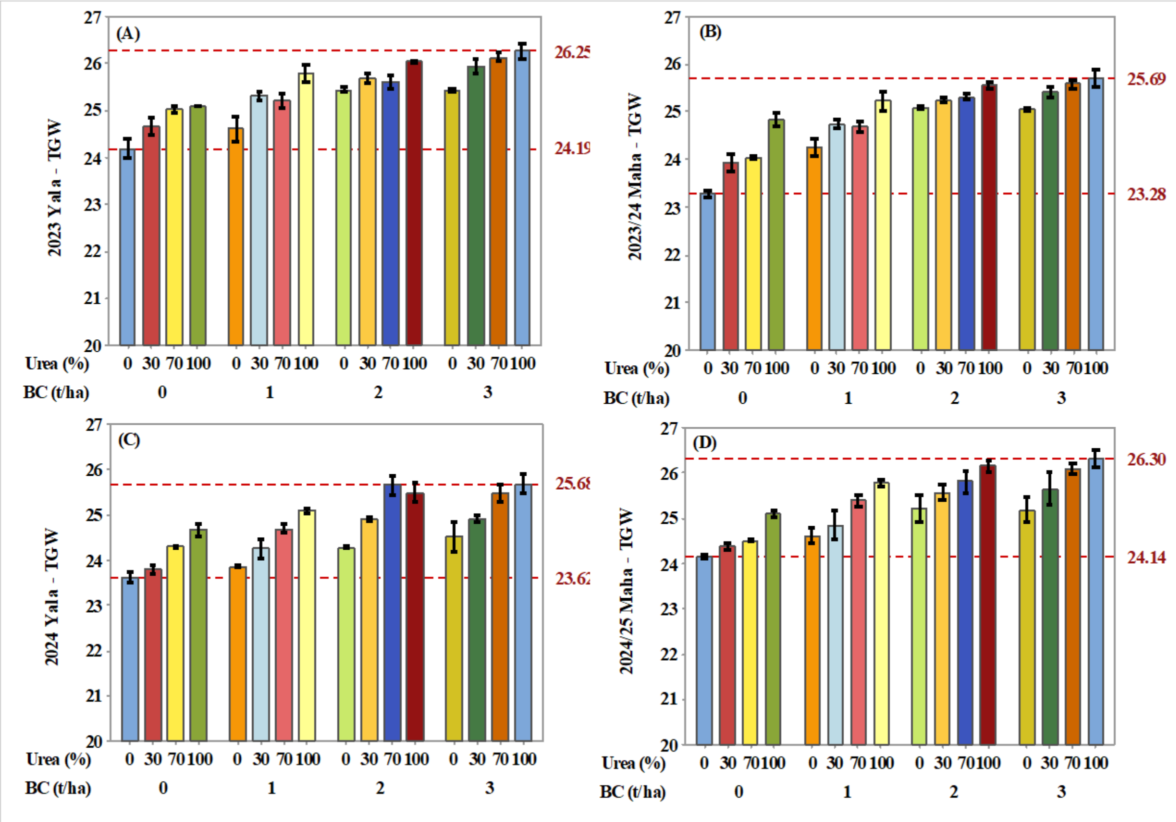

Figure 4: Effect of rice husk biochar and urea levels on 1000 grain weight (TGW) in four consecutive seasons. Four graphs represent four seasons. Error bars represent the standard error of the mean. Numbers from 0-3 and 0, 30, 70 and 100 in x-axis represent the four biochar and urea levels added respectively. BC, rice husk biochar; U, urea; S, season. Main effects of BC and S were significant (P<0.01), whereas main effect U and interactions BC×U, BC×S, U×S, BC×U×S were not significant.

The co-application of rice husk biochar and urea generally shows a tendency to increase the TGW of rice, particularly at optimal application rates, although this effect is not always statistically significant across all studies, and in some contexts, TGW is noted to be highly genetically controlled. Both pot and field experiments conducted by Ali *et al.*, (2020a) and Ali *et al.* (2021) showed an increment of TGW. In the field experiment the increment was only 6.4%. A study conducted in poor soils by Oladele *et al.* (2019) observed significant very high (16%) improvement in TGW indicating biochar performs well in poor soils characteristic of a large proportion of upland soils in sub-Saharan Africa, which are highly weathered, acidic, and possess low activity clays (such as kaolinite) with low native fertility. Similar to the present study, the application of 3 t/ha rice husk biochar in the experiment conducted by Kartika *et al.* (2018) also observed significant increment of TGW. However, Gowthami *et al.* (2022) reported no significant influence on TGW with the application of biochar and urea. According to them one possible reason cited for the lack of significant influence on TGW is believed to be largely genetically controlled, minimizing the role of management practices in manipulating this parameter.

A contrasting outcome has been reported by Koyama *et al.* (2016) and Chen *et al.* (2023) with the application of rice husk charcoal. They observed significantly increased brown rice yield due to a higher grain number and percentage of ripened grains but resulted in a decrease in the TGW. Similarly, a six-season experiment conducted by Huang *et al.* (2019) observed decreased TGW in first three seasons. Poor assimilate partitioning to the grain filling although the total biomass is increased has been mentioned as the reason for this reduction.

Table 2: Grain thickness and equivalent diameter of rice grains as influenced by different rice husk biochar and urea fertilization levels over 2023 *Yala* to 2024/25 *Maha*

Treat.	GT mm 2023 *Yala*	GT mm 2023/24 *Maha*	GT mm 2024 *Yala*	GT mm 2024/25 *Maha*	ED mm 2023 *Yala*	ED mm 2023/24 *Maha*	ED mm 2024 *Yala*	ED mm 2024/25 *Maha*
BC0U0	1.61^{g}	1.66^{g}	1.60^{g}	1.63^{g}	2.59^{f}	2.59^{h}	2.58^{e}	2.66^{h}
BC0U30	1.64^{fg}	1.72^{ef}	1.65^{ef}	1.66^{f}	2.64^{de}	2.61^{gh}	2.60^{de}	2.72^{fg}
BC0U70	1.67^{ef}	1.73^{ef}	1.67^{ef}	1.69^{e}	2.64^{de}	2.62^{fgh}	2.63^{cd}	2.73^{fg}
BC0U100	1.68^{ef}	1.74^{de}	1.67^{de}	1.70^{de}	2.61^{ef}	2.70^{bc}	2.63^{cd}	2.78^{cde}
BC1U0	1.68^{ef}	1.69^{fg}	1.67^{de}	1.71^{de}	2.64^{de}	2.60^{gh}	6.63^{cd}	2.70^{gh}
BC1U30	1.69^{de}	1.78^{cd}	1.72^{cd}	1.71^{de}	2.65^{de}	2.60^{gh}	2.55^{f}	2.70^{gh}
BC1U70	1.69^{de}	1.78^{cd}	1.73^{bc}	1.72^{d}	2.71^{abc}	2.64^{fg}	2.57^{ef}	2.70^{gh}
BC1U100	1.73^{cd}	1.80^{bc}	1.73^{bc}	1.79^{c}	2.71^{abc}	2.66^{ef}	2.60^{de}	2.73^{fg}
BC2U0	1.69^{de}	1.74^{de}	1.63^{fg}	1.81^{bc}	2.69^{bc}	2.67^{d}	2.65^{bc}	2.75^{def}
BC2U30	1.71^{c}	1.79^{bc}	1.72^{cd}	1.80^{c}	2.69^{bc}	2.69^{c}	2.60^{de}	2.80^{bcd}
BC2U70	1.75^{bc}	1.80^{bc}	1.75^{bc}	1.82^{bc}	2.71^{abc}	2.70^{bc}	2.65^{bc}	2.80^{bcd}
BC2U100	1.75^{bc}	1.79^{bc}	1.75^{bc}	1.80^{c}	2.72^{abc}	2.72^{b}	2.65^{bc}	2.78^{b}
BC3U0	1.75^{bc}	1.72^{ef}	1.66^{ef}	1.69^{de}	2.69^{bc}	2.67^{def}	2.60^{de}	2.74^{efg}
BC3U30	1.77^{abc}	1.79^{bc}	1.75^{bc}	1.79^{c}	2.69^{bc}	2.71^{bcd}	2.65^{bc}	2.78^{cde}
BC3U70	1.80^{a}	1.83^{ab}	1.77^{ab}	1.83^{ab}	2.74^{ab}	2.74^{ab}	2.70^{a}	2.80^{bcd}
BC3U100	1.80^{a}	1.85^{a}	1.79^{a}	1.86^{a}	2.75^{a}	2.76^{a}	2.70^{a}	2.83^{a}
BC		s^{**}					s^{*}	
U		ns					ns	
S		s^{**}					s^{*}	
BC×U		ns					ns	
U×S		ns					s^{*}	
BC×S		s^{*}					ns	
BC×U×S		ns					ns	

Note: GT, grain thickness; ED, equivalent diameter; BC, biochar; U, urea; S, season; BC0, BC1, BC2 and BC3, represent 0, 1, 2 and 3t/ha biochar levels respectively while U0, U30, U70 and U100 represent 0, 30, 70 and 100% urea levels based on the recommended rates respectively. S, season; Values followed by the same letters, within the columns, are not significantly different at P<0.05. * Indicate the significant difference at P<0.05 and ** indicate P<0.01.

However, in later seasons number of panicles increased (Huang *et al.,* 2019), indicating the residual effect of biochar. The overall tendency for rice husk biochar and N co-application to increase TGW is supported by general improvements in nutrient use efficiency (Ali *et al.,* 2020b), soil quality parameters and root morphological attributes (Ali *et al.,* 2020a), leaf physiological traits and increased N accumulation (Ali *et al.,* 2021).

Figure 5 illustrates the changes in grain length (GL), width (GW) and length-to-width ratio (L/W R) in each season. For all these parameters biochar and urea levels significantly affect but there is no seasonal effect. The effects of rice husk biochar and urea on rice grain morphometric traits specifically GT, ED, GSA, GSP, and GV are not directly reported in previous studies. However, extensive data on GW, GL, and the L/W R, have been reported in many studies. Chen *et al*. (2023) observed decreased and no significant difference of GL in two seasonal experiments respectively. In the same experiment GW was significantly increased in both years. They have also observed negative correlations between GL and head milled rice rate and GW and amylose content. But in the present study there is a weak positive relationship (0.11) between GW and AMY. Anwari *et al.* (2019) also observed positive relationship between rough rice rate and L/W R. Ali *et al.* (2022) has also observed significant increment of GL under biochar amendments. However, they observed no significant difference in GW and grain area in the same experiment. The L/W R is a critical indicator of grain shape (short, medium, or long grain). The meta-analysis conducted by He *et al.* (2024) showed a general positive effect of biochar on grain L/W R (overall 1.4%). In specific conditions like rice varieties, soil conditions and amount of N fertilizer application the increment was more pronounced (He *et al.,* 2024).

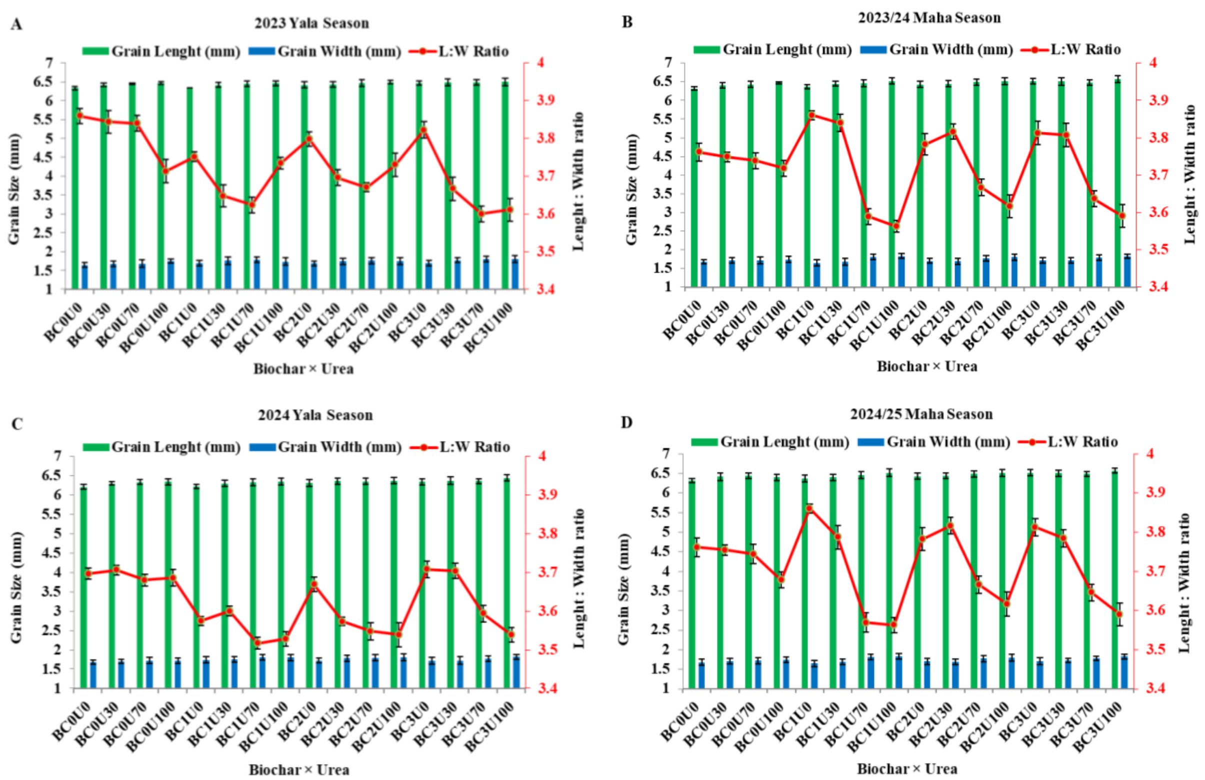

Figure 5: Effect of rice husk biochar and urea levels on grain length, grain width and grain length-to-width ratio four consecutive seasons. Four graphs represent four seasons. Error bars represent the standard error of the mean. BC, rice husk biochar; U, urea. For grain length and length-to-width ratio, main effects were significant (P<0.05), whereas all interactions were not significant. For grain width main effect BC and S were significant (P<0.05), whereas main effect U and all interactions were not significant.

The improvements in grain physical properties are associated with the physiological and nutritional benefits conferred by the rice husk biochar and N co-application, which enhances the capacity for grain filling. The combined application improves soil characteristics which increases nutrient availability and N uptake (Ali *et al.,* 2021; Ning *et al.,* 2022). These enhanced soil and nutrient conditions, along with improved root morphological attributes and photosynthetic rates, consequently, improve overall growth (Ali *et al.,* 2022b), leading to better filling of the grains and improved morphometric traits (Ali *et al.,* 2022b).

Further rice husk biochar is a source of plant-available Si. The increase in Si content in various rice tissues positively correlated with the higher grain yields observed in many studies (Olanrewaju *et al.,* 2024). While these attributes are not directly linked to grain characters, silicon plays a role in structural strength and promotes effective photosynthesis (Olanrewaju *et al.,* 2024; Zhao *et al.,* 2023), which supports the production of heavy, well-filled grains.

Figure 2 presents the fitted and forecasted responses of rice GL, GW and TGW (Figure 2A, 2B and 2C) beyond 3 t/ha rice husk biochar level. Fitted lines exhibited a steady and nearly linear increase in GL and GW, although the rate of improvement diminishes at higher levels. These results indicate positive response to the higher concentration of rice husk biochar. Figure 3 illustrates the Pearson correlation matrix among rice grain nutritional and morphometric traits measured in the current study. Among many attributes strong to weak positive correlations were observed. Positive correlations were observed among TGW with GL ($r = 0.39$), GW ($r = 0.28$), and GV ($r = 0.64$). These correlations suggest that heavier grains were associated with larger and more voluminous kernels. Similarly, GL showed a moderate association with GW ($r = 0.41$) and GT ($r = 0.51$), which confirms that the expansion of one dimension was generally accompanied by proportional increases in others.

Comparative effectiveness of rice husk biochar and partially burnt paddy husk on rice grain quality

As shown in Table 04, the comparison of rice husk biochar and partially burnt paddy husk demonstrated that rice husk biochar was more effective in improving grain quality traits. The Department of Agriculture, Sri Lanka recommends applying partially burnt paddy husk at a rate of 625 kg/ha (Yaya programme, 2019). Since partially burnt paddy husk is produced through uncontrolled incomplete burning with ample amount of oxygen, its carbon conversion efficiency is as low as 55% and ash content is high (Asadi *et al.*, 2021). Therefore, partially burnt paddy husk is considered a mixture of ash and high-carbon char. In this study, higher rice husk biochar rates (especially 2 and 3 t/ha) consistently outperformed partially burnt paddy husk in improving nutritional and physical grain traits. These results indicate that rice husk biochar is a more efficient and environmentally sustainable alternative to partially burnt paddy husk for use in low-country dry zone rice ecosystems in Sri Lanka.

Table 3: Grain sphericity, volume and surface area as influenced by different rice husk biochar and urea fertilization levels over 2023 *Yala* to 2024/25 *Maha*

Treat.	GSP % 2023 *Yala*	GSP % 2023/24 *Maha*	GSP % 2024 *Yala*	GSP % 2024/25 *Maha*	GV (mm^3) 2023 *Yala*	GV (mm^3) 2023/24 *Maha*	GV (mm^3) 2024 *Yala*	GV (mm^3) 2024/25 *Maha*	GSA (mm^2) 2023 *Yala*	GSA (mm^2) 2023/24 *Maha*	GSA (mm^2) 2024 *Yala*	GSA (mm^2) 2024/25 *Maha*
BC0U0	39.92^{f}	40.04^{e}	39.73^{g}	38.75^{g}	9.46^{h}	9.48^{h}	9.14^{g}	9.42^{j}	2.82^{g}	3.01^{g}	2.99^{g}	3.17^{gh}
BC0U30	40.05^{ef}	40.17^{de}	39.86^{fg}	38.85^{fg}	10.13^{g}	10.11^{g}	10.01^{f}	10.10^{i}	2.94^{f}	3.08^{f}	3.06^{fg}	3.21^{fg}
BC0U70	40.63^{bc}	40.43^{bcd}	40.12^{cdef}	39.22^{cde}	10.32^{defg}	10.31^{efg}	10.17^{ef}	10.27^{fgh}	3.00^{ef}	3.13^{ef}	3.12^{ef}	3.26^{ef}
BC0U100	40.68^{c}	40.58^{bc}	40.44^{bc}	39.33^{cd}	10.59^{cde}	10.31^{efg}	10.47^{cde}	10.27^{fgh}	3.02^{def}	3.16^{de}	3.14^{def}	3.30^{de}
BC1U0	40.07^{ef}	40.35^{cde}	40.20^{cde}	39.06^{defg}	10.13^{g}	10.42^{def}	10.28^{def}	10.11^{i}	3.04^{def}	3.07^{f}	3.16^{de}	3.30^{de}
BC1U30	40.15^{ef}	40.27^{cde}	39.96^{efg}	39.08^{defg}	10.32^{defg}	10.24^{fg}	10.10^{f}	10.23^{ghi}	3.07^{cde}	3.17^{de}	3.19^{de}	3.11^{h}
BC1U70	40.18^{ef}	40.30^{cde}	39.99^{efg}	39.05^{defg}	10.20^{fg}	10.51^{cde}	10.20^{ef}	10.50^{de}	3.09^{cd}	3.20^{cde}	3.24^{cd}	3.43^{bc}
BC1U100	40.31^{cde}	40.43^{bcd}	40.12^{cdef}	38.91^{efg}	10.74^{bc}	10.66^{cd}	10.25^{ef}	10.59^{d}	3.11^{bcd}	3.25^{cd}	3.24^{cd}	3.43^{bc}
BC2U0	40.26^{def}	40.49^{bcd}	40.07^{defg}	39.01^{defg}	10.27^{efg}	10.15^{fg}	10.01^{f}	10.14^{hi}	3.06^{de}	3.20^{cde}	3.18^{de}	3.23^{efg}
BC2U30	40.28^{cde}	40.45^{bcd}	40.09^{cdef}	39.03^{defg}	10.54^{cdef}	10.22^{fg}	10.27^{def}	10.40^{ef}	3.09^{cd}	3.25^{cd}	3.20^{de}	3.33^{d}
BC2U70	40.23ef	40.50^{bcd}	40.05^{efg}	39.19^{cdef}	10.64^{bcde}	10.41^{def}	10.08^{f}	10.62^{cd}	3.11^{bcd}	3.33^{b}	3.19^{de}	3.38^{bc}
BC2U100	40.61^{bcd}	40.55^{bc}	40.42^{bcd}	39.48^{bc}	10.62^{bcde}	10.76^{bc}	10.62^{bc}	10.36^{efg}	3.16^{abc}	3.34^{b}	3.28^{bc}	3.40^{b}
BC3U0	40.36^{bcde}	40.48^{bcd}	40.42^{bcd}	39.32cd	10.70^{bcd}	10.72^{c}	10.58^{bcd}	10.23^{ghi}	3.00^{ef}	3.14^{ef}	3.12^{ef}	3.26^{ef}
BC3U30	40.61^{bcd}	40.73^{b}	40.16^{cdef}	39.1^{2cdef}	10.75^{bc}	10.77^{bc}	10.63^{bc}	10.75^{c}	3.11^{bcd}	3.24^{bc}	3.23^{cd}	3.37^{cd}
BC3U70	41.28^{a}	40.75^{ab}	41.09^{a}	39.81^{ab}	11.00^{ab}	11.02^{b}	10.88^{ab}	11.01^{b}	3.18^{ba}	3.34^{b}	3.33^{ab}	3.47^{ab}
BC3U100	41.14^{a}	40.89^{a}	40.95^{a}	40.00^{a}	11.29^{a}	11.31^{a}	11.17^{a}	11.30^{a}	3.25^{a}	3.39^{a}	3.37^{a}	3.51^{a}
BC		s*				s**				s*		

U	ns	s**	ns
S	s*	s**	s*
BC×U	ns	ns	ns
U×S	ns	ns	ns
BC×S	ns	ns	ns
BC×U×S	ns	ns	ns

Note: GSP, grain sphericity; GV, grain volume; GSA, grain surface area; BC, biochar; U, urea; S, season; BC0, BC1, BC2 and BC3, represent 0, 1, 2 and 3t/ha biochar levels respectively while U0, U30, U70 and U100 represent 0, 30, 70 and 100% urea levels based on the recommended rates respectively. S, season; Values followed by the same letters, within the columns, are not significantly different at P<0.05. * Indicate the significant difference at P<0.05 and ** indicate P<0.01.

Table 4: Dunnet's comparison of milling properties of rice

Treatment	GL (mm)	GW (mm)	GT (mm)	L/W R	ED (mm)	GSP (%)	GV (mm^3)	GSA (mm^2)	TGW (g)	AO ($\times 10^{-2}$ μM/mL)	Fe (μg/g)	ASH (%)	AMY (%)
PB	6.30	1.66	1.73	3.71	2.68	40.06	10.62	3.14	24.63	8.35	18.83	53.04	27.69
BC0	6.33	1.67	1.73	3.72	2.65	39.95	10.68	3.12	24.41	8.33	18.70	49.14	27.15
BC1	6.36	1.69	1.74	3.73	2.65	39.92	10.60	3.20	24.90	8.34	19.48*	54.86	28.12
BC2	6.47*	1.74*	1.75	3.76*	2.73*	40.05	10.63	3.21	25.47*	8.35	19.93*	60.19*	28.29*
BC3	6.51*	1.76*	1.78*	3.78*	2.72*	40.51*	11.13*	3.27*	25.59*	8.34	19.89*	62.52*	28.56*

Note: Means labeled with * are significantly different from the control treatment mean at P< 0.05, GL - grain length, GW - grain width, GT - grain thickness, L/W R - grain length-to-width ratio, ED - equivalent diameter, GSP - grain sphericity, GV - grain volume, GSA - grain surface area, TGW - thousand grain weight, AO - antioxidant capacity, Fe - Ferrous content, ASH - ash content, AMY - amylose content, PB - Partially burnt paddy husk (the control treatment for Dunnett's test analysis), BC-rice husk biochar.

CONCLUSIONS

This multi-season field study shows that a single application of rice husk biochar has a strong and lasting effect on At 362 rice grain nutritional and morphometric traits over four consecutive cropping seasons grown under low-country wet zone climatic conditions. Biochar significantly improved grain AMY, AO, Fe, ASH, and morphometric traits while stabilizing TGW under changing seasonal conditions. The clear main and interaction effects of biochar, compared to the slight response to urea, highlight the outstanding long-term soil improvement and nutrient-holding ability of rice husk biochar. Furthermore, the results suggest that the single application of rice husk biochar is more beneficial than the application of partially burnt paddy husk. Future research should focus on long-term dynamics of rice husk biochar and nutrient interactions under varying climatic and soil conditions.

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AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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