Research Article

Evaluating the Performance of Local Pakistani Rice Cultivars Under Reduced N Dose

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Abstract | Rice cultivation relies heavily on nitrogen-intensive varieties to meet food demand. However, the excessive application of nitrogen fertilizers leads to environmental degradation, emphasizing the need to optimize nitrogen use efficiency (NUE) at recommended fertilizer levels to promote sustainable agriculture. As nitrogen is the key limiting factor in rice production, reducing its overuse without compromising yields remains a critical agricultural goal. This study analyzed the morpho-physiological traits of 10 Pakistani local rice genotypes for NUE at N50 and N100 doses. Results showed a significant increment in morpho-physiological traits with nitrogen dose. Grain weight (GW) exhibited a highly significant positive correlation with NUE across all genotypes at both N50 and N100 levels ($r_{10} = 1.00, P \leq 0.001$). In contrast, grain nitrogen content (GNC) and straw nitrogen content (SNC) showed significant correlation with NUE under N50 ($r_{10} = 0.77, P \leq 0.01$; $r_{10} = 0.73, P \leq 0.05$, respectively), while in N100, only GNC showed significant correlation ($r_{10} = 0.68, P \leq 0.05$). Under N100 treatment, the genotypes IR-6 and Super Gold-2019 exhibited higher grain yield. However, at N50, IR-6 lagged behind Super Gold-2019 and Punjab Basmati. A reduction in grain yield of 33.24% in Punjab Basmati and 30.44% in Super Gold-2019 was observed at N50 compared to N100. The strong association between GNC and SNC with NUE underscores the potential of these morphophysiological traits as genetic markers, making their inclusion in cultivar breeding programs crucial for efficiently monitoring and enhancing NUE.

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Introduction

Sustainable agriculture is crucial in addressing food security and environmental challenges, particularly in rice cultivation. Practices such as irrigation and fertilization boost growth, but higher doses affect plant-available soil carbon (C) and nitrogen (N) in paddies (Maris *et al*., 2015). The rate of chemical N fertilizer application is also approximately 37-fold, reaching 30 million tons of pure N annually, or around one-third of the worldwide N applied (FAOSTAT, 2022). Because of the growing human population, changing food patterns driven by rising wealth, and the increased use of biofuels, the need for agricultural production is estimated to double globally by 2050 (Tilman *et al*., 2011; Ray *et al*., 2013). The most promising strategy for ensuring food security with limited environmental impact is to increase crop yields rather than clear new lands for food production (Foley *et al*., 2011). High-yielding rice varieties and high fertilizer use, particularly N, have significantly raised rice production over the last four decades. Still, N overuse has resulted in environmental problems such as global warming, ozone layer depletion, and eutrophication, which are causing concern worldwide. Due to these problems, the N rates applied to rice must be reduced, without impacting yield. Rice has the lowest N utilization efficiency (NUE) among all the cereals. So, more N-efficient varieties that demand less water, labor, and fewer pesticides urgently need to be developed now (Prasad *et al*., 2017), which is possible by checking the response of regional cultivars to N dose.

Nitrogen, as an essential nutrient in crop production, is one of the main yield-controlling factors (Agha *et al*., 2004; Ahmad *et al*., 2009). Protein formation is an integral part of the dual functional and structural nature of N, which helps plants to synthesize chemical energy from solar energy by photosynthesis. Although excess supply accelerates plant growth and photosynthetic activity, it can delay crop maturity by interacting with other nutrients in changing soil ecological parameters. Optimized N management enhances crop maturity and energy-use efficiency, resulting in earlier harvests with lower moisture requirements (15.5%) and water savings (Havlin *et al*., 2016). Losses via ammonia volatilization, denitrification, and nitrate leaching, which accounted for 10 to 60% of the total N supplied, had damaging consequences for the environment and impacted yield (Guo *et al*., 2017).

Since the 1960s, numerous experiments have been conducted to enhance rice production and nitrogen use efficiency (NUE) (Peng *et al*., 2009; Li *et al*., 2018). Research has revealed that rice cultivars differ in their response to nitrogen (N) input, highlighting the potential to identify and develop cultivars with superior NUE. Generally, nitrogen application should be managed throughout the growing season based on panicle size and growth stage for each cultivar to achieve higher yields and improved NUE (Ladha *et al*., 2005; Zhang *et al*., 2009; Huang *et al*., 2018). Grain NUE is often used as an indicator to evaluate variations in cultivar responses to nitrogen fertilization (Huang *et al*., 2018; Wei *et al*., 2021). Previous studies have demonstrated that rice cultivars exhibit distinct responses to nitrogen management practices across different seasons and years, both in terms of yield and NUE. However, further evaluation of local rice cultivars under various nitrogen treatments remains necessary (Peng *et al*., 2006; Wu *et al*., 2016). Moreover, contradictory results were also found, indicating that increasing nitrogen dosage alone does not necessarily ensure efficient production (Lee, 2021). Instead, applying nitrogen in multiple splits throughout the growth cycle has been shown to enhance NUE by synchronizing nitrogen supply with plant demand and minimizing nitrogen losses (Kaushal *et al*., 2010; Kamruzzaman *et al*., 2014; Jeong *et al*., 2014). Understanding rice growth and development under varying nitrogen (N) availability is crucial for enhancing nitrogen use efficiency (NUE) and reducing excessive fertilizer application in agricultural systems (Evans and Clarke, 2019).

With recently rising crop demands, N optimization is an essential consideration for crop cultivation in Pakistan, given rising fertilizer costs, declining soil fertility, and growing environmental concerns. In many rice-growing regions, excessive N application has led to nutrient imbalances, reduced efficiency, and contamination of surface and groundwater. At the same time, erratic rainfall patterns and limited water availability have intensified the need for efficient nutrient management. By optimizing N use, farmers can sustain high yields while reducing production costs and mitigating greenhouse gas (GHG) emissions. Therefore, developing and promoting nitrogen-efficient rice cultivars suited to local conditions is essential to ensure sustainable rice production and long-term food security in Pakistan. In light of current challenges, this study aims to: (1) evaluate the response of selected rice cultivars' morphophysiological traits to a reduced nitrogen dose N50; (2) assess the impact of reduced nitrogen application on grain yield; and (3) identify and recommend the most nitrogen-efficient cultivars (enhanced NUE) suitable for cultivation under reduced nitrogen input conditions in Pakistan. To test these objectives, a field experiment was conducted to examine the relationships among plant morphophysiological parameters, yield performance, and NUE across 10 rice cultivars subjected to two nitrogen application levels (N50 and N100). Plant morphophysiological traits, such as leaf area index, tiller dynamics, root architecture, and chlorophyll content, directly influence nitrogen uptake, assimilation, and remobilization efficiency, which are the core physiological components of NUE. Cultivars exhibiting higher photosynthetic NUE and greater post-anthesis nitrogen remobilization from vegetative tissues to grains typically achieve superior agronomic NUE under both limited and adequate nitrogen supply. Therefore, genotypic variations in these traits under contrasting N levels (N50 and N100) can reveal key mechanisms driving differences in N uptake efficiency (NUpE), N utilization efficiency (NUtE), and overall yield performance across the ten rice cultivars tested. The findings are expected to provide valuable insights into optimizing nitrogen management strategies for rice cultivation while minimizing environmental impacts. Furthermore, identifying nitrogen-efficient cultivars will help promote sustainable rice production in Pakistan.

Materials and Methods

Study design

This study was conducted at the Directorate of Farms, University of Agriculture, Faisalabad, Pakistan. This area has an average seasonal temperature of 29.7±4.79°C, humidity (45.79±8.87%), and rainfall (36.05±56.66mm). The experiment was designed with two treatments (T1 = 85 kg N ha^{-1} (50% RD of N) and T2 = 170 kg N ha^{-1} (100% RD of N)) and three replications, using a randomized complete block design (RCBD) with a total of six experimental plots. The study area soil was categorized as clay loam in texture, pH of 8.50, electrical conductivity (EC) of 1.31 dS/m, soil total nitrogen (TN) of 0.08%, available phosphorus (AP) of 9.50 mg/kg, and potassium (K) of 187mg/kg.

Ten local rice cultivars were selected as shown in Table 1. Nitrogen was applied in three splits: basal, first topdressing, and second topdressing according to the treatment plan to assess the efficiency of different nitrogen levels at various growth stages. As per the treatment strategy, urea and diammonium phosphate (DAP) served as sources of nitrogen (N) and phosphorus (P), respectively. In contrast, sulfate of potash (SOP) served as a source of potassium (K).

Table 1: *Local rice cultivars included in the study.*

Name	Year of release	Yield potential (maund per acre)	Type
Rajbhog	Traditional	60-70	Coarse
Raj Munndo	Traditional	75-80	Coarse
KS-282	1982	100	Coarse
Kissan Basmati	2016	72	Aromatic
IR-6	1971	80	Coarse
Punjab Basmati	2016	75	aromatic
Basmati-515	2011	70	Basmati
KSK-434	2013	105	Coarse
PK-386	2013	70	Fine
Super Gold 2019	2019	70	Fine

Morphophysiological traits measurement

Phenological stages were recorded through field observations. Panicle initiation (PI) was noted when the flag leaf sheath started to swell, indicating panicle development. Flower initiation (FI) was identified by the emergence of the first floret from the boot leaf. The height of the plant (PH) was determined using an iron meter rod from the base of the stem to the uppermost tip of the panicle (excluding awn) at the maturity stage. The length of the panicles (PL) was measured from the point where they emerge above the soil to the end of the plant's panicle using an iron meter rod. After harvesting, plants from each plot were gathered, and tillers were counted to determine the total number per plant (n=3) at the maximum tillering stage. On-site leaf area (cm2) was determined by taking the length and width of representatives and multiplying them by the correction factor (0.75) (Palaniswamy and Gomez, 1974). The number of productive panicles per plant was counted manually at harvest. The leaf chlorophyll content (*SPAD value*) was recorded after 45 (at tillering; TL), 60 (at panicle initiation; PI), and 70 (near heading; HD) days after (seedling) transplanting (DAT). About three fully expanded leaves from a randomly selected plant were included for measuring chlorophyll contents with a

SPAD meter after calibration (Hussain *et al*., 2000).

At harvest, three plants were selected from each plot for the determination of grain yield (g/plant) (grains per plant of the plot), and 100-grain weight (weight of 100 grains). A digital balance was used and expressed as grams per plant. Straw dry weight (g) was measured after the plant was harvested at maturity and oven-dried at 65 °C to a constant weight using a digital balance. Grain and straw samples were first air-dried, then oven-dried at 65 °C, pulverized, and prepared for chemical analysis. A 0.5 g plant sample was digested with 10mL of sulfuric acid and left to stand overnight. Digestion was then carried out on a hot plate at 300-350 °C for 4-5 hours until the solution became colorless. After cooling, the digested samples were filtered using Whatman No. 42 filter paper. the filtrate was collected in plastic bottles, and the volume was adjusted to 50mL with distilled water. The prepared samples were then subjected to chemical analysis. Root biomass was found after carefully uprooting representative hills, gently washing the roots free of soil, and separating them from the shoots. The cleaned roots are then oven-dried at 70°C for 48-72 hours until constant weight, weighed, and expressed as g/plant, representing below-ground biomass (BGB). The above-ground biomass (AGB) was calculated as the sum of grain weight and straw dry weight (DW).

Nitrogen content was analyzed with the Kjeldahl distillation method. For this 4% boric acid and 40% sodium hydroxide (NaOH) solutions were prepared, along with a mixed indicator solution containing bromocresol green and methyl red. 5mL of the digested sample was transferred into a distillation flask, and 10mL of 40% NaOH was added to neutralize the acid and release ammonia. The flask was connected to a distillation unit. A receiving flask containing 5mL of 4% boric acid and 2-3 drops of mixed indicator was placed to collect the distillate. Distillation was carried out until the distillate volume reached approximately 40-50mL, as indicated by a color change (red to green or blue), showing ammonia capture. The distillate was then titrated with a standard acid to determine nitrogen content (Johansson, 1988). The protein content was calculated by multiplying N-content by 6.25 (Krul, 2019).

$$Protein_{grain+straw}\ (\%) = N\ (\%) \times 6.25$$

The Nitrogen Use Efficiency (NUE) was determined using the following formula (Mérigout *et al*., 2008).

$$NUE\% = \frac{N_{grain+straw}}{N_r} \times 100$$

Where, $N_{(grain+straw)}$ represent the N accumulation in grains and straw, and N_r represents the applied N dose.

Statistical analysis
The statistical analysis was conducted using a randomized complete block design (RCBD) in a factorial configuration at the 5% significance level (Steel *et al*., 1997). Two-way analysis of variance (ANOVA) with Fisher's LSD was performed to check the statistical significance of the parameters. To assess the strength of the significant relation between the variables, Pearson's correlation was used, and the impact of NUE on individual rice plant features was evaluated using linear regression with ANOVA (single factor) to determine the P-value in OriginPro 2024 (10.1) and MS Excel 2021 (21.08), respectively. To group cultivars with similar responses based on featured traits, a heatmap with dendrograms was constructed using morpho-physiological parameter data in R (v.4.2.3) (pheatmap package) using the Euclidean distance metric.

Results

Cumulative results for cultivars: Morpho-physiological traits
Cultivars' phenological and morphological traits dynamics across changing N dose: Punjab Basmati, KSK-434, and Super Gold-2019 showed a more promising PH at N50 than at N100. Kissan Basmati resulted in a lower PH of 142cm at N100. Punjab Basmati, KSK-434, and Super Gold-2019 showed 17.05, 15.22, and 15.13% reductions in PH, respectively, at the N50 level compared to N100 (Figure 1a). Basmati-515 resulted in the lowest PL at 23cm with N100. Reduction of 15.23, 16.16, and 15.96% in the panicle length was observed in Rajbhog, Kissan Basmati, and Super Gold-2019, respectively under N50 (Figure 1b). Mean values for PI and FI increase from 93.69-106.33 and 95.40-114.20 (days) with the increased N dose (N50 to N100), respectively (Figure 1c, d). Super Gold 2019 showed the highest FI (122 days), and Basmati-515, 118 PI (days) at N100, whereas Basmati-515, 102, and 107 (days) for PI and FI at N50, respectively. Cultivars Rajbhog, Kissan

Basmati, and Super Gold-2019 showed higher PI values at the N50 level. KSK-434 showed a maximum of 16 tillers at N100 (Figure 1e). More than 32 tillers were observed at N100 treatment in KSK-434 and Super Gold-2019 varieties. These cultivars also showed promising tillering at the N50 level compared with N100. KS-282 resulted in the lowest 10 number of tillers at N100. KSK-434 and Super Gold-2019 showed 19.51% and 16.28%, respectively, with low tiller counts (19.51% and 16.28%, respectively) under reduced N. Nitrogen optimization led to significant variation in key agronomic traits, with the NOT, LA, and NOP ranging from 11.07 to 14.27 cm^2, 45.23 to 57.80 cm^2, and 11.67 to 15.10 cm^2, respectively, across increasing nitrogen doses. Super Gold-2019, PK-386, and KSK-434 resulted in a greater NOP in N100 than in 50% N50 (Figure 1f). In the goal of high yield with reduced N dose, Super Gold-2019 and KSK-434 performed best at N50. ANOVA showed only a significant Interaction between treatment × cultivars for PI, FI, SPC, and NUE.

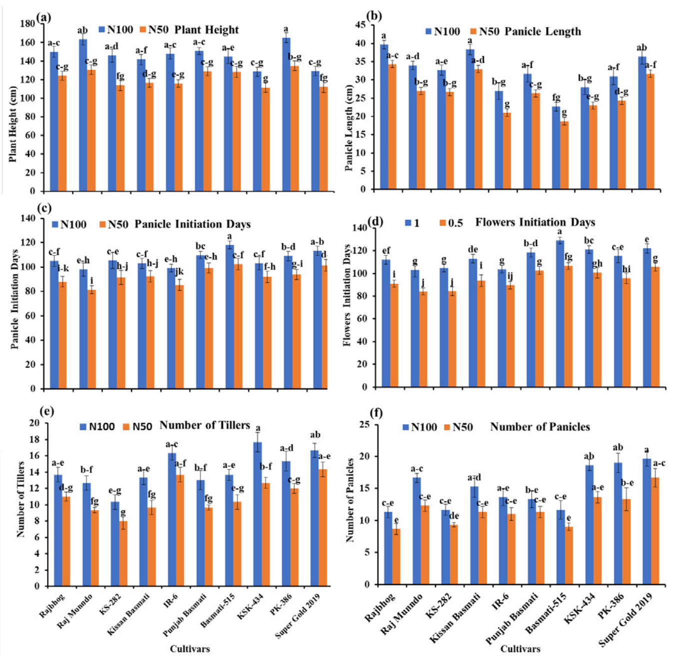

Figure 1: *Cultivars' phenological and morphological traits dynamics across changing N dose. Small letters represent the least significant differences (LSD, p<0.05) across various cultivars with changing N doses.*

Photosynthetic indicators

Higher SPAD values (higher chlorophyll/N) resulted in greater leaf expansion and leaf area (LA) because nitrogen is required for cell division and growth. Punjab Basmati, PK-386, and Rajbhog showed higher LA (cm^2) in N100 than in N50. Basmati-515 had the lowest LA (44 cm^2) at N100 compared to others (Figure 2a). SPAD readings, which indicate chlorophyll content, showed increasing trends with nitrogen levels. SPAD45 increased from 36.59 to 45.42, and SPAD60 rose from 39.09 to 47.92 as N application increased from N50 to N100. KSK-434 exhibited the highest SPAD values at both SPAD45 (53.67) (TL stage) and SPAD60 (55.67) (PI stage), respectively (Figure 2c). Under an N100 dose at the HD stage, KSK-434 exhibits the highest SPAD value, 59, significantly outperforming all other cultivars. Basmati-515, Punjab Basmati, IR-6, and Kissan Basmati and KS-282 cluster in an intermediate range 50–56, while Rajbhog, Raj Mondo, and Super Gold 2019 show moderate values around 52–54 (Figure 2d). Reducing the fertilizer dose to N50 markedly lowers SPAD values across all cultivars, but the magnitude of decline varies widely. Basmati-515 and PK-386 prove most resilient, retaining relatively high chlorophyll levels, whereas Super Gold 2019 displays extreme sensitivity, plummeting from 53 to 30, the lowest value recorded. Rajbhog and Raj Mondo also suffer large drops, while the remaining cultivars show moderate reductions. Overall, KSK-434 excels

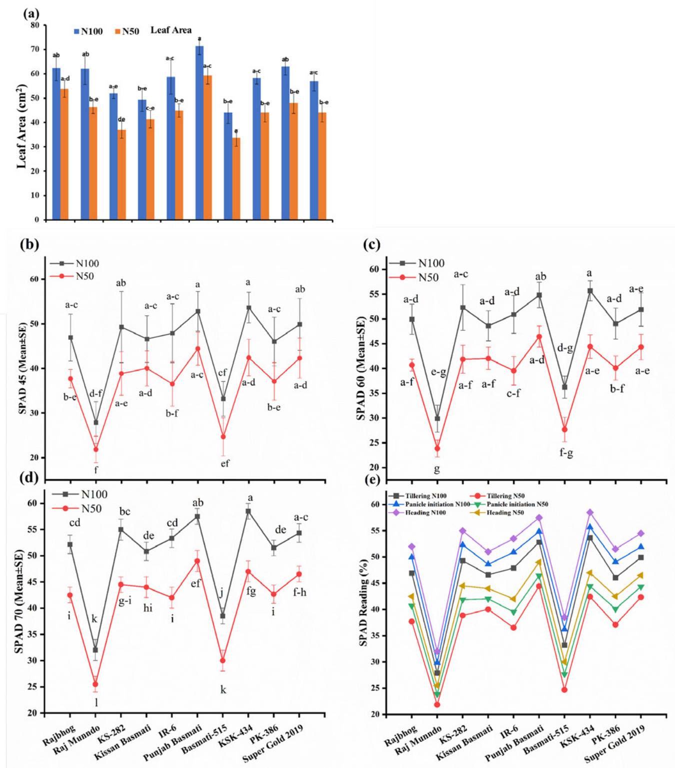

Figure 2: *Leaf area (cm^2) (a), cultivars SPAD values at late tillering (45 DAT) (b), panicle initiation (60 DAT) (c), and heading (70 DAT) (d). Cultivars' cumulative percentage variation across N100 and N50 at three different stages and times (e). Small letters represent (LSD, p<0.05) with mean ±SE, across various cultivars with changing N doses at N50 and N100.*

under N100; Basmati-515 and PK-386 perform best under nutrient stress (N50); and Super Gold 2019 emerges as the most fertilizer-responsive and vulnerable cultivar when nitrogen supply is limited (Figure 2e).

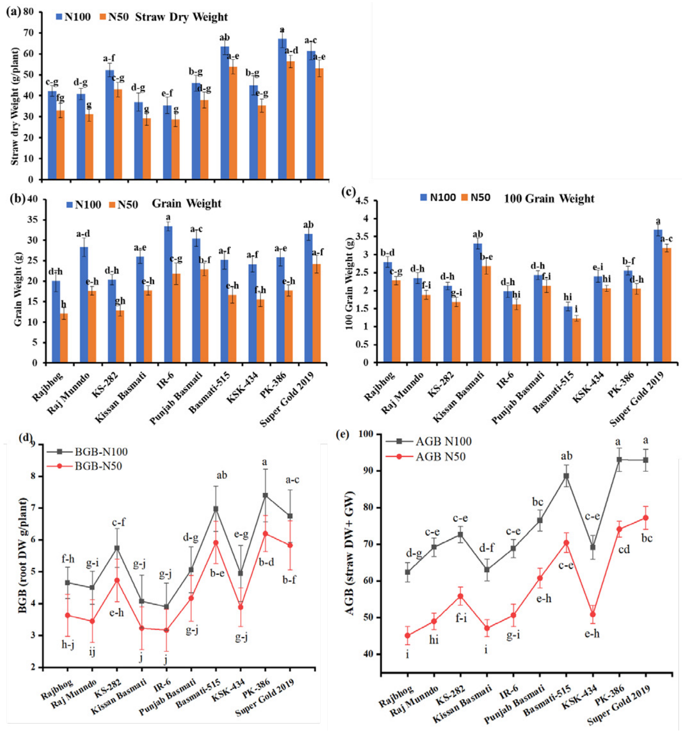

Figure 3: *Cultivars straw dry weight (g/g/plant) (a) photosynthetic indicators, biomass accumulation, and grain yield (b,c) and below-ground (root biomass g/g/plant) and above-ground photosynthetic (straw DW + grain weight)components across changing N dose. Small letters represent (LSD, p<0.05), across various cultivars with changing N doses at 50 and 100.*

Biomass accumulation and grain yields

The highest straw yield was observed at N100 treatment in Basmati-515, PK-386, and Super Gold-2019 cultivars (Figure 3a). Basmati-515 and PK-386 also showed higher straw yield at N50 levels. But these values were lower than the N100 straw yield values. Basmati-515 and PK-386 exhibited higher straw weight at N50 than at N100. IR-6 resulted in a low 35 g straw yield (Figure 3a). Punjab Basmati and Super Gold-2019 resulted in 19.90% and 30.44% in grain yield, respectively, at the N50 level as compared to the N100 level. This is due to high N levels, which lead to more chlorophyll formation and, consequently, greater photosynthesis, resulting in better plant growth and yield. At N50, Punjab Basmati had a GW of 23.87 (g/plant). At the same time, IR-6 recorded a maximum GW of 33.40 g/plant at N100 (Figure 3b). However, the 100-grain weight (100GW) was less responsive to nitrogen, with values ranging between 2.08 and 2.52 (g/plant) across varieties (Figure 3c). Punjab Basmati, KSK-434, and Super Gold-2019, these cultivars exhibited encouraging results in 100GW at N50 level. Super Gold-2019 showed the best response to N50 in terms of AGB (straw DW+ grain weight), whereas PK-386 showed the best response in terms of BGB (root biomass g/plant) (Figure 3d, e). Basmati-515 had the lowest grain yield among all cultivars.

Cultivars' nutritional quality and NUE

GPC increased from 18.11±2.39 to 23.54±2.72 (%), with Kissan Basmati showing 20.21 at N50 and IR-6 reaching 26.82 at N100 (Figure 4a). GNC ranged from 2.9±0.37 to 3.7±0.38 (%), with Punjab Basmati showing 20.43% at N50 and IR-6 reaching 25.82% at N100 (Figure 4b). Similarly, SPC ranged from 7.32±0.72 to 9.60±0.63 (%), with Super Gold 2019 reaching 8.17 at N50 and PK-386 achieving a maximum of 10.67 at N100 (Figure 4c). SNC ranged from 1.07±0.22 to 1.43±0.17 (%), with Super Gold 2019 showing a value of 1.30% at N50, and IR-6 reaching 1.5 at N100 (Figure 4d) These results demonstrate the strong positive effect of nitrogen fertilization on physiological traits across rice varieties. Nitrogen fertilization significantly varied NUE among cultivars. The results showed that N at the two levels, N50 and N100, significantly affected NUE (Figure 4e). NUE exceeded 62% in Super Gold-2019 cultivars under the N100 treatment. Punjab Basmati and Super Gold-2019 showed higher NUE at the N50 level. Rajbhog resulted in lower NUE at N50. 33.38% and 34.78% of NUE were observed in Punjab Basmati and Super Gold-2019, respectively, at the N50 level. Super Gold 2019 performed better than other cultivars in the following order at: Rajbhog < Raj Munndo < KS-282 < KSK-434 < IR-6 < Basmati-515 < Kissan Basmati < PK-386 < Punjab Basmati < Super Gold 2019. Super Gold 2019, Punjab Basmati, PK-386, Kissan Basmati, and Basmati-515 originated primarily from their higher nitrogen utilization efficiency (NUtE), driven by greater post-anthesis nitrogen remobilization from leaves and stems to the grain, higher photosynthetic nitrogen-use efficiency, and sustained green leaf area duration under both N50 and N100 (Figure 2). In contrast, the traditional aromatic landraces Rajbhog and Raj Munndo, along with the older inbred KS-282, displayed the lowest NUE mainly because of poor nitrogen uptake efficiency (NUpE) under limited N supply (N50), excessive vegetative growth (luxury N uptake) at N100, and lower harvest index, resulting in reduced partitioning of assimilated N to the grain. Intermediate performers such as IR-6, KSK-434, and Basmati-515 balanced moderate uptake capacity with reasonable internal utilization efficiency. In contrast, modern high-yielding non-aromatic hybrids and inbred (Punjab Basmati, Super Gold 2019, PK-386) combined enhanced root foraging ability, deeper root systems, and higher expression of ammonium and nitrate transporters (OsAMT1 and OsNRT2 families) with efficient N remobilization traits (up-regulated OsNAC and OsGLN genes during grain filling), enabling them to produce significantly more grain per unit of nitrogen absorbed or applied across both low and optimum nitrogen environments (Lee *et al*., 2020b; Hu *et al*., 2023).

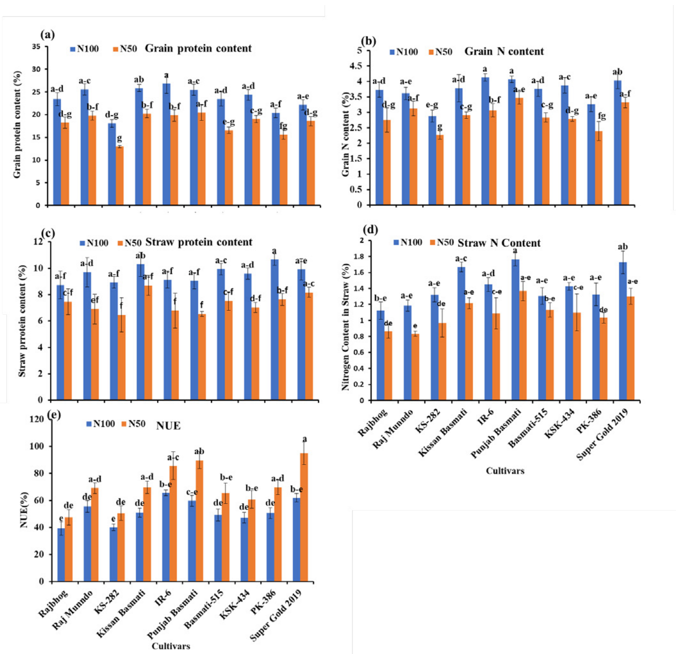

Figure 4: *Cultivars' nutritional quality and NUE across changing N dose. Small letters represent the (LSD, p<0.05), across various cultivars with changing N doses at 50 and 100.*

Traits symphony for cultivars in response to N dose and NUE

Correlation analysis: Correlation analysis (Figure 5) showed that several traits were highly significantly positively correlated with N treatments. Interestingly, GW correlations at N50 and N100 (r_{10}=1.00, P≤0.001) were highly significantly positively correlated with NUE. Whereas GNC and SNC were correlated considerably (r_{10}=0.77, p≤0.01, r_{10}=0.73 p≤0.05) with NUE at N50, respectively, and at N100, only GNC is significantly correlated (r_{10} =0.68, p≤0.05) with NUE.

NUE relation with cultivars morphophysiological features

Environmental sustainability goals include a 50% reduction in N fertilizer application. In our results, NUE was highly significantly positively (*R=1, P=0.00*, *R=1, P=0.00*) correlated with GW at both N50 and N100 doses for all genotypes (cultivars), respectively (Figure 6a). Super Gold 2019 (10), Punjab Basmati (6), and Kissan Basmati (5) resulted a higher significant positive correlation (GW) at N50. In contrast, Kissan Basmati (5), Super Gold 2019 (10), and Punjab Basmati (6) showed higher GW (g) at N100. In the case of 100GW Super Gold 2019 (10), Kissan Basmati (4), and Rajbhog (1) form non-significant (p=0.2991, p=0.1776) consistent correlation peaks (R=0.36, R=0.13) at N50 and N100 with NUE, respectively. KS-282(3) consistently exhibited the lowest GW and 100GW at both doses, forming the base of the correlation line, but Rajbhog (1) outcompeted all other genotypes in 100GW, except Kissan Basmati (4). Similarly, GNC also showed a significantly correlated result (R=0.77, p=0.0091) (Figure 6b). Super Gold 2019 (10) showed a higher NOT at N50 (R=0.50) than N100 (R=0.43) in which Super Gold 2019 (10) lags KSK-434 (8). Overall, GW showed a highly significant correlation with both doses, whereas SNC and GNC were strongly positively correlated with only N100.

A high N fertilization rate significantly (R = -0.04) reduced PI days (Figure 7a). Cultivars Punjab Basmati (6) and Super Gold-2019 (10) showed higher values at the N50 level. However, these values were lower than those on N100 PI days. Raj Mundo (2) showed the lowest PI (98) days at N100. For phenological and photosynthetic indicators, only SPAD54 showed a weak, significant correlation (R=0.0002, p=0.0094) with N100 for KSK-434 (8) (Figure 7b). FI also showed a weak to moderate, non-significant correlation for Super Gold 2019 (10) under the N50 dose. Results showed a nonsignificant correlation at both N100 and 50 doses for PH and PL. PK-386 (9) showed the highest nonsignificant correlation for PH and PL. At both N100 and N50 levels, Super Gold 2019 clearly outperformed all other cultivars, achieving the highest above-ground and below-ground biomass and the best total biomass production (Figure 8). The relationship between root (BGB) and shoot (AGB) biomass was powerful and highly significant under both high (R^2= 0.86, p = 0.0001) and low nitrogen (R^2= 0.89, p= 0.00004), with the correlation

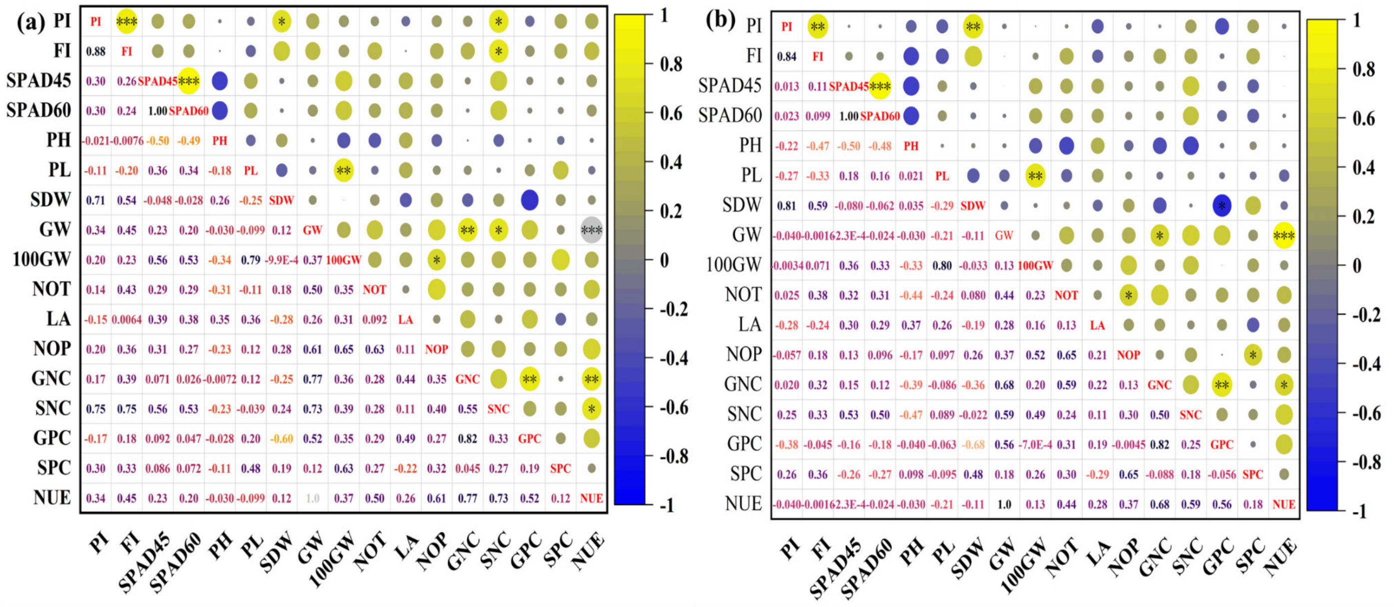

Figure 5: *Pearson correlation among morphophysiological features at N50 (a) and N100 (b). The lower triangle shows the correlation (r) values, and the upper triangle shows the circle size and color representing the significance level between variables at N50 and N100, respectively. PI: Panicle Initiation, FI: Flower initiation, SPAD45:SPAD value 45 days, SPAD60: SPAD value at 60 days, PH: Plant Height, PL: Plant Length, SRW: Straw Dry Weigh, GW: Grain Weight, 100GW:100 Grain Weigh, NOT, No of Tillers, LA: Leaf Area, NOP: No of panicles; GPC: Grain Nitrogen Content; SNC: Straw Nitrogen Content; GPC: Grain Protein Content; SPC: Straw Protein Content, NUE: Nitrogen Use Efficiency, PI: Panicle Initiation, FI: Flower initiation, SPAD45: SPAD value 45 days, SPAD60: SPAD value at 60 days. "****"- p≤0.001, "***"- p≤0.01," **" - p≤0.05, "*"- p≤0.1.*

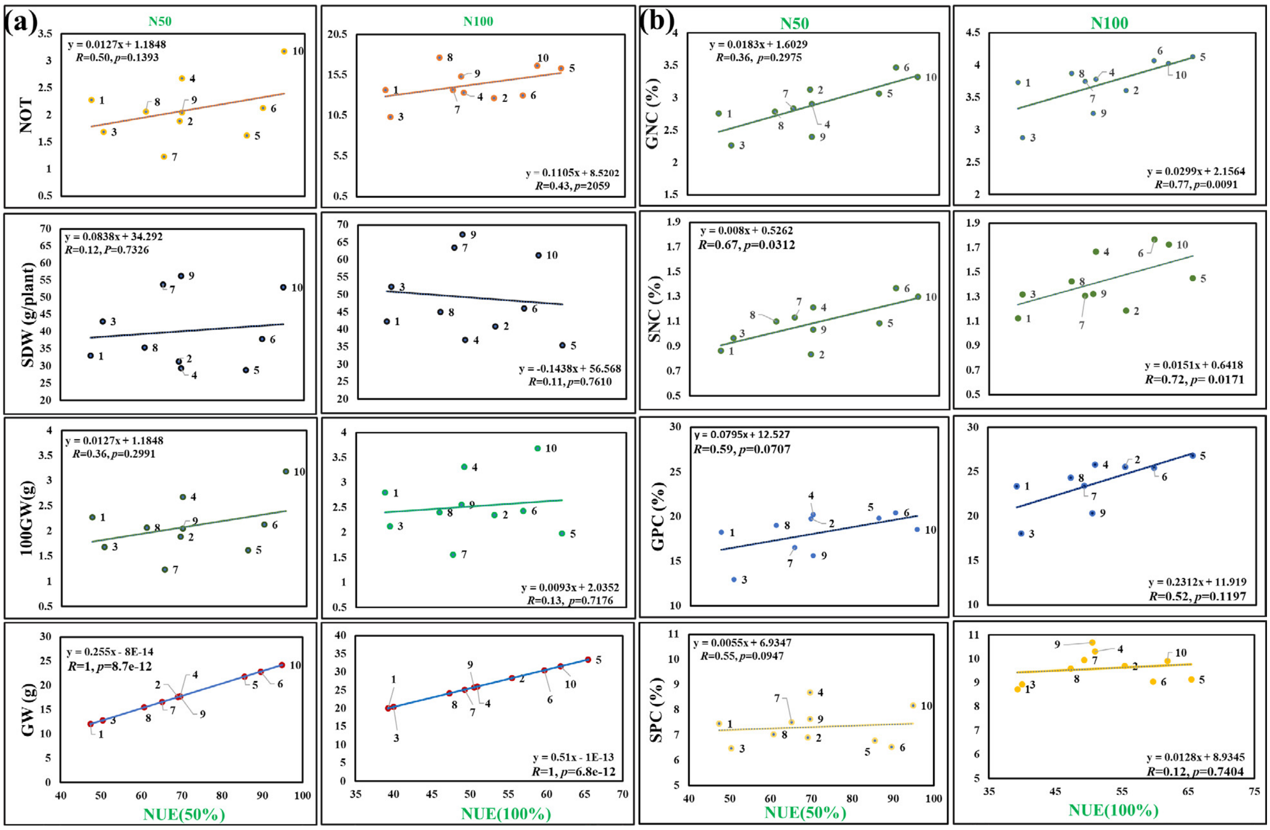

Figure 6: *Relation between NUE and (a) Grain Weight (GW), 100 Grain Weight(100GW), Straw Dry Weigh (SRW), No of Tillers (NOT) different rice genotypes, (b) GNC, Grain Nitrogen Content; SNC, Straw Nitrogen Content; SPC, Straw Protein Content, GPC, Grain Protein Content. 1, Rajbhog; 2, Raj Munndo; 3, KS-282; 4, Kissan Basmati; 5, IR-6; 6, Punjab Basmati; 7, Basmati-515; 8, KSK-434; 9, PK-386; 10, Super Gold 2019.*

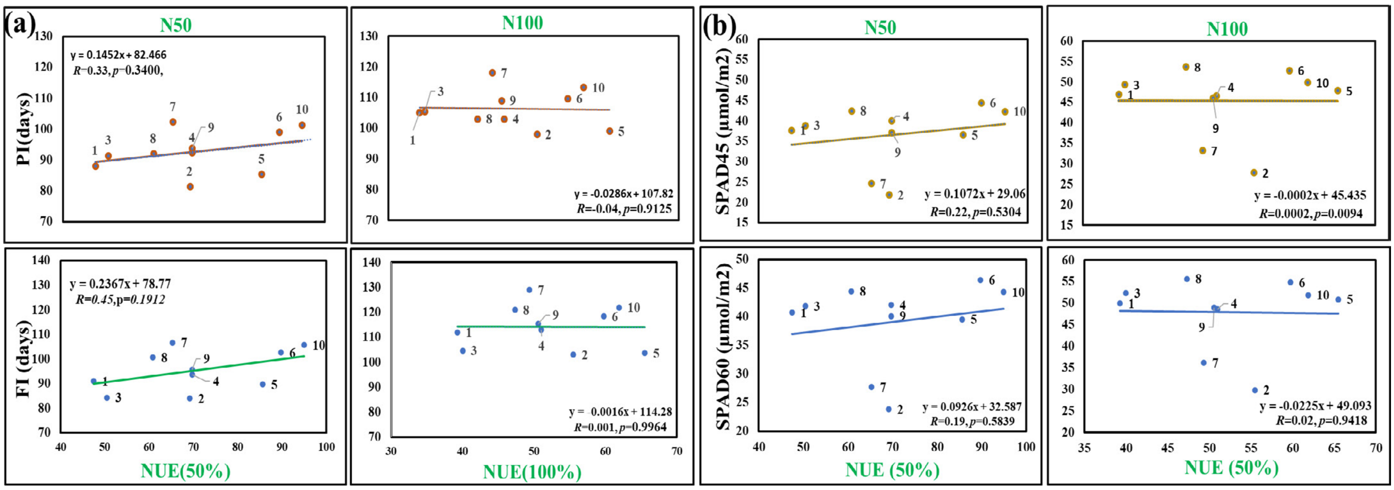

Figure 7: *Relationship between Nitrogen use efficiency (NUE) and (a) flower initiation (FI) and panicle initiation (PI), (b) SPAD value at 45 days (µmol/m2) and SPAD value at 60 days (µmol/m2) of different rice genotypes. 1, Rajbhog; 2, Raj Munndo; 3, KS-282; 4, Kissan Basmati; 5, IR-6; 6, Punjab Basmati; 7, Basmati-515; 8, KSK-434; 9, PK-386; 10, Super Gold 2019.*

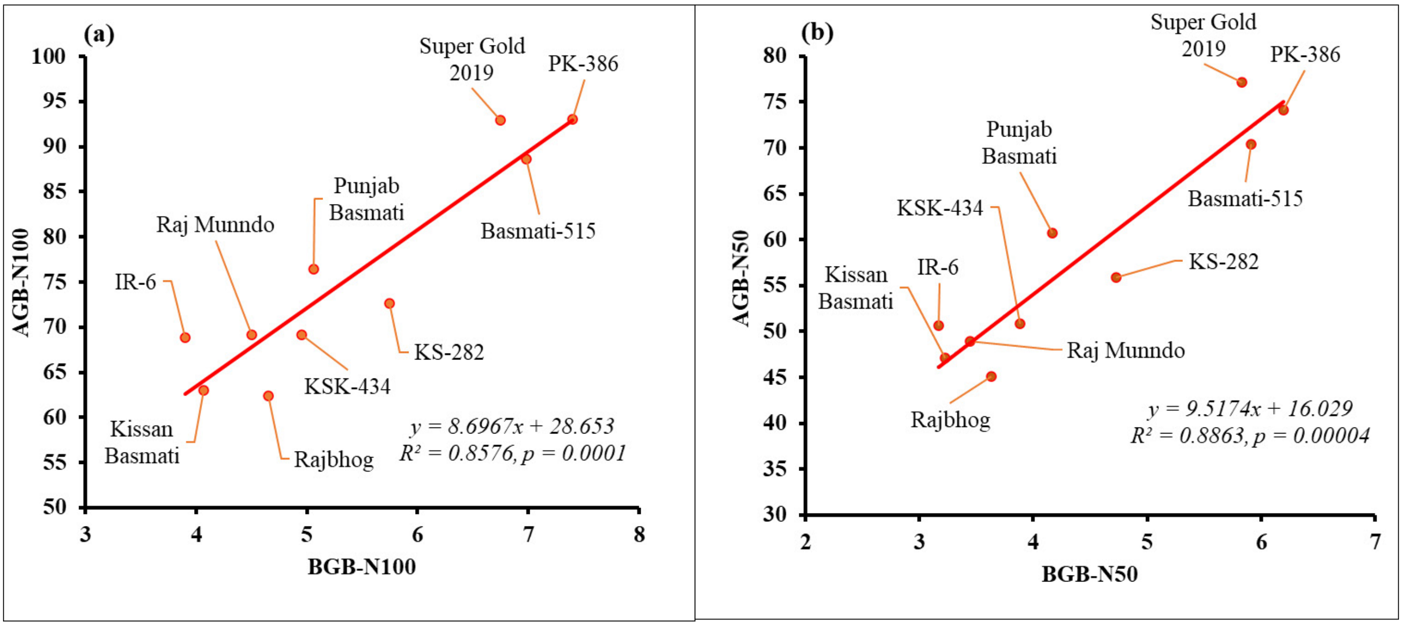

Figure 8: *Relationship between above-ground biomass (ABG) and below-ground biomass (BGB) at N100 (a) and N50(b), respectively.*

being even tighter at low N. This indicates that Super Gold 2019 is the most efficient and stable cultivar for biomass accumulation across nitrogen conditions, making it the top choice for both optimal and nitrogen-stressed environments.

Overall, nitrogen fertilization significantly affected NUE across cultivars. The results showed that N at the two levels, N50 and N100, significantly affected NUE. Super Gold-2019 and Punjab Basmati performed best because they have superior nitrogen uptake efficiency and, especially under limited supply, nitrogen utilization efficiency (higher biomass produced per unit of N absorbed). At low nitrogen (N50), these cultivars maintained or even increased NUE by 33–35% compared to N100, indicating stronger root foraging capacity, better N remobilization from vegetative tissues, and more efficient photosynthetic N use to support growth when soil N is scarce. In contrast, cultivars like Rajbhog exhibited poor NUE at N50 due to weaker root systems and lower metabolic efficiency, making them highly dependent on high soil N availability.

Discussion

Effect of nitrogen dose on growth, physiological parameters, and Chlorophyll content: In the context of N optimization in local Pakistani rice cultivars, our results showed that PI, FI, and SPC were significantly influenced by N dose, with varying N levels, as per our first objective. Our study revealed that increased N concentration significantly affects photosynthetic yield, chlorophyll content, grain weight, and N content (Figures 7 and 8). Higher chlorophyll levels, indicating greater N uptake, were associated with increased dry biomass. According to Khalofah *et al*. (2021) N fertilizer rates affect SPAD readings because of differences in N uptake efficiency among cultivars. Different cultivars have different abilities to uptake N, Cultivars that absorb/uptake more N have high chlorophyll contents, which means a higher photosynthetic rate. Krouma *et al*. (2006) found similar findings concerning chlorophyll contents, in which leaves' chlorophyll content was significantly increased by N fertilization. After 45 Days from transplanting the nursery to the field, Super Gold-2019 showed a decrease of only 18%. After 60 days of transplanting, a similar 18% decrement was observed in Punjab Basmati at the N50 treatment compared to N100 (Figure 2). As N is the key factor in the chlorophyll molecule. Hence, an increase in chlorophyll content in the plant.

According to Murooka *et al*. (2002), N fertilizer rates affect rice plant growth and leaf area formation because of differences in N uptake efficiency among the analyzed. Enhanced NUE led to increased development of root physiological and morphological traits, which are closely associated with yield. As a result, N application is critical for increasing crop productivity. A weaker straw might impede plant growth and reduce yields if rice is deficient in N. It is possible due to N's role in stimulating cell division, leading to increased panicle formation in rice during the productive stage (Ghoneim *et al*., 2018). Low nitrate reductase (NR) activity of a cultivar resulted in higher nitrate concentration (Anjana *et al*., 2007). A study by Chen *et al*. (2004) showed that increased nitrate supply increased the metabolic pool (MP) and the storage pool (SP) in leaf blades. Santi *et al*. (2013) also found higher shoot nitrate concentrations in nonlegume plants. Such approaches typically increased dry weight (Hakeem *et al*., 2011). This could be due to N application, which significantly affects the protein and amino acid composition of grains. Increasing N application can significantly increase protein content in grains.

Nitrogen uptake, accumulation, and its impact on biomass and yield

An increased N dose increased GNC and SNC, resulting in enhanced growth and yield.GW showed a highly significant N content at both doses, whereas SNC and GNC showed a strong positive correlation with only N100. Plants absorb and convert N into amino acids and other nitrogenous compounds, determining final yield (Kant *et al*., 2011). The yield of grain was positively correlated with N accumulation, demonstrating that the grain itself was an essential sink for N (Koutroubas *et al*., 2004). Efficient nitrogen (N) uptake, allocation, and assimilation are crucial for optimal plant growth, with nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) being the primary inorganic N forms available to plants (Kumari *et al*., 2022). N is a crucial component of plant protein, which is responsible for all the plant's fundamental functions. This is because plant metabolism relies heavily on N, and greater N accumulation results in higher biomass production.

Performance of cultivars at reduced N level

IR-6 and Super Gold-2019 experienced less than a 20% decrease in tiller number at N50 compared to N100, likely due to enhanced photosynthesis from higher N absorption, which improves dry mass production. The Rajbhog cultivar showed only a 16% decrease in leaf area at the N50 level compared with N100. Punjab Basmati and Super Gold-2019 showed the least variation in grain yield between the N50 and N100 treatments. From our research, we observed a reduction of less than 18% in straw weight for the Punjab Basmati and Super Gold-2019 cultivars at the N50 treatment compared with N100. Only a 16% and 17% decrement was also observed in the panicle length and NOP in cultivars Kissan Basmati and Punjab Basmati, respectively, at the N50 level. There was only a 16% lower N content in a grain of the Raj Mundo cultivar at the 50% N treatment compared with the N100 level. Results of our study show minimal improvement in the NUE of the Raj Mundo cultivar at the N50 level compared with N100. Liu *et al*. (2021) also reported contradictory results regarding excessive N supply on morpho-physiological parameters. Factors such as root biomass, morphology, and physiological capacity for N uptake significantly influence total N uptake (Glass, 2003). Enhanced NUE led to increased growth of root physiological and morphological traits, which are closely associated with yield (Yang *et al*., 2012; Ju *et al*., 2015). In previous studies, it has been shown that this was mainly attributed to root biomass, root length, and root tips (Peng *et al*., 2008; Mishra and Salokhe, 2011; Yang *et al*., 2012; Wu and Cheng, 2014). Root characteristics, including biomass, length, tips, and oxidizing activity, are crucial for yield and NUE; therefore, there remains a need to study these traits, as well as additional root traits. N fertilizer rates affect NUE because of differences in N uptake efficiency among genotypes (Lam *et al*., 2003).

Importance of N for cultivars' productivity and environmental benefits

Consistent N supply is essential for rice to achieve its full growth potential, as adequate soil N levels enhance photosynthesis, plant growth, and overall yield (Ajala *et al*., 2019; Lee *et al*., 2020a). Enhancing NUE not only improves fertilizer value but also boosts crop production, conserves energy, and mitigates environmental impacts (Yu-Hua *et al*., 2007). Improved NUE also augments disease resistance, further benefiting crop performance (Duan *et al*., 2014). Low-cost production and higher returns on investment may be achieved through cultivar selection for improved nitrogen utilization and minimal losses (Hakeem *et al*., 2011). When the rice plant receives a suitable amount of N, its growth and yield improve (Yuan, 2017). Due to morphological improvements and heterosis utilization, coupled with high N fertilizer applications, super hybrid rice cultivars display increased biomass, larger panicle size, and larger sink size, resulting in higher yield than common rice cultivars (Zhang *et al*., 2009).

Punjab Basmati and Super Gold-2019 achieved the highest grain N levels, while Kissan Basmati and IR-6 had the highest crude protein concentrations. An application has a significant effect on the protein and amino acid composition of grains; increasing N application can significantly increase protein content. A study by Li *et al*. (2006) found that although an appropriate N application may raise rice NUE, an excessive one would reduce rice NUE and the NHI (nitrogen harvest index) because the majority of N taken up was deposited in straws rather than grains (Cheng *et al*., 2011). Above the critical range, N application results in low NUE and high environmental pollution (Yang *et al*., 2017).

Overall, this study assessed the ability of various rice cultivars to optimize NUE across differing N availability. A negative correlation was found between plant height and SPAD value at both N doses. Panicle length was positively correlated with NUE at N100, and grain weight was positively correlated with NUE at N50. Punjab Basmati and Super Gold-2019 demonstrated N efficiency, performing comparably at both N50 and N100 levels, making them promising candidates for future research, in line with our third hypothesis.

Conclusion

This study aimed to evaluate the impact of nitrogen (N) levels on the yield of ten local rice cultivars of Pakistan. Key physiological and agronomic parameters, including plant height, tiller number, panicle count, leaf area, chlorophyll content, 100-grain weight, grain yield, and straw yield, were assessed. The results revealed significant differences among cultivars for both N levels. Punjab Basmati and Super Gold-2019 exhibited superior performance in agronomic traits across both treatments. Positive correlations were observed between plant height, chlorophyll content, and biomass with NUE at both N levels. The study highlighted considerable genetic variation among the cultivars, with Punjab Basmati and Super Gold-2019 showing to higher N efficiency and better growth and yield at N100 than at N50. These cultivars demonstrated higher grain N content and crude protein concentrations, with Kissan Basmati also showing high straw N concentrations. Overall, Super Gold-2019 emerged as the most N-efficient variety, effectively utilizing N at both levels. Rice cultivars with high NUE will increase grain yield while reducing environmental costs and promoting low-input, sustainable rice cultivation.

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Novelty Statement

This study innovatively examined the performance of 10 local rice cultivars under differentiated N doses.

Author's Contribution

Waleed Raza: Original draft, writing, validation, data

curation, illustrations, review and editing, validation, and designed manuscript.
Muhammad Usman: Review, writing, formal analysis, validation, and editing,
Zeeshan Ahmad: Writing original draft, illustrations, review, and editing, validation.
Azan Saeed: Writing, review, validation and editing.
Muhammad Jamshed and Azhar Sohail Shahzad: Review, validation, writing and editing.
Faseeha Fatima and Amel Ayari-Akkari: Validation, writing, and editing
All authors contributed to the manuscript writing.

Data availability
Data will be made available on request.

Generative AI and AI-assisted technology statement
Except for grammarly, no AI tool was used during the preparation of this manu.

Conflict of interest
The authors have declared no conflict of interest.

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