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Growth and metabolic changes in rice varieties under low-nitrogen condition

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ABSTRACT

Nitrogen (N) is a crucial element for optimal plant development and productivity, yet its inefficient utilisation in crops like rice contributes to environmental degradation and high input costs. This study investigates the physiological and biochemical responses of two contrasting rice (Oryza sativa L.) genotypes—Vikramarya (N-efficient, V1) and Aditya (N-inefficient, V2) under limited (50% RDN) and optimal (100% RDN) N conditions at the rate of 60 kg N per ha and 120 kg N per ha, respectively. Plants were grown in field conditions. The assessments were conducted at four developmental stages: 6th tiller, flag leaf, booting, and panicle. Key physiological parameters including plant height, biomass, net photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO_2, and leaf chlorophyll content were measured. Biochemical analyses encompassed the nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate synthase activity and reduced nitrogen content. Results revealed significant inter-varietal differences in growth, carbon assimilation, and nitrogen metabolism. V1 consistently exhibited superior physiological performance and higher N assimilatory enzyme activities across all stages, especially under low-N conditions. These findings underscore the relevance of physiological and biochemical markers in assessing nitrogen use efficiency and highlight V1 as a promising candidate for crop improvement programs targeting sustainable rice cultivation with reduced N inputs.

Keywords: Nitrogen use efficiency; Rice (*Oryza sativa* L.); Low-N; Carbon assimilation; Nitrogen assimilation

INTRODUCTION

Nitrogen (N) is one of the most vital macronutrients required for the development and advancement of crops, which is also a crucial part of ATP, amino acids, chlorophyll, and nucleic acids (Arya *et al.,* 2019). N has a major role in controlling crop production and biomass accumulation in plants by directly influencing physiological processes such as respiration, photosynthesis, and protein synthesis (Lawlor, 2002; Masclaux-Daubresse *et al.,* 2010). N accessibility is one of the most important elements influencing production in rice (*Oryza sativa* L.), which supports more than fifty percent of the world populace (Fageria, 2007). Regardless of its significance, average nitrogen use efficiency (NUE) of rice is only about 30–40% (Ladha *et al.,* 2005). This means that a large amount of N that has been utilised goes missing through leaching, volatilisation, and denitrification, which contributes to environmental deterioration. In intensive rice farming platforms, improper and unbalanced N fertiliser usage is a prevalent trend that causes greenhouse groundwater pollution, gas emissions, and soil acidification (Zhang *et al.,* 2015). Accordingly, creating rice varieties with higher NUE is a sustainable way to lessen reliance on fertilisers while preserving or increasing production (Kant *et al.,* 2011). The capacity of a plant to efficiently absorb, digest, and use available N is known as NUE. It is an intricate feature governed by several biochemical, physiological, and molecular processes (Hirel *et al.,* 2007). Physiological and biochemical markers are useful instruments for describing how plants react when N levels are low. According to Rao *et al.* (2013), the accessibility of N has an immediate effect on growth characteristics including plant height and biomass. N status is also closely related to carbon assimilation parameters, such as net photosynthetic rate (P_n), stomatal conductance (g_s), intercellular CO_2 (C_i), and transpiration rate (T_r), as N is involved in Rubisco production and chlorophyll content (Evans, 1989; Yamori *et al.,* 2005). On the molecular level, nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), and glutamate synthase (GOGAT) are the four main enzymes that control N metabolism in plants (Miller *et al.,* 2008). NR and NiR facilitates the sequential reduction of NO_3^- to NO_2^- and NO_2^- to NH_4^+,

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correspondingly, while GS and GOGAT play critical roles in ammonium assimilation and amino acid biosynthesis (Lea and Azevedo, 2006). The activity levels of these enzymes are sensitive indicators of total NUE of plants and the effectiveness of their N uptake.

A number of variables affect the generation of rice crop. The crop output may be significantly impacted by the plant population and nutrient availability (Khutso *et al.,* 2024). While conventional and scientific breeding methods have produced several high-yielding rice varieties, little is known about how responsive they are to N, especially in nitrogen-limiting environments (low-N). In addition to providing genetic resources for breeding efforts aimed at NUE augmentation, the screening and characterisation of rice genotypes with varying NUE give important insights into varietal adaptation techniques (Abdelrahman *et al.,* 2018). In our previous research involving more than 20 rice varieties, the varieties Vikramarya (V1) and Aditya (V2) were recognised as nitrogen-efficient (N-efficient) and nitrogen-inefficient (N-inefficient), correspondingly, based on comparative growth and yield analyses under variable N treatments. These two distinct genotypes provide a great model system for investigating the physiological and biochemical processes linked to NUE (Tantray *et al.,* 2022).

The objective of this experiment is to look into the physiological and biochemical responses of V1 and V2 rice varieties under low and optimal nitrogen conditions. Specifically, we evaluate growth parameters (such as plant height and biomass), carbon assimilation characteristics (including P_n, g_s, C_i, and T_r), and activities of key nitrogen metabolism enzymes (NR, NiR, GS, and GOGAT) at four developmental stages. This thorough investigation seeks to understand varietal variations in N response and to discover physiological and biochemical indicators linked with high NUE. The outcomes of this study will help to further improve our comprehension of plant N physiology and assist breeding techniques for producing N-efficient rice varieties, which is critical for agricultural sustainability in the face of changing climates and growing input costs.

MATERIALS AND METHODS

Plant sample

Vikramarya (V1) and Aditya (V2) varieties of rice were used in this study to evaluate the physiological analysis of the leaves. These varieties were identified as N-efficient and N-inefficient, correspondingly, through screening of more than 20 different varieties of rice in our earlier experiment and were procured from Genetics Division, ICAR-IARI, New Delhi (Tantray *et al.,* 2022; Bashir *et al.,* 2023). V1 variety displays semi-dwarfism, elongated stout grains, white, resistant to gall midge (GM), rice tungro virus (RTV), and green leafhopper (GLH) with an expected productivity of 50 Q/ha whereas V2 was found to be semi-dwarf; the grain is elongated and sturdy, resilient to bold, less susceptible to RTV, brown spot (BS) and bacterial leaf blight (BLB), vulnerable to GM, and brown planthopper (BPH); and the expected yield is 33–40 Q ha^{-1} (Bashir *et al.,* 2023).

Field conditions for plant growth

In the kharif season, the evaluation was conducted in IARI, Delhi, India (June-October 2022). The coordinates of the place are 28.08º N latitude and 77.12º E longitude. The elevation above mean sea level is 750 feet (228.61 metres). It has a transitional temperate and arid-prone climate. During the growing season (July to September), the average daily maximum temperature varies from 32.2 ºC–40 ºC, while the average daily minimum temperature ranges from 12.2 ºC–27.5 ºC. There is much rain from June to September, with an average rainfall of 500 mm.

Treatment information

The varieties were grown in accordance with the N recommended doses (RD) and was enhanced with additional vital nutrients to provide the best possible rice development. The recommended dose of nitrogen is 120 kg/ha (Singh Shivay *et al.,* 2014; Yadav *et al.,* 2014). They were raised with low-N and optimum-N supply. Nitrogen was added in the urea formulation in three split doses.

Samplings

Four development stages (6th tiller, flag leaf, booting and panicle stage) were chosen for tests that were stage-specific. After four weeks of rice plant transplanting, the inter-varietal responsiveness was assessed based on physiological performance (growth and carbon assimilatory parameters) and biochemical analysis (nitrogen assimilatory parameters) at the chosen development stages. The measurement of growth traits were taken around 9:00-11:00 AM. As soon as possible, the leaves were submerged in liquid N and transferred to a -80º deep freezer until they were ready for biochemical analysis. They were lyophilised using (Labconco, FreeZone 4.5 L, USA) to preserve the leaf material for a long time (Tantray *et al.,* 2022).

Assessment of plant growth parameters

Plant height was calculated in the field using a metre scale in centimetre (cm) by placing the base of the scale at the soil surface next to the main stem. The plant length was recorded from the ground level to the tip of the tallest leaf or inflorescence. Plant biomass (g/plant) was calculated after 72 hours of drying in an oven at 65 ºC.

Assessment of carbon assimilatory parameters and leaf chlorophyll content

The Infra-Red Gas Analyzer is the core of the photosynthetic system, monitoring CO_2 and H_2O levels (IRGA) (CID-340, Photosynthesis System, Bio-Science, USA). It was utilised to estimate carbon assimilatory traits. Carbon assimilatory parameters reflect the physiological capacity of the plant to fix carbon. A light intensity of around 800 µmol m^{-2} s^{-1}, relative humidity of about 70%, air temperature of about 28 °C, and a CO_2 content of about 380 µmol/mol were all present throughout the analysis, which were carried out in an ordinary field. Maximum measurement precision was ensured by only collecting data when the IRGA detected photosynthetically active radiation (PAR) over 700 µmol m^{-2} s^{-1}. The Soil Plant Analysis Development (SPAD) chlorophyll meter was utilised to quantify leaf chlorophyll in the intact and fully grown leaves in terms of relative chlorophyll content during morning hours. The data is shown as SPAD values, which are a measure of the relative chlorophyll concentration.

Determination of Biochemical Parameters

The NR, NiR, GS, and GOGAT activity and reduced nitrogen content were among the biochemical characteristics that were assessed in rice leaf samples in four developmental stages of both rice varieties under low and optimum-N conditions.

In vitro NR activity

The enzyme extraction procedure used the Ahmad and Abdin (1999) approach. One g freshly obtained leaf material was homogenised in 3 mL cold isolation buffer (phosphate buffer of 0.1 M concentration having pH 7.5), including 5 mM cysteine–HCl, 0.3% PVP, 5 mM EDTA, and 100 mM sodium phosphate buffer. After passing the homogenate through muslin fabric, it was centrifugated at 4°C and 10,000 rpm for 15 minutes. Analysis of the enzyme activity was done using the resultant supernatant. The supernatant was pipetted to a fresh tube and placed on ice till the enzyme assessment was performed. The activity of enzyme was conducted using the methodology established by Campbell (1978). The reaction solution (3 mL) comprised enzyme extract (0.5 mL), NADH (50 µM, 0.5 mL), potassium nitrate (0.5 mL), and potassium phosphate buffer (100 µM, 0.5 mL, pH 7.5). Enzyme extract was only introduced to study samples to initiate the enzyme reaction. In the control solution, the extract was replaced with deionised water. After 30 min of incubation at 33 °C, zinc acetate (1 M, 0.2 Ml) followed by 75% ethanol (1.8 mL) was added to stop the combination. The precipitate formed was removed by centrifugating at room temperature for 5 minutes at 2000 rpm. The supernatant was obtained and *N*-(1-naphthyl) ethylene diamine solution [0.02% (w/v)] and sulphanilamide solution [1% (w/v)] were introduced to it. For the reaction to produce a pink hue due to diazotisation, it was incubated for 15 min. The absorbance of the reaction solution was quantified at 540 nm compared to a control using a UV–VIS spectrophotometer (Model EI-2371, Electronics India).

In vitro NiR activity

Gupta and Beevers (1984) approach was followed for enzyme extraction. 3 mL isolation buffer (phosphate buffer 0.1 M having pH 7.5) containing cysteine–HCl (1 mM), sodium phosphate (100 mM, pH 8.8), and EDTA was used to homogenise 1 g of leaf tissue. After centrifuging the homogenate at 4 °C for 30 min at 10,000 rpm, the resulting solution was utilised to assess the activity of enzymes. The technique outlined by Ida and Morita (1973) was utilised to evaluate the performance of NiR enzyme. The reaction mixture (final volume: 3 mL) was made up of equal parts of the enzyme extract, Tris–HCl buffer (100 µmol having pH 7.5), $NaNO_2$ (3 µmol), and methyl viologen (2 µmol). The test sample was mixed with freshly created sodium dithionite (24 µmol, 0.3 mL) dissolved in sodium bicarbonate (0.2 M) to initiate the reaction. The solution was incubated for 20 min at 30 °C. The test tube was shaken to halt the process until the decreased blue hue of methyl viologen vanished entirely. To measure the amount of nitrite, a aliquot of 0.1 mL of the reaction solution was mixed with sulfanilamide (1% in 3N HCl, 1 mL) and 0.02% NEDD (1 mL). After adjusting the amount with distilled water to a total volume of 6 mL, the pink hue was left to develop in the dark for 20 min. The absorbance was measured at 540 nm using spectrophotometer. Each sample was compared to a blank, containing all the solutions except enzyme extract.

In vitro GS activity

The technique established by McNally *et al.* (1983) was utilised for the assessment of GS activity. The leaf samples were collected and then extracted in Tris–HCl buffer (5 mL, pH 7.5) containing $MgCl_2$ (1 mM), β-mercaptoethanol (10 mM), Tris–HCl (25 mM), and DTT (1 mM) were used to homogenise 1 g of leaf tissue that had been powdered in liquid nitrogen. The pulverised leaf samples were collected in Oak Ridge tubes and stored on ice after being extracted using Tris–HCl buffer in a pre-chilled pestle and mortar. Then, it was centrifugated at 4 °C for 10 min at 5,000 rpm. The resulting solution was obtained and centrifugated again at 4 °C for 15 min at 12,000 rpm. This upper layer is obtained in separate tubes to estimate GS activity. The Rhodes *et al.* (1975) approach was used to measure glutamine synthetase (GS) activity. The reaction solution included Tris-HCl buffer (100 µmol having pH 8), sodium glutamate (250 µmol), $MgSO_4$ (100 µmol), L-cysteine (10 µmol), ATP (10 µmol), hydroxylamine (10 µmol), and the enzyme extract (final volume 3 mL). Enzyme extract was only introduced to experimental samples to initiate the reaction; in the control sample, the enzyme extract was replaced with deionised water. The solution was incubated for 30 min. at 37 °C. One mL of ferric chloride reagent, which was made up of equal volumes of $FeCl_3 \cdot 6H_2O$ in 0.2 N HCl (10%), TCA (24%), and HCl (50%) was added to stop the reaction by forming γ-glutamyl hydroxamate (GH). The absorbance was quantified at 540 nm.

In vitro GOGAT activity

The Mohanty and Fletcher (1980) methodology was used for extraction. 1 g of leaf material was crushed in liquid N by adding extraction buffer (5 mL) which contained EDTA (10 mM), $MgCl_2$ (10 mM), Tris–HCl (100 mM, pH7.5), Sucrose (0.2 M), KCl (10 mM), and β-mercaptoethanol (10 mM). The reaction was centrifugated at 4 ºC for 10 min at 10,000 rpm. The resultant was obtained and again centrifuged at 12,000 rpm for 15 min at 4 °C. The resulting solution was pipetted to a fresh tube and placed on ice till an enzyme test was performed. The enzyme activity was evaluated using the technique developed by Fowler *et al.* (1974). All the reagents viz., α-ketoglutarate (10 µmol), Tris–HCl buffer (75 µmol), NADH (0.3 µmol), L-glutamine (15 µmol), and enzyme extract (3 mL) were mixed. The extract of enzyme was only introduced to the test samples to start the reaction, whereas the extract was replaced with deionised water in the blank sample.

Just before obtaining an absorbance reading, NADH (1.5 mM) was introduced to a cuvette containing the reaction mix. The absorbance was then quantified using spectrophotometer at 340 nm for 60 s.

Estimation of reduced nitrogen content (RNC)

Dried leaf samples were finely ground into a uniform powder, and 5 mg of the

powdered material was used for elemental analysis. 5 mg of the homogeneously crushed powdered dried leaf material was utilised for elemental analysis. A CHNS elemental analyser (Elementar Analyzer systeme, Germany) used the standard combustion concept to estimate the nitrogen concentration. The nitrogen in the sample was transformed into nitrogen gas or nitrogen oxides after burning at 1000 °C. Nitrogen was quantified using a temperature-sensitive detector maintained at 290 °C. The N content was measured in mg/g dry weight DW.

Statistical evaluation

Statistical assessment was done for the experiment and data was generally represented as mean ± standard error (SE). The mean and SE were calculated by Microsoft Excel 2016, using average and standard error function after selecting raw data. The data on various parameters were subjected to one-way analysis of variance (ANOVA) using IBM® SPSS® Statistics version 20. The significance of differences among mean were calculated using duncan's multiple range tests (DMRT) at $P < 0.05$. The percent variation (PV) was calculated by the following formula,

PV = Treatment – Control/ Control × 100

RESULTS

The physiological and biochemical traits (growth, carbon assimilatory, and nitrogen assimilatory parameters) of rice plants were measured at four key developmental stages: 6th tiller, flag leaf emergence, booting, and panicle emergence. Both rice varieties, Vikramarya (V1) and Aditya (V2), were grown under two nitrogen treatments: 100% recommended dose of nitrogen (N-100%) and 50% nitrogen (N-50%) to assess varietal response to nitrogen availability. N-100% is considered as the control and N-50% as the treatment.

Low nitrogen supply alters growth traits in plants

For growth analysis, two key morphological parameters—plant height and plant biomass—were evaluated across developmental stages. The results revealed clear varietal differences in growth performance influenced by N levels (Figure 1). Plant height progressively hightened from the 6th tiller stage to the panicle initiation in both rice varieties; however, the extent of growth was significantly influenced by N availability and genotype. V1 consistently maintained higher plant height under both N treatments, while V2 exhibited significant reductions under low-N. When compared among treatment conditions, the plant height was dropped substantially 26.51, 20 and 17.62% at flag leaf, booting and panicle stages respectively under limited-N than the plant grown under opt-N in V2.

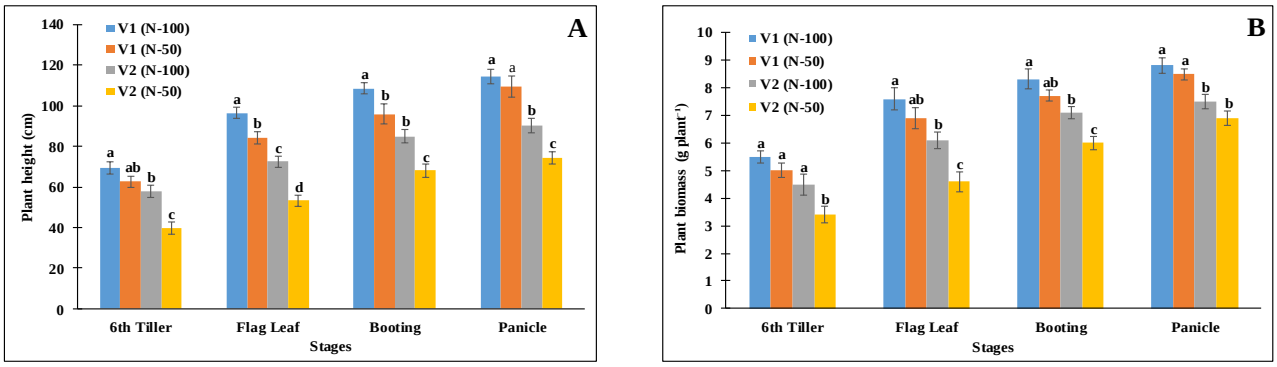

Figure 1: Changes in plant growth of V1 and V2 rice varieties at four developmental stages under low and optimum-N conditions. Bars represent plant height (A) and plant biomass (B). Data are mean ± standard error (SE) for each treatment (n = 3). Different letters (a, b, c, d) above the bars show statistically significant differences among treatment according to Duncan's multiple range test (P < 0.05)

Plant biomass accumulation revealed striking differences between the two rice varieties under varying N conditions, especially as the plants progressed through developmental stages. The N-efficient genotype V1 displayed a steady and robust increase in biomass from the

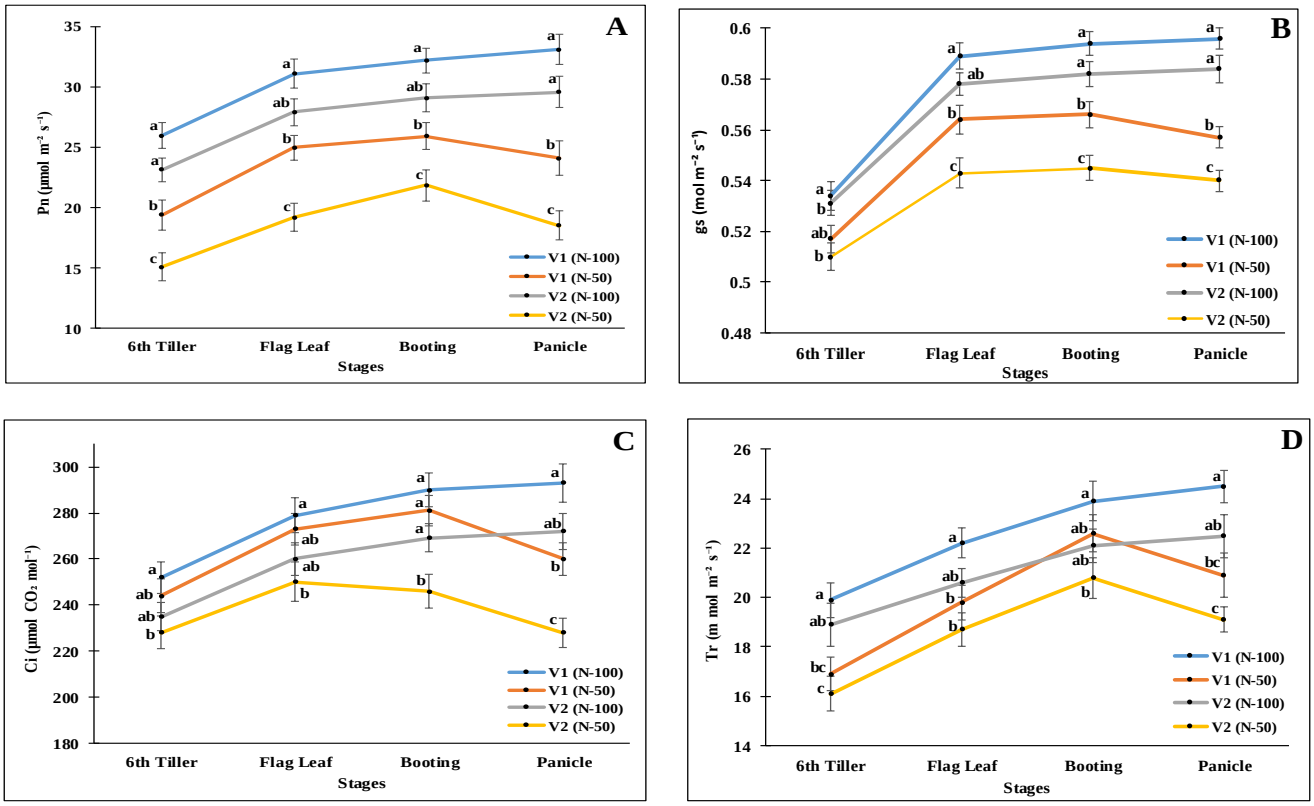

Figure 2: Changes in gas–exchange traits of V1 and V2 rice varieties at four developmental stages under low and optimum-N conditions. Bars represent net photosynthetic rate (A), stomatal conductance (B), intercellular CO_2 conc. (C), and transpiration rate (D). Data are mean±standard error (SE) for each treatment (n=3). Different letters (a,b,c,d) above the bars show statistically significant differences among treatments according to Duncan's multiple range test (P<0.05)

6th tiller to the panicle stage under both N treatments. Even under low-N, V1 maintained its biomass accumulation pattern with only minor fluctuations, suggesting strong adaptive growth capacity. The percent decrease in biomass in V1 due to reduced N was 9.09% at 6th tiller, 9.21% at flag leaf, 7.33% at booting, and 3.63% at panicle stage. In contrast, V2 showed a clear N-dependent response: while biomass increased progressively from early to late stages under opt-N, this upward trend was significantly dampened under N-deficient conditions. The drop in V2 biomass under low-N was most pronounced at later stages, reflecting compromised assimilate production and allocation. Aditya showed sharper decline by 24.44% at 6th tiller, 24.59% at flag leaf, 15.49% at booting, and 8% at panicle stage. These results demonstrate that V1 maintains higher biomass and exhibits less reduction under N stress compared to V2, particularly as the plant transitions from vegetative to reproductive stages. The divergence in performance between the two varieties became especially evident at the booting and panicle stages, where V1 sustained high biomass values, whereas V2 exhibited a sharp reduction.

Gas-exchange and chlorophyll content under low-N

The carbon assimilatory parameters and chlorophyll content were measured at four growth stages in varieties V1 and V2 under low and opt-N treatment (Figure 2). The P_n of both varieties under varying N treatment reveals critical physiological and agronomic insights. For V1, the transition from opt to low-N fertilisation caused progressive declines in P_n by 25.28% at the 6th tiller stage, 19.70% at flag leaf, 19.52% during booting, and 27.20% at panicle formation. This pattern indicates that while V1 maintains relatively stable P_n during vegetative phases (19.52% to 25.28% reduction), its photosynthetic machinery becomes more N-dependent during reproductive development, with the sharpest decline occurring at panicle initiation. V2, however, exhibited greater sensitivity to N limitation, with P_n reductions intensifying across growth stages by 34.69% at 6th tiller, 31.16% at flag leaf, 24.84% during booting, and 37.29% at panicle. Notably, photosynthetic collapse of V2 at panicle formation (37.29%) surpasses even its

early-growth vulnerability, suggesting that N metabolism in this variety is particularly critical during reproductive organ development. The analysis of g_s across developmental stages reveals nuanced responses to N availability in V1 and V2 rice varieties. Under opt-N, V1 exhibited progressive g_s increases from 6th tiller stage to panicle formation, while V2 showed a similar trajectory. Low-N induced gradual declines, with g_s of V1 decreasing by 3.18% (6th tiller), 4.24% (flag leaf), 4.71% (booting), and 6.54% (panicle), whereas V2 demonstrated greater sensitivity at later stages: 3.95%, 6.06%, 6.36%, and 7.53% respectively. Notably, both varieties experienced maximal stomatal limitation at panicle initiation, where gs reduction in Aditya (7.53%) surpassed reduction in Vikramarya (6.54%), mirrors their photosynthetic rate patterns. Low-N induced progressive C_i declines, with reductions of V1 intensifying from 3.17% (6th tiller) to 11.26% at panicle. C_i in V2 displayed sharper sensitivity, particularly during reproductive phases and decreased by 2.98% (6th tiller), 3.85% (flag leaf), 8.55% (booting), and 16.18% (panicle). Both varieties experienced maximal C_i reduction at panicle initiation, with 16.18% decline in V2 underscoring heightened metabolic stress during grain development. The T_r dynamics under N limitation reveal distinct varietal strategies in water-use regulation. For V1, T_r declined by 5.44% at the booting and 14.69% at panicle leaf initiation under Low-N, reflecting moderate stomatal sensitivity during early growth. In contrast, V2 exhibited pronounced T_r reductions across all stages by 14.81% at 6th tiller, 9.22% at flag leaf, 5.88% at booting, and 15.11% at panicle stage. The data unveils a U-shaped stress response in V2, with maximal T_r decline at vegetative initiation (14.81%) and reproductive transition (15.11%), while booting-stage resilience (5.88%) suggests transient hydraulic adjustments.

The chlorophyll content dynamics, measured via SPAD values, reveal distinct varietal responses to N availability (Figure 3). V1 under opt-N showed progressive chlorophyll accumulation from 6th tiller stage to panicle formation, while low-N induced reductions of 11.18% at 6th tiller and 8.99% at flag leaf, reflecting moderate N-dependent chlorophyll retention during vegetative growth. In contrast, V2 exhibited consistent declines across all stages under low-N by 11.67% at 6th tiller, 11.21% at flag leaf, 11.40% at booting, and a sharp 20.67% decline at panicle initiation stage.

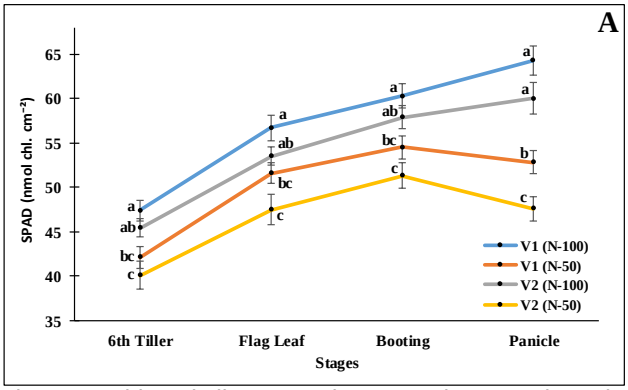

Figure 3: Chlorophyll content decreases in V1 and V2 rice varieties under limited-N administration than optimal N treatment. SPAD = chlorophyll content (A). Each curve point in each graph represents the mean ± SE at each growth stage (n = 3). Different letters above the graphs demonstrate statistically significant differences among treatment means according to DMRT test ($P < 0.05$)

Low nitrogen supply alters enzymatic activity and reduced nitrogen levels (RNC)

The two distinct rice varieties were examined for enzymatic performance at limited nitrogen levels of treatment at four chosen developmental stages. The functions of key enzymes in nitrogen assimilation (NR, NiR, GS, and GOGAT), as well as reduced nitrogen content were examined (Figure 4). The NR activity of leaves were measured in varieties V1 and V2 exposed to low-N condition when compared with opt-N.V2 showed pronounced reductions of NR activity across all stages from 6th tiller (21.05%), flag leaf (22.73%), booting (20.21%) and panicle (25.00%) grown under low-N relative to V2 grown under opt-N condition. Nevertheless, in V1, a rise in activity was noted at the initial stage at 6th tiller, flag stage, and booting stage and showed slope from booting (20.18%) and panicle stage (28.18%) under low-N than V1 grown under opt-N. NiR activity measurements across developmental stages and N regimes reveal clear contrasts between the two rice varieties. For V1, NiR activity showed a decrease of 22.08% at the 6th tiller stage. At the flag leaf stage, NiR activity showed a reduction of 15.69%. In V2, the pattern of decline was evident at every stage. At the 6th tiller, NiR activity decreased by (27.00%) at flag leaf, (17.62%) at booting, (24.43%) and (30.00%) at panicle. The GS activity data unveil distinct N-responsive strategies between V1 and V2 rice varieties. Under opt-N, V1 exhibited

progressive GS activation, rising from 1.41 µmol/g FW/h at the 6th tiller to 1.69 at panicle, while V2 showed a slower ascent from 1.20 to 1. Under low-N, GS activity of V1 declined moderately at early stages (7.80% at 6th tiller, 10.13% at flag leaf, but partially recovered at panicle (8.28%). In contrast, V2 suffered sharp, stage-agnostic declines of 8.33% at 6th tiller, 9.70% at flag leaf, and a catastrophic 14.81% collapse at panicle under low-N. Under opt-N, V1 exhibited progressive GOGAT activation, rising from 0.57 µmol/g FW/h at the 6th tiller to 0.83 at panicle formation. Low-N caused moderate declines of 10.53% (6th tiller), 9.23% (flag leaf), and 20.48% (panicle). Notably, GOGAT activity of V1 under low-N increased by 7.14% from booting to panicle, suggesting compensatory upregulation during reproductive N demands. In contrast, V2 suffered severe reductions across all stages under low-N having 8.69% (6th tiller), 15.09% (flag leaf), and a catastrophic 29.17% collapse at (panicle).

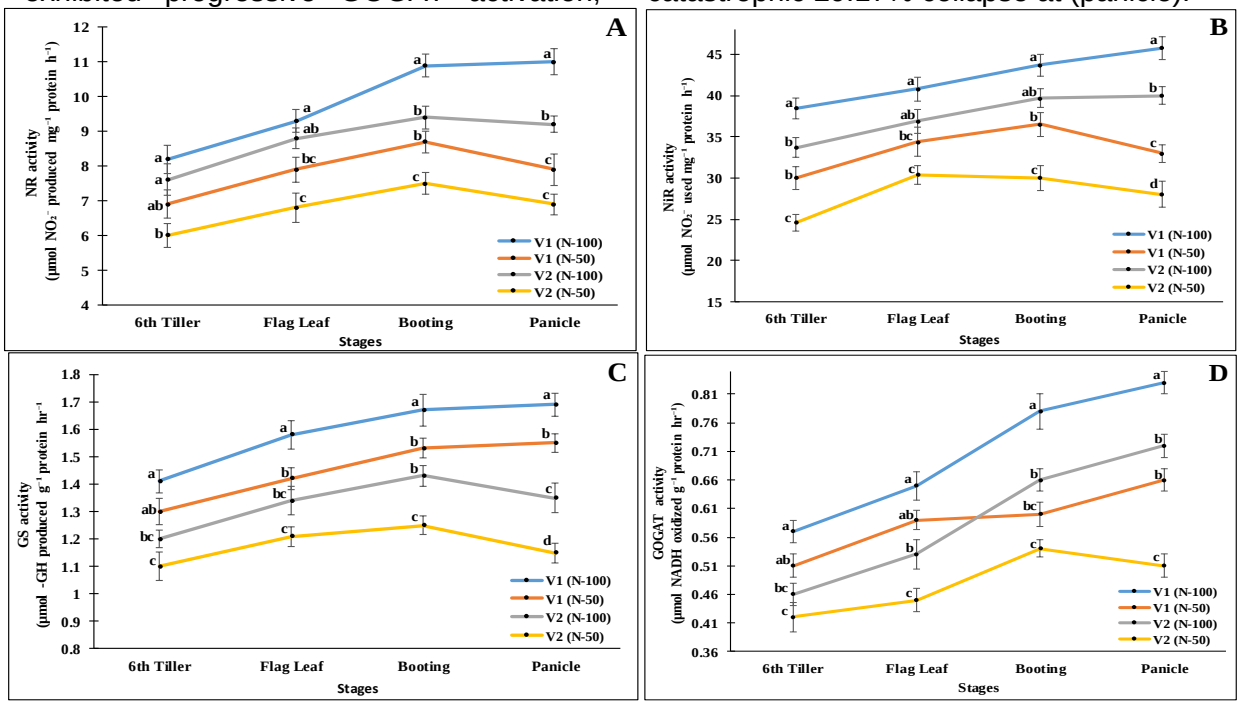

Figure 4: Effect of nitrogen treatment on enzyme activity in rice varieties V1 and V2 grown under N-50% and N-100% levels. NR activity (A) NiR activity (B). GS activity (C) and GOGAT activity (D). Each graph represents mean ± SE (n=3) of each treatment. Different letters above the graph show statistically significant differences among treatment means according to Duncan's multiple range test ($P < 0.05$)

The reduced nitrogen content (RNC) of leaves statistics of both varieties evaluated under low-N conditions (Figure 5). RNC in leaves increases in both varieties when the N level is optimum; however, it decreases in low nitrogen-treated varieties as the plant matures. The analysis of RNC across developmental stages reveals distinct metabolic strategies in V1 and V2 under N limitation. V1 exhibited progressive N depletion under low-N, with reductions intensifying toward reproductive stages exhibiting 10.81% at 6th tiller, 17.78% at flag leaf, 18.87% at booting, and a sharp 23.64% collapse at panicle. In contrast, V2 displayed mixed resilience having moderate declines at early stages 8.57% at 6th tiller, 7.14% at flag leaf but escalating losses at booting (13.04%) and panicle stages (20.83%).

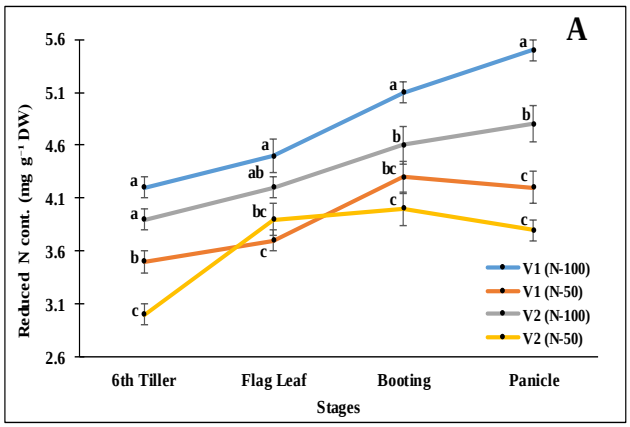

Figure 5: Effect of reduced nitrogen content activity in rice varieties V1 and V2 grown under N-50% and N-100% levels. Reduced nitrogen content (RNC) (A). Each graph represents mean ± SE (n=3) of each treatment. Different letters above the graph demonstrate statistically significant differences among treatment means according to DMRT test ($P < 0.05$)

DISCUSSION

Understanding physiological and biochemical responses of rice varieties under N-limiting circumstances is critical for producing NUE variants. In the present study, two contrasting rice varieties viz., V1 and V2 were analysed under low and optimum nitrogen conditions across four growth stages to elucidate their responses in terms of growth, carbon assimilation, and nitrogen metabolism. The following discussion expands on the findings, focussing on the variations observed especially under low-N situations, which are crucial for detecting NUE traits. Plant height is an important morphological characteristic that represents total plant health and is heavily controlled by N availability. Under opt-N circumstances, both varieties increased plant height significantly at all stages, demonstrating the importance of N in stimulating cell division and elongation (Wu *et al.,* 2020). But under low-N conditions, both varieties saw a considerable loss in plant height, with V2 showing a steeper decline. V1 retained comparatively greater plant height under N-deficiency, especially during the flag leaf and panicle initiation stages, indicating an enhanced adaptive mechanism to low-N supply. This outcome corroborates the findings of Wang *et al.* (2022), who showed that N-efficient varieties exhibit a lower inhibition of ascending growth under N stress due to more effective internal N redistribution and signalling. Plant biomass is an accurate measure of plant growth and NUE. As anticipated, opt-N treatment increased biomass accumulation in both varieties, but V1 consistently produced more biomass than V2. Under low-N conditions, biomass decreased substantially in both varieties, but V1 showed less reduction across all stages, especially during booting and panicle initiation phases. These findings indicate that V1 retains improved carbon partitioning and nutrient utilisation efficiency under low-N stress. Efficient biomass distribution, particularly in roots, is crucial for nutrient foraging under stressful situations (Kumar *et al.,* 2021). The tendency of V1 variety to maintain greater root biomass in low-N environments may allow for higher N absorption and assimilation, supporting its categorization as an N-efficient genotype.

Photosynthesis is intrinsically connected to N status, as N is an essential component of Rubisco and chlorophyll. Both genotypes demonstrated better P_n under opt-N, with V1 regularly surpassing V2. Under low-N conditions, V2 showed a significant drop in P_n, notably during the panicle initiation and flowering phases. The highly steady P_n of V1 during N deprivation indicates a good internal N economy and preservation of photosynthetic machinery. Tantray *et al.* (2020) found that N-efficient rice varieties may withstand greater P_n levels in low-N environments by retaining chlorophyll content and Rubisco activity. Nitrogen intake greatly altered g_s. Under opt-N, both varieties showed high g_s levels, but under low-N, V2 showed a more dramatic drop, particularly during reproductive phases. In contrast, V1 had a considerably lower g, indicating improved stomatal control. Stomatal conductance, which controls CO_2 input and water loss, is a crucial factor in determining water efficiency. The increase g_s of V1 under low-N conditions may enhance CO_2 absorption and photosynthetic performance, supporting findings by Li *et al.* (2023). Transpiration rate (T_r) measures stomatal activity and is affected by g_s and vapour pressure imbalance. Under opt-N, T_r was higher in both varieties, with V2 showing a minor decline at the booting stage but a quick increase at the panicle stage. Under low-N conditions, V1 showed reasonably steady T_r levels until booting, after which they dropped dramatically, whereas V2 showed severe drops, particularly during flowering. Maintaining transpiration during N deficit may improve nutrient transfer and leaf cooling. The capacity of V1 to maintain T_r in low-N conditions suggests greater hydraulic conductance and physiological flexibility, which is analogous to the findings published by Kumari *et al.* (2021), who linked stable T_r to improved NUE in rice. C_i reflects the equilibrium of CO_2 between demand and supply in the leaf mesophyll. Here, it was noticed startling observations under opt-N, V1 demonstrated ideal C_i levels, however V2 also showed a consistent increase throughout all stages, but their C_i is lower than V2 under low-N up until booting and then increases at the panicle stage. However, in low-N conditions, C_i reduced in V1 during early development stages, indicating greater CO_2 fixation through effective photosynthesis. V2, on the other hand, demonstrated higher C_i, particularly at the flag leaf, which might be attributed to lower Rubisco

activity and photosynthetic efficiency. The opposing C_i patterns indicate greater physiological adaptation of V1 to low-N. Recent findings (Makino, 2021) suggest that NUE breeding should prioritise efficient photosynthetic CO_2 assimilation under N stress.SPAD value offers a quick and non-invasive estimation of leaf chlorophyll levels and is highly connected to nitrogen concentration in the leaves (Wang *et al.,* 2014). In this study, both varieties had greater SPAD values under opt-N circumstances, indicating adequate chlorophyll concentration and robust photosynthetic machinery. Under low-N circumstances, SPAD measurements decreased significantly, notably in V2. This reduction was most noticeable during the panicle initiation and blooming periods, which are crucial for biomass buildup and grain growth. Sustaining chlorophyll levels during stress is critical for carbon absorption and energy generation. The capacity of V1 to maintain greater SPAD values in low-N environments might be attributed to fast internal N remobilization and improved chloroplast structure protection (Liu *et al.,* 2025). The observed trend supports its better net photosynthetic rate and growth performance under stress.

Nitrate reductase (NR) is a crucial enzyme in nitrate absorption that is extremely sensitive to N levels. Under opt-N, both cultivars demonstrated strong NR activity with V1 showing significantly more activity. Under low-N conditions, NR activity decreased in both, but V1 maintained considerably higher activity than V2, notably during the panicle initiation and flowering periods. This tendency shows that V1 may better digest available nitrate even when supplies are restricted. Higher NR activity under stress circumstances improves internal N cycling, as documented by Sathee *et al.* (2021). Nitrite reductase (NiR) is an enzyme that participates in the second step of nitrate absorption in plants. NiR efficiency is crucial for maintaining cellular N homeostasis, especially when N is limited (Hakeem *et al.,* 2011). In the ongoing research, both varieties of rice displayed increased NiR activity under opt-N circumstances across all development stages, indicating that there was enough nitrate substrate and cellular energy for nitrate reduction. Under low-N stress, both varieties showed a considerable decrease in NiR activity, with V2 displaying a steeper loss, notably during the panicle initiation and blooming stages. Several investigations have demonstrated that genotypes with higher NUE maintain NiR activity under N-deprived circumstances, ensuring ongoing N absorption despite external constraints (Reddy *et al.,* 2004; Hakeem *et al.,* 2013). Glutamine synthetase (GS) is essential for ammonium absorption and keeps nitrogen levels stable. Under opt-N, both varieties had increased GS activity. Under low-N conditions, V1 had much more GS activity than V2, particularly during active vegetative and reproductive phases. The continuous GS activity of V1 implies improved N uptake and reallocation under stress, which supports efficient biomass production. According to Ma *et al.* (2022), GS overexpression during N deficit is a sign of NUE. Glutamate synthase (GOGAT) collaborates with GS in the absorption of ammonium. GOGAT activity, like GS, reduced under low-N conditions in both varieties. However, V1 declined less than V2, indicating that N metabolism was better maintained under stress. The increased GOGAT activity in V1 may contribute to enhanced amino acid biosynthesis and protein turnover, hence promoting growth and development in low-N environments (Castillo *et al.,* 2000). Reduced nitrogen content (RNC) in plant tissues indicates the effectiveness of nitrogen absorption, assimilation, and redistribution. The capability of crop to transform inorganic nitrogen (NO_3^- and NO_2^-) into physiologically useful forms is reflected in its composition, which predominantly includes ammonium (NH_4^+), amino acids, amides, and other nitrogenous metabolites (Liu *et al.,*2022). The RNC increased progressively across developmental stages in all treatments, with significantly higher values under opt-N conditions. V1 possesses a more robust N assimilation and retention system, enabling it to maintain N metabolism even under nutrient-limited conditions. In contrast, the reduced performance of V2 under low-N indicates weaker N assimilation efficiency. These results emphasize the superior NUE of V1, aligning with its improved growth and physiological traits under limited nitrogen input.

The biochemical and physiological outcomes from this investigation give strong backing for the engagement of nitrogen metabolism in influencing the levels of rice NUE. The greater growth and N absorption capabilities of V1 indicate that its higher NUE is most likely related to its capacity for efficiently utilise accessible N for both growth and metabolic activities. The higher activity of N absorption

enzymes in V1 indicates effective N utilisation, whilst more efficient photosynthetic machinery contributes to increased carbon fixation and biomass production. V2, on the other hand, fails to absorb N effectively, as demonstrated by decreased activity of NR, GS, and GOGAT, which has a direct influence on its growth and photosynthetic efficiency under limited nitrogen conditions.

CONCLUSION

This study has provided valuable insights into the physiological and biochemical mechanisms that govern NUE in rice. The results clearly indicate that V1, a N-efficient variety, has better growth, photosynthetic efficiency, and N absorption capability than V2, a N-inefficient variety. The changes in enzyme activity, carbon absorption, and growth parameters highlight the intricate interplay of N supply, plant physiology, and metabolic pathways. Further study into the genetic and molecular underpinnings of these features will be required to improve N efficiency in rice and other crops, eventually leading to increased food security and sustainable farming practices.

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