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Assessing the genetic variability and trait interactions for nitrogen use efficiency in rice

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Abstract

In major cereals, excessive use of nitrogen fertilizers and low nitrogen use efficiency adversely affect land, water and food systems. Developing nitrogen-efficient cereal varieties reduces fertilizer dependence, lowers costs and minimizes environmental pollution while maintaining yield stability. These varieties enhance nitrogen uptake and assimilation, ensuring sustainable food production in low-nitrogen soils. The study aimed to assess the genetic variability for nitrogen use efficiency among 160 rice genotypes by evaluating their performance under three different nitrogen levels -N0 (0N), N50 (50% recommended dose of nitrogen (RDN) and N100 (100% RDN), during *rabi* season. The physiological and yield traits were recorded at the active tillering and flowering stages. The results indicated that the application of different nitrogen levels significantly affected the physiological traits such as chlorophyll index (SPAD value), leaf greenness index (NDVI), light-adapted PSII quantum yield (Fv'/Fm'), photosynthetic rate at both the stages. Also, there was a significant variation observed in yield traits such as the number of productive tillers, spikelets per panicle, 100-grain weight, spikelet fertility and grain yield among the rice genotypes under different N levels. Correlation analysis showed a significant positive relationship between Fv'/Fm' and photosynthetic rate with grain yield under N50 and N100 levels. Hierarchical clustering analysis identified the five high-yielding genotypes such as IRG91, IRG140, IRG302, IRG374 and IRG375, that performed significantly well under N50 in terms of physiological and yield traits compared to the N0 level and the reduction in yield was significantly less over N100. Future research should focus on identifying the key genes and pathways associated with NUE in rice.

Keywords

food security; grain yield; nitrogen use efficiency; physiological traits; rice007A

Introduction

Rice (*Oryza sativa* L.) belongs to the Poaceae family and has a panicle-type inflorescence. Its fruit is a caryopsis, where the seed coat is fused with the pericarp (1). Rice is an important staple crop of the Asian continent and it ranks first among the world's most extensively cultivated cereals. A significant portion of the global population (60%) depends on rice for their daily nutrition (2). The Asia-Pacific region, which includes over 100 countries, produces more than 90% of the world's rice. However, several factors affect rice production, including climate, soil conditions, water availability and management practices such as cultivar selection, sowing dates, weed and nutrient management. Among the essential nutrients, nitrogen (N) is one of the major and vital nutrients that plays a major role in influencing rice production in both upland and lowland ecosystems. According to the USDA Foreign Agricultural Service (2023/2024) (3), global rice production is projected at 515.87 million metric tons, with China (144.62 MMT) and India (137.83 MMT) as the top producers.

In the past fifty years, field crop yields have doubled, largely due to a sevenfold increase in global nitrogenous fertilizer use (4). The global fertilizer application surged from 10.8 to 109 million metric tons from 1960 to 2019, with projections suggesting it could reach 249 million metric tons by 2050 (5). Approximately 16% of the world's nitrogenous fertilizer is used for rice production (6). The excess application of nitrogenous fertilizer is practiced in China and India, which together account for nearly 50% of global rice production and consumption (7). However, nitrogen use efficiency in rice farming remains low, at only 20-50% (8). Approximately 60% of soil nitrogen is lost through leaching, runoff, denitrification, volatilization and microbial consumption. This surplus nitrogen contributes to greenhouse gas emissions (such as N_2O, NO and NO_2), biodiversity loss, soil acidification, groundwater contamination and surface water eutrophication (9,10), which can exacerbate climate change. Addressing these challenges requires efficient resource use and proactive measures in agricultural production to manage the future climate risks without causing damage to the ecosystem (11).

In addition to crop production practices aimed at enhancing nitrogen use efficiency (NUE), the development of nitrogen-efficient rice varieties is essential for mitigating global climate change impacts (12). Since much of the genetic potential for improving NUE remains untapped within crop germplasm, research should prioritize its exploration and utilization, rather than solely focusing on yield improvement under high nitrogen conditions (13). Therefore, breeding rice varieties that maintain yield stability under reduced nitrogen inputs is crucial for promoting environmentally sustainable and cost-effective agriculture (14,15). This strategy not only minimizes fertilizer application and cultivation costs for farmers but also reduces environmental risks.

Several studies have investigated genotype-specific responses to nitrogen availability using diverse experimental approaches. Srikanth et al. (16) investigated genetic variability among 14 rice genotypes in response to graded nitrogen levels (N0, N50, N100 and N150) across four seasons in a split plot design to assess the agro-morphological traits, grain yield, flag leaf characteristics, photosynthetic pigment content, flag leaf gas exchange parameters and chlorophyll fluorescence traits. Similarly, Padhan et al. (17) evaluated 30 diverse rice genotypes under two nitrogen treatments i.e., N-deficient (N0, no external nitrogen) and N-sufficient (N120, 120 kg N ha^{-1}), during the 2019 and 2020 Kharif seasons to explore mechanisms governing reproductive stage nitrogen remobilization and partitioning to grain. Wang et al. (18) screened various rice genotypes, including super hybrid rice, ordinary hybrid rice and commercial *indica,* under different nitrogen application rates (60 and 120 kg N ha^{-1} in 2012; 0, 120 and 210 kg N ha^{-1} in 2013) in split plot arrangement. These studies collectively highlight the importance of identifying and utilizing rice genotypes with improved NUE to develop climate-resilient and resource-efficient varieties.

Nitrogen use efficiency is a complex phenomenon influenced by a range of edaphic, climatic and management factors. It requires management through an integrated approach that encompasses agronomic, physiological and molecular perspectives (19). NUE can be enhanced agronomically up to a certain level, beyond which biological crop improvement alone can break the barrier for further improvement (20). Based on this insight, research on the biological basis of N-response and NUE in diverse crops gained momentum (21). Screening diverse germplasm for high NUE under low nitrogen conditions offers a pathway to minimize fertilizer dependence while maintaining yields. Recent studies have shown that nitrogen-efficient varieties (NEVs) outperform nitrogen-inefficient varieties (NIVs) in terms of both grain yield and NUE, primarily due to enhanced root and shoot activity (22,23). Nevertheless, the specific plant traits linked to N-efficient rice varieties remain largely unexplored (24). Identifying high-yielding varieties that perform well under different nitrogen levels from existing germplasm and understanding their physiological and molecular mechanisms, is crucial for developing nitrogen efficient varieties. Hence, the present study was formulated with the collection of 160 rice genotypes to identify the genotypic variability for NUE and to assess the relationship between physiological traits and grain yield under different nitrogen levels. A set of 500 diverse rice lines, specifically Indian rice lines from the 3K Panel, for which whole genome sequence (WGS) information available, were assembled for this study. The single nucleotide polymorphisms (SNPs) present among these 500 lines were retrieved to analyze genetic diversity and population structure. Based on these analyses, a subset of 160 unique lines, forming an association mapping panel that effectively represents the original genetic diversity, were filtered and selected for the study.

Materials and Methods

Plant material and seasons

A total of 160 rice genotypes from the rice 3K- panel (Table S1) were selected to study the genetic variability for NUE under different nitrogen levels. All the rice genotypes belong to the rice association mapping panel. The experiment was conducted during the *Rabi*-2023 at the wetland farm of the Tamil Nadu Agricultural University, Coimbatore.

The weather parameters such as temperature, relative humidity and precipitation prevailed during the cropping period were recorded. The mean minimum and maximum temperatures were 19.5, 29.2^{0}C respectively, the mean relative humidity was 79.6% and the mean precipitation was 3.37 mm.

Soil analysis and experimental details

The experimental soil was clay in texture, slightly alkaline (pH 8.025), non-saline (EC- 0.36 dS/m). The organic carbon content was medium (0.72%), soil available N was low (193.5 kg/ha), available P was medium (18 kg/ha) and available K was high (721kg/ha). The experiment was conducted in split plot design with three replications where nitrogen levels were assigned to the main plots and genotypes to the sub plots. The treatments were randomly allocated to ensure homogeneity. The split plot design was chosen for its practical advantages and statistical efficiency. Applying nitrogen treatments uniformly across small plots can be challenging, therefore, nitrogen levels were assigned to larger main plots to minimize variability in application. This design effectively manages field variability, particularly soil heterogeneity, by reducing the impact of large-scale variations in fertility. The recommended dose of fertilizer given for rice was 150:50:50 N: P: K kg/ha. The required amount of urea was calculated according to the plot size, which is given in Table 1. The nitrogen treatments were as follows: T1-0 N, T2- 50% recommended dose of nitrogen (RDN) and T3- 100% RDN. The plot size was 175.87 square metres/treatment. The urea was applied in four equal split doses as basal (before transplanting), active tillering, panicle initiation and heading stage in T2 and T3 as per the treatment schedule of fertilizer application. Single superphosphate was applied as a basal as phosphorus source and muriate of potash was applied in four splits as that urea, in all the treatments. All other cultural practices and crop protection measures were taken as per the Crop Production Guide (2020) published by Directorate of Agriculture and TNAU, Tamil Nadu, India.

The seeds of 160 rice genotypes were sown during 4th week of October 2023 and transplanted 21 days after sowing in November 2023. In the main field, the seedlings were transplanted with a spacing of 20 × 15 cm. A selective pre-emergence herbicide, Butanil S (1.25 kg), was mixed with the soil and broadcast across the field. Gap filling was carried out on the 10th day after transplanting. The treatment plots were irrigated uniformly with 2 cm water depth up to the panicle initiation stage, after which a 5 cm water depth was maintained. Crop protection practices were implemented following the recommended package of practices for rice cultivation. The field view of T1, T2 and T3 are given in Fig.1A, Fig.1B and Fig.1C.

Analysis of physiological traits

The physiological traits were measured in the youngest fully expanded fifth leaf of primary tillers at the active tillering stage and in the flag leaf during the flowering stage. The observations were recorded between 10:00 AM and 12:30 PM. The chlorophyll index was measured using SPAD meter (Minolta SPAD 502, Japan) and expressed in SPAD units. NDVI was measured using a GreenSeeker handheld crop sensor (N-Tech Industries, Inc., Ukiah, CA, USA). The Greenseeker sensor was placed about 60 cm above the plant canopy to record measurements. The chlorophyll fluorescence traits such as light-adapted PSII quantum yield (Fv'/Fm') were measured using a chlorophyll fluorometer (OS5p+, Opti Sciences, Hudson, NH) (25). Photosynthetic rate (Pn, µmol. CO_2 $m^{-2}sec^{-1}$) was measured using CI-340 handheld photosynthesis system (CID BioSciences Inc).

Fig. 1A. Field View of T1 (0 RDN) for 160 genotypes.

Fig. 1B. Field View of T2 (50% RDN) for 160 genotypes.

Fig. 1C. Field View of T3 (100% RDN) for 160 genotypes.

Table 1. Details of urea application across four split doses

S.No	N levels	Basal	Tillering	Panicle initiation	Heading stage	Total
1	T1	-	-	-	-	-
2	T2	0.71 Kg	0.71 Kg	0.71 Kg	0.71 Kg	2.85 Kg
3	T3	1.42 Kg	1.42 Kg	1.42 Kg	1.42 Kg	5.71 Kg

Yield traits

The yield parameters such as number of productive tillers, grains per panicle, hundred seed weight(g), spikelet fertility (%) and single plant yield (g) were measured.

1.Number of productive tillers

The number of panicles producing tillers from tagged plants of each genotype was counted.

2.100 grain weight

100 grain weight was measured by weighing the well-developed 100 grains of three plants of each genotype. It was expressed in g plant^{-1}.

3.Total number of grains per panicle

The total number of filled and unfilled grains per panicle was determined by counting the filled grains from three panicles of tagged plants for each genotype. The average of these values was then calculated to represent the number of filled grains per panicle.

4.Spikelet fertility (%)

The spikelet fertility was calculated by

$$\text{Spikelet fertility (\%)} = \frac{\text{Total number of grains - Number of unfilled grains}}{\text{Total number of grains per panicle}}$$

5.Grain yield per plant

Panicles from the selected individual plants of each genotype were used to determine grain yield per plant. The panicles were threshed, cleaned, dried and the final grain weight was recorded. Grain yield per plant was expressed in grams per plant (g/plant).

Statistical Analysis

Two -way analysis of variance (ANOVA) was performed using the R software (version 4.3) using the agricolae package to assess the significance of nitrogen dosage and genotype effects on physiological traits and yield traits. The Least Significant Difference (LSD) method was applied for pair wise comparisons at a 5% significance level (26). The correlation analysis was performed in R software (version 4.3) using corrplot package. Hierarchical clustering analysis was also performed using ward's method using Euclidean distance to classify genotypes based on trait similarities.

Results

Effects of nitrogen dosage on physiological traits of rice genotypes

The physiological traits increased from active tillering stage to flowering stage. The traits such as SPAD, NDVI, Fv'/Fm' and photosynthetic rate showed an increase in both range and mean values with respect to increase in nitrogen dosage (N0 to N100). The application of 100% RDN (T3) recorded the highest value of all the physiological traits at both the stages of observation. However, the plants received 50% reduced nitrogen level recorded significantly higher for the physiological traits values compared to the N0 level.

Physiological traits at active tillering stage

The analysis of variance revealed that treatments and genotypes exhibited highly significant differences ($P<0.01$) across all measured traits. Additionally, the interaction between genotype and treatment was also found to be highly significant ($P<0.01$), consistently influencing all traits at both growth stage (Table S2). Under N0 level, the rice genotypes showed a significant reduction in SPAD values, ranging from 22.8 to 42.25 with an average of 33.83. In contrast, under the N50 level, SPAD values ranged from 25.25 to 45.9, with an average of 37.36, while the highest values were recorded under N100, ranging from 29.95 to 49.55, with an average of 41.12. The same trend was also observed for NDVI, light-adapted PSII quantum yield and photosynthetic rate in the rice genotypes grown under different nitrogen regimes. Under N0 level, the NDVI values ranged from 0.15 to 0.615 with an average of 0.38 and for the N50 level, the values ranged from 0.215 to 0.67 with an average of 0.51, whereas in N100 the values were significantly higher ranging from 0.35 to 0.74 with an average of 0.61 (Table 2). The Fv'/Fm' and photosynthetic rates were drastically reduced under the N0 level compared to N50 and N100. Compared to N100, the Fv'/Fm' values were reduced by 7.46 to 16.4% in N50 and N0 respectively. The photosynthetic rate was decreased by 14.1 to 28.7% compared to N50 & N0 respectively.

Physiological traits at flowering stage

A significant reduction was observed in SPAD values, NDVI, light -adapted PSII quantum yield and photosynthetic rate in rice genotypes due to the reduced doses of nitrogen ie. N0 and N50 level of nitrogen compared to the N100 level at the flowering stage. The analysis of variance highlighted significant differences ($P<0.01$) among treatments and genotypes for all traits. Moreover, a highly significant Genotype × Treatment interaction ($P<0.01$) was observed across all traits at both growth stages (Table S2). The SPAD values ranged from 26.75 to 45.6, with an average of 37.09 under N0 level. However, the SPAD values were significantly higher in N50 over N0 level, which ranged from 34.8 to 48 and an average of 42.3, while N100 exhibited the highest range of 39.25 to 49.85 with an average of 46.08 (Table 2). The same trend was observed for NDVI, photosynthetic rate and Fv'/Fm' in rice genotypes under varying levels of nitrogen. Relative to N100, Fv'/Fm' values decreased by 6.6% under N50 and by 15% under N0. Similarly, the photosynthetic rate was reduced by 18.8% under N50 and by 32.8% under N0.

Effects of nitrogen dosage on yield traits of rice genotypes

The number of productive tillers, grains per panicle, hundred seed weight, spikelet fertility and grain yield exhibited significant variation in all the treatments and among the genotypes ($P<0.01$). Significant interactions ($P<0.01$) for the Genotypes *Treatment are also observed for the yield traits (Table S3).

***Productive Tillers* -** The number of productive tillers varies from 2 to 6.5 under N0, 2.5 to 11 under N50 and 5 to 14 under N100.The average number of productive tillers increases from 4.12 at N0 to 6.41 at N50 and 8.70 at N100. Nitrogen application boosts the number of productive tillers, with N100 producing the highest tiller count (Table3).

Table 2. Range and mean values of physiological traits for 160 genotypes under different nitrogen levels

		Active tillering stage		Flowering stage	
Traits	**Treatments**	**Range**	**Mean**	**Range**	**Mean**
SPAD	N0	22.8-42.25	33.83	26.75-45.6	37.09
	N50	25.25-45.9	37.36	34.8-48	42.3
	N100	29.95-49.55	41.1	39.25-49.85	46.08
NDVI	N0	0.155-0.615	0.38	0.215-0.675	0.47
	N50	0.215-0.67	0.51	0.36-0.76	0.60
	N100	0.35-0.74	0.61	0.545-0.8	0.69
Fv'/Fm'	N0	0.434-0.636	0.56	0.525-0.664	0.599
	N50	0.5-0.68	0.62	0.608-0.706	0.658
	N100	0.62-0.697	0.67	0.644-0.724	0.705
Pn (µmol. CO_2 m^{-2} sec^{-1})	N0	11.3 – 23.8	15.6	13.25-24.65	17.8
	N50	13.05 – 25.95	18.8	15.5-31.4	21.5
	N100	14.45 – 32.1	21.9	18.2-35.7	26.5

***Number of Grains per Panicle* -** The grains per panicle range from 29 to 139 in N0, 43 to 156.5 in N50 and 49 to 205 in N100.The average number of grains increases from 49.12 (N0) to 75.36 (N50) and further to 95.56 (N100). Higher nitrogen levels result in an increased grain count per panicle (Table3).

***Hundred seed Weight (g)* -** The weight of 100 seeds varies from 1.15-2.6 g (N0), 1.24-2.78 g (N50) and 1.34 -2.85 g (N100). The average weight increases from 1.70 g (N0) to 1.91 g (N50) and 2.08 g (N100). Nitrogen improves grain size or density, with seeds becoming heavier under higher nitrogen levels (Table3).

***Spikelet Fertility (%)* -** Spikelet fertility ranges from 38.9%-84.6% (N0), 52.35%-85.92% (N50) and 67% - 90.46% (N100). The average spikelet fertility rises from 64.43% (N0) to 73.9% (N50) and 81.3% (N100). Increased nitrogen enhances the ability of spikelets to develop into grains, contributing to higher yields (Table3).

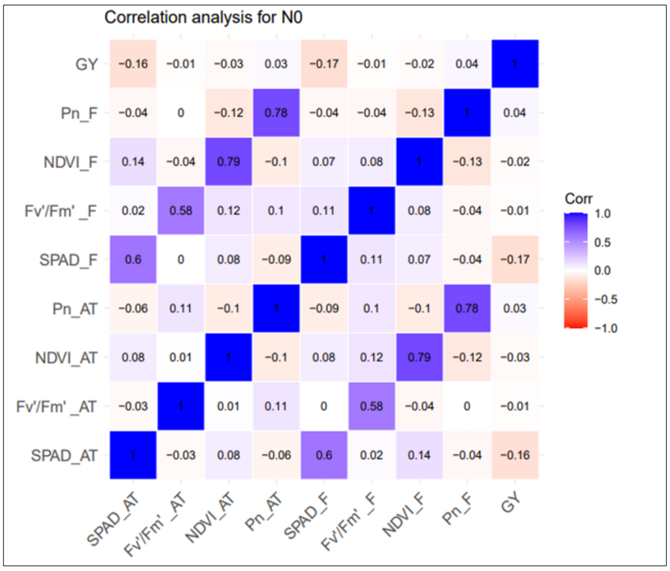
***Yield (g/plant)* -** The yield per plant varies from 1.09-6.94 g (N0), 2.67-15.05 g (N50) and 5.89-29.18 g (N100).The average yield increases from 2.19 g/plant (N0) to 6.85 g/plant (N50) and 14.15 g/plant (N100). Higher nitrogen levels significantly enhance plant yield, with N100 leading to the highest productivity (Table3).

Table 3. Range and mean values of yield traits for the 160 genotypes under different nitrogen levels

Yield traits			
Traits	**Treatments**	**Range**	**Mean**
Productive tillers	N0	2-6.5	4.12
	N50	2.5-11	6.41
	N100	5-14	8.70
No. of grains/panicle	N0	29-139	49.12
	N50	43-156.5	75.36
	N100	49-205	95.56
100 seed weight (g)	N0	1.15-2.6	1.70
	N50	1.24-2.78	1.91
	N100	1.34-2.85	2.08
Spikelet fertility (%)	N0	38.9 - 84.6	64.43
	N50	52.35-85.92	73.9
	N100	67-90.46	81.3
Yield (g/plant)	N0	1.09 - 6.94	2.19
	N50	2.67 - 15.05	6.85
	N100	5.89 - 29.18	14.15

Correlation analysis of physiological and yield traits

Correlation coefficient analysis was performed to identify the interrelation among the traits for N0 (Fig.2), N50 (Fig.3) & N100 (Fig.4) levels. The correlation analysis revealed that the intensity of association between physiological and yield traits increased when the quantity of nitrogen increases. At N50 & N100 level, the photosynthetic rate and light-adapted PSII quantum yield are positively associated with grain yield at both active tillering and flowering stage. The physiological traits such as SPAD, NDVI, Fv'/Fm' and Photosynthetic rate are positively correlated with grain yield at N100.



	SPAD_AT	Fv'/Fm' _AT	NDVI_AT	Pn_AT	SPAD_F	Fv'/Fm' _F	NDVI_F	Pn_F	GY
GY	−0.16	−0.01	−0.03	0.03	−0.17	−0.01	−0.02	0.04	1
Pn_F	−0.04	0	−0.12	0.78	−0.04	−0.04	−0.13	1	0.04
NDVI_F	0.14	−0.04	0.79	−0.1	0.07	0.08	1	−0.13	−0.02
Fv'/Fm' _F	0.02	0.58	0.12	0.1	0.11	1	0.08	−0.04	−0.01
SPAD_F	0.6	0	0.08	−0.09	1	0.11	0.07	−0.04	−0.17
Pn_AT	−0.06	0.11	−0.1	1	−0.09	0.1	−0.1	0.78	0.03
NDVI_AT	0.08	0.01	1	−0.1	0.08	0.12	0.79	−0.12	−0.03
Fv'/Fm' _AT	−0.03	1	0.01	0.11	0	0.58	−0.04	0	−0.01
SPAD_AT	1	−0.03	0.08	−0.06	0.6	0.02	0.14	−0.04	−0.16

Fig. 2. Correlation analysis of N0 for 160 genotypes. SPAD_AT - Chlorophyll index at active tillering stage; NDVI_AT - leaf greenness index at active tillering stage; Fv'/Fm'_AT - light-adapted PSII quantum yield at active tillering stage; Pn _AT-Photosynthetic rate at active tillering stage; SPAD_F- Chlorophyll index at flowering stage; NDVI_F - leaf greenness index at flowering stage; Fv'/Fm'_F - light-adapted PSII quantum yield at flowering stage; Pn_F - Photosynthetic rate at flowering stage; GY- grain yield.

Hierarchical Clustering of 160 genotypes under different nitrogen levels

The hierarchical clustering analysis of 160 genotypes categorized into three distinct groups based on physiological traits at the active tillering and flowering stages, along with grain yield, highlighting differences in nitrogen use efficiency.

Under N50 level (Fig 5), the rice genotypes were classified into 3 major clusters such as Cluster I - 59, Cluster II- 36, Cluster III- 65. Cluster I genotype exhibited moderate SPAD, NDVI and photosynthetic rates, maintaining relatively

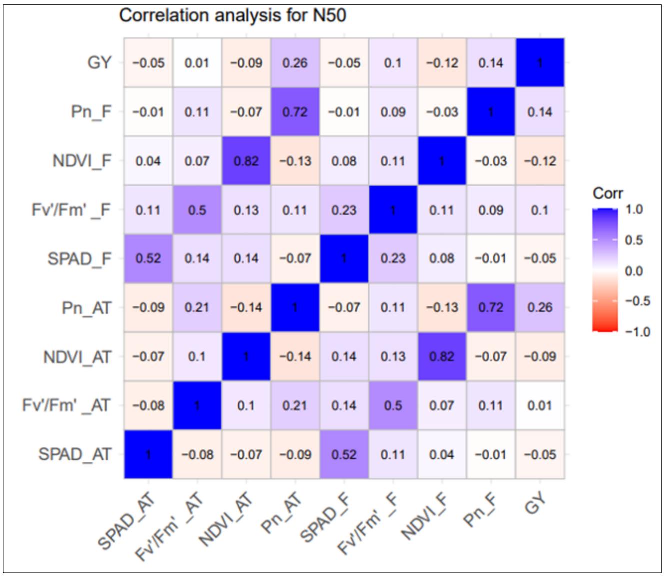


Fig. 3. Correlation analysis of N50 for 160 genotypes. SPAD_AT - Chlorophyll index at active tillering stage; NDVI_AT - leaf greenness index at active tillering stage; Fv'/Fm'_AT - light-adapted PSII quantum yield at active tillering stage; Pn _AT- Photosynthetic rate at active tillering stage; SPAD_F- Chlorophyll index at flowering stage; NDVI_F - leaf greenness index at flowering stage; Fv'/Fm'_F - light-adapted PSII quantum yield at flowering stage; Pn_F -Photosynthetic rate at flowering stage; GY- grain yield.

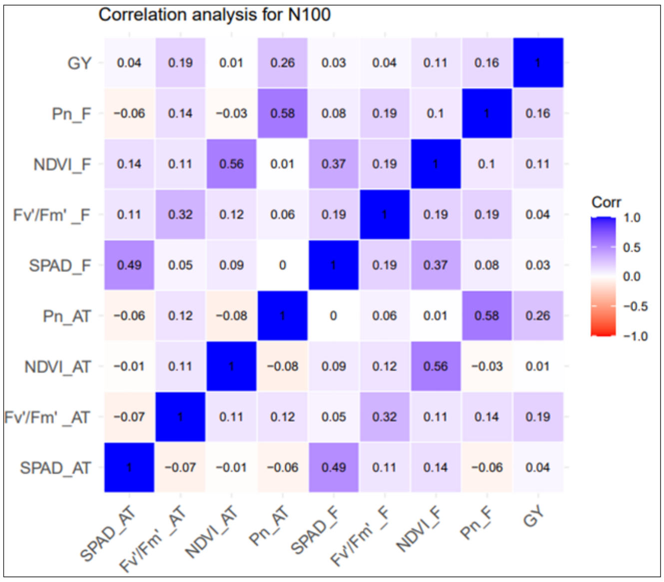


Fig. 4. Correlation analysis of N100 for 160 genotypes. SPAD_AT - Chlorophyll index at active tillering stage; NDVI_AT - leaf greenness index at active tillering stage; Fv'/Fm'_AT - light-adapted PSII quantum yield at active tillering stage; Pn _AT- Photosynthetic rate at active tillering stage; SPAD_F- Chlorophyll index at flowering stage; NDVI_F - leaf greenness index at flowering stage; Fv'/Fm'_F - light-adapted PSII quantum yield at flowering stage; Pn_F -Photosynthetic rate at flowering stage; GY- grain yield.

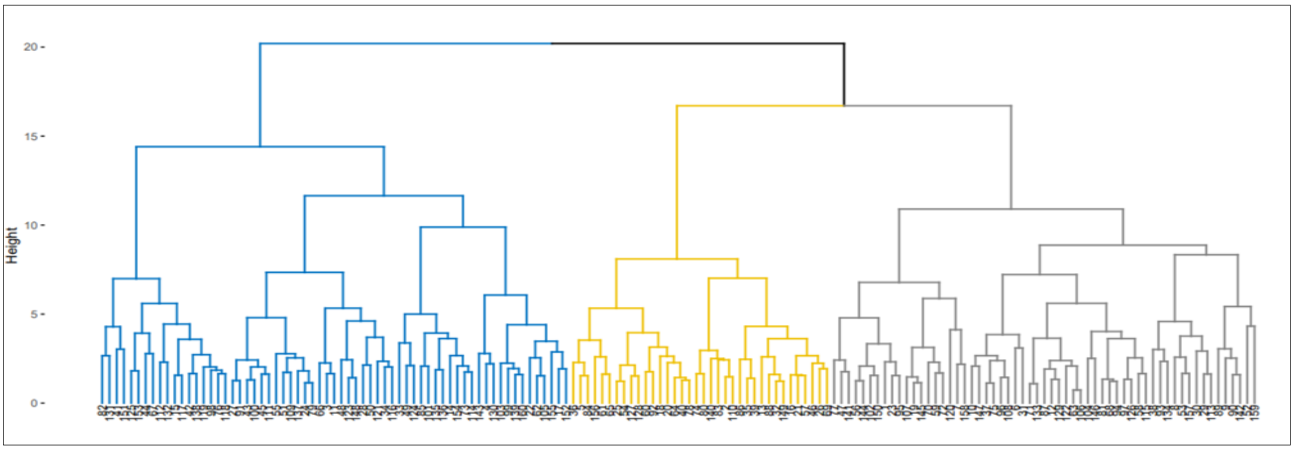


Fig. 5. Hierarchical clustering of 160 genotypes under N50.

stable physiological traits across growth stages. However, their grain yield (6.42 t/ha) remained intermediate, suggesting limitations in biomass partitioning. Cluster II genotypes had the highest SPAD, NDVI and Fv'/Fm' values, indicating superior nitrogen assimilation and chlorophyll retention. Despite their high photosynthetic rates, their grain yield (5.60 t/ha) was lower than Cluster III, suggesting that these genotypes prioritized vegetative growth over grain production. In contrast, Cluster III genotypes demonstrated the highest photosynthetic efficiency, achieving the highest grain yield (7.95 t/ha). Though their SPAD and NDVI values were moderate, their superior carbon fixation efficiency enabled better biomass conversion into yield.

Under N100 levels (Fig.6), the genotypes were classified into three major clusters such as Cluster I-65, Cluster II-65 and Cluster III-30. Cluster I genotype exhibited the highest SPAD values, indicating better nitrogen uptake, chlorophyll retention and moderate NDVI and photosynthetic rates. However, their grain yield (13.11 t/ha) was lower than Cluster II, suggesting limitations in biomass partitioning. Cluster II genotypes demonstrated the highest NDVI, Fv'/Fm' and photosynthetic rates, reflecting superior light interception and carbon assimilation efficiency. This translated into the highest grain yield (16.35 t/ha), indicating their superior nitrogen use efficiency and potential for high yield breeding programs. In contrast, Cluster III genotypes exhibited the lowest SPAD, NDVI and photosynthetic rates, suggesting weaker nitrogen assimilation and lower photosynthetic efficiency, resulting in the lowest grain yield (11.64 t/ha). The significant differences observed across clusters highlight the strong correlation between nitrogen uptake, photosynthetic efficiency and grain yield.

From the hierarchical clustering analysis, the genotypes such as IRG91, IRG140, IRG302, IRG374, IRG375 were selected which are higher yielders under N50level and the reduction in yield was significantly less over the recommended dose of nitrogen (N100). These genotypes have optimum physiological traits at the active tillering and flowering stage which are tabulated in Table 4. This indicates that these genotypes exhibit efficient nitrogen use, maintaining productivity even with reduced nitrogen input.

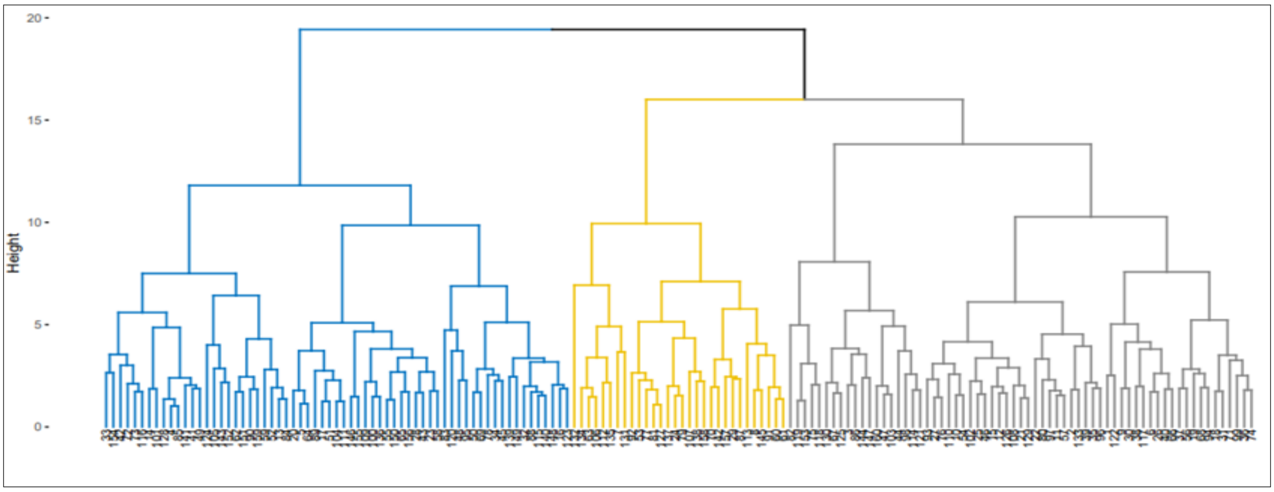


Fig. 6. Hierarchical clustering of 160 genotypes under N100.

Table 4. Physiological and yield values of selected five genotypes

S. No	Genotypes	Yield @ N50	SPAD_AT	NDVI_AT	Fv'/Fm'_AT	Pn_AT	SPAD_F	NDVI_F	Fv'/Fm'_F	Pn_F
1	IRG302	15.05	39.75	0.575	0.598	23.5	41.45	0.61	0.659	25.05
2	IRG374	12.76	37.15	0.425	0.623	25.95	39	0.58	0.630	26.2
3	IRG140	12.56	37.95	0.61	0.617	19.8	38.65	0.65	0.657	24.4
4	IRG375	12.22	37.9	0.435	0.672	18.25	43.3	0.59	0.684	21.75
5	IRG91	11.96	36.55	0.545	0.612	24.5	37.4	0.68	0.6575	31.4

Discussion

Nitrogen is one of the major nutrients that significantly limit the crop production when it is deficient. However, excessive use of nitrogenous fertilizers negatively impacts the ecosystem. Therefore, efficient resource utilization and sustainable crop production should be key focuses of current research. Apart from the conventional and agronomic management options, reducing excessive fertilizer usage requires a thorough understanding of the performance of genotypes under low nitrogen conditions, which could pave way for the development of genotypes with high nitrogen use efficiency (NUE) that would sustain the grain yield. While screening rice genotypes for high NUE, the underlying mechanism for high yield under low nitrogen levels must be identified and this would be a critical priority for improving NUE (27). This study aimed at identifying genetic variability under different nitrogen levels (N0, N50 and N100) and to identify high yielding genotypes among160 genotypes under low nitrogen levels. The physiological traits at active tillering and flowering stages and yield traits were recorded. The results indicated a significant reduction in the physiological and yield traits between the genotypes due to the absence or 50% reduction in the nitrogen compared to the recommended dose of nitrogen application. However, it was observed that in some of the genotypes, the quantum of reduction in the values of physiological traits and yield was significantly less between 100% and 50% nitrogen levels.

Nitrogen is essential in regulating various physiological and biochemical processes in plants. In our study, we observed an increase in physiological traits such as SPAD, NDVI, Fv'/Fm' and photosynthetic rate with higher nitrogen levels (0N, 50N, 100N) during both the active tillering and flowering stages. These findings align with those of Liang et al. (28), who reported that rice leaf SPAD values, stem nitrate concentrations, soluble proteins, photosynthetic rates, stomatal conductance and total nitrogen concentrations all increased with rising nitrogen levels.Yang et al. (29) demonstrated that adequate nitrogen significantly enhances nitrate reductase activity, protein content and chlorophyll content, which are closely associated with SPAD values across different nitrogen levels.

SPAD value increased with higher nitrogen levels at both tillering and flowering stages, with the highest chlorophyll content observed under 100% RDN and the lowest under 0% RDN (T1). The mean total chlorophyll content increased from 36.93 (N0) to 41.00 (N100) during tillering and from 41.09 (N0) to 41.85 (N100) during flowering (30). Consistent with these findings, our study observed an increasing trend in SPAD values at both the active tillering and flowering stages with the application of nitrogen, ranging from N0 to N100, which might be due to the increased chlorophyll content. Swain and Sandeep (31), Rajesh et al. (32) and Devika et al. (33) similarly highlighted that increased nitrogen application improves SPAD readings, reflecting higher chlorophyll content. These results underscore the importance of nitrogen in enhancing chlorophyll synthesis and photosynthetic efficiency, contributing to improved plant growth and productivity.

Nitrogen plays a crucial role in the allocation of the photosynthetic apparatus, influencing both photosynthesis (Pn) and the quantum yield (Fv/Fm) in plants. In conditions of low nitrogen, quantum yield becomes a key limiting factor for photosynthesis (34). The optimal nitrogen application rates can enhance PSII activity, photochemical efficiency and the proportion of open PSII reaction centers in rice leaves (35-37). Srikanth et al. (16) reported that the maximum quantum yield of PSII increased with increasing nitrogen levels, reaching 0.792 at N0, 0.802 at N50, 0.815 at N100 and 0.82 at N150 in rice genotypes. In our study, the light-adapted quantum yield of PS II (Fv'/Fm') increased for an increase in nitrogen level at both the active tillering and flowering stages which enabled plants to utilize captured light energy more effectively during photosynthesis, thereby increasing the quantum efficiency and photosynthetic rate of PSII.

Photosynthesis forms the basis of dry matter production in plants. In rice, around 90% of the grain yield is derived from the photosynthetic activity of leaves after flowering, particularly from the flag leaf (38). Optimal nitrogen application has been demonstrated to enhance enzyme activity and chlorophyll content in plant leaves, thereby improving the plant's photosynthetic performance (39). Devika et al. (33) reported significantly higher mean photosynthetic rates with increased nitrogen application, which supports the strong role of nitrogen in improving photosynthetic efficiency. Srikanth et al. (16) reported that the photosynthetic rate increased with increasing nitrogen levels, reaching 15.7 at N0, 19.3 at N50, 23.7 at N100 and 25.4 at N150 in rice genotypes. Consistent with these findings, our study observed an increase in photosynthetic rates with higher nitrogen levels, due to increased chlorophyll content and quantum yield of PSII contributing to enhanced grain yield. Additionally, the increase in NDVI values at higher nitrogen levels in our study, particularly at the N100 level, reflects a significant increase in canopy greenness.

Nitrogen application plays a critical role in enhancing yield-related traits in rice. Artacho et al. (40) demonstrated a linear relationship between nitrogen fertilization and an increase in the number of panicles per square meter. Yoshinaga et al. (41) reported a significant increase in spikelets per panicle with higher nitrogen application. Chaturvedi (42) reported that nitrogen application led to an increased protein content, which subsequently enhanced grain weight. Additionally, nitrogen application boosts spikelet fertility by improving the plant's vigor and reproductive development, resulting in a higher proportion of fertile spikelets and ultimately increasing grain yield (43). Previous studies have reported that increased nitrogen application enhances grain yield, primarily due to improved tillering, a greater number of panicles and grains (33,44) and increased total dry matter accumulation (44).

Rai (30) recorded the highest grain yield at 100% recommended dose of nitrogen followed by 50% RDN and 0 RDN, which is similar with our findings. In our study, yield traits such as the number of productive tillers, grains per panicle, hundred seed weight, spikelet fertility and single plant yield were positively influenced by higher nitrogen levels. The improved yield under higher nitrogen application may be attributed to more efficient nitrogen and nutrient absorption, which enhances dry matter production and its translocation from source to sink (33).

The correlation analysis showed that the maximum quantum yield of PS II and photosynthetic rate are positively associated with grain yield under N50 and N100.Srikanth et al (16) reported that traits such as the maximum quantum efficiency of PSII (Fv/Fm) have been positively associated with grain yield, especially under reduced N application.

The genetic behavior of rice genotypes under different nitrogen levels, as assessed by Metwally et al. (45) Hukum and Amit (46) and Rajesh et al. (32) highlights the critical role of morpho-physiological and yield traits, including photosynthetic rate, nitrate reductase activity, SPAD values, panicle production and grain yield. Kim and Kim (47) identified specific genetic resources with superior nitrogen use efficiency (NUE), characterized by a higher number of panicles per plant and spikelets per panicle under both normal and low nitrogen conditions. These findings emphasize the importance of genetic resources in improving adaptation to nitrogen-deficient environments and underscores the interconnected nature of morpho-physiological and yield traits in optimizing rice productivity under low-nitrogen conditions.

In the present study, a population of 160 genotypes was selected to study the genotypic variability under different nitrogen levels. The physiological traits at the active tillering and flowering stage and the yield traits were recorded. From the hierarchical clustering, the genotypes such as IRG91, IRG140, IRG302, IRG374, IRG375, which are higher yielders under both N50 & N100, having optimum physiological traits at both the stages were identified. The selected 5 genotypes serve as valuable genetic resources for breeding rice varieties with tolerance to low nitrogen levels. Further physiological and molecular characterization under low nitrogen levels is essential to unravel the molecular mechanisms behind the high nitrogen use efficiency and yield under low nitrogen levels.

Identifying genetic variability in genotypes under different nitrogen levels offers significant advantages, such as improving crop breeding for higher nitrogen use efficiency (48), enhancing sustainability by reducing fertilizer dependence and increasing crop performance under varying environmental conditions (49). This knowledge helps develop resilient crops that optimize nitrogen use, ultimately contributing to more sustainable agricultural practices. However, the process is resource-intensive, requiring large-scale field trials, genetic screenings and lab analyses, which can be costly and time-consuming (50). Additionally, the complexity of genetic interactions and environmental variability complicates the identification of genetic mechanisms and trade-offs between nitrogen efficiency and other traits may arise. These challenges, combined with limited understanding of the underlying genetic pathways, make the task of generalizing findings across all crops or conditions difficult (49).

Conclusion

The exploration of genetic variation in rice under different nitrogen levels underscores the importance of identifying and utilizing genotypes having yield stability under reduced nitrogen application. Physiological traits such as SPAD, NDVI, Fv'/Fm' and photosynthetic rate can be used as indicators in breeding programs, helping to select genotypes that perform well under reduced nitrogen levels. The five genotypes identified under reduced nitrogen application provide a solid foundation for breeding programs aimed at developing rice varieties that maintain high productivity while minimizing nitrogen fertilizer dependence. The available genetic stocks offer valuable resources for dissecting genes and pathways associated with NUE. Future research on the selected five genotypes can focus on identifying key genes and pathways for nitrogen use efficiency through genomic, transcriptomic and metabolomic analyses. These genotypes can be utilized to develop resilient hybrids and optimize agronomic practices for sustainable productivity. Long-term field trials will further validate their adaptability and performance across diverse environments. High-throughput phenotyping technologies like remote sensing could accelerate genotype selection based on nitrogen-related traits. Additionally, studying genotype-soil-environment interactions could optimize nitrogen management for specific regions. Lastly, the impact of climate change on nitrogen availability and rice productivity should be considered, as rising temperatures, droughts and floods may further challenge nitrogen use. Exploring the resilience of these genotypes under such conditions would be critical for long-term agricultural sustainability. Integrating these research areas could promote sustainable and efficient rice cultivation practices, strengthening food security and minimizing the environmental footprint of rice farming.

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Authors' contributions

SD collected the articles and wrote the first draft. SA, AK and DM edited the manuscript. RV, RM, PR and MS assisted in correcting the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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