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RESEARCH ARTICLE

Screening rice genotypes for nitrogen efficiency under graded nitrogen application

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Abstract

The global application of nitrogen (N) fertilizers continues to rise in efforts to bolster agricultural productivity. However, this surge in usage has led to significant N losses, resulting in low nitrogen use efficiency (NUE) among genotypes and consequent water and air pollution. Although many studies advocate reducing N fertilizer usage, research on screening rice genotypes under graded N application is limited. This study aimed to screen rice genotypes to identify N use efficient cultivars under varying nitrogen levels: N0, N50, N100 and N150. The study also sought to identify key physiological traits linked to grain yield under reduced N conditions. Grain yield decreased by 45.2 % at N0 and 21.4 % at N50 while increasing by 22.3 % at N150 compared to N100. Under reduced N application (N0 and N50), MTU-1010, Vasumati, DRR Dhan-58, Varadhan, Brown Gora SB 92, Tulasi, BV-1692 and DRRH2 exhibited least reduction in grain yield, over N100. Notably, parameters such as ΦPSII (actual quantum yield of PSII), ETR (electron transport rate) and qP (coefficient of photochemical quenching) displayed a robust positive association with grain yield under reduced N application compared to the recommended (N100) and high (N150) N application. This underscores the significance of PSII photochemistry in enhancing grain production under limited N. Consequently, leaf chlorophyll fluorescence traits emerge as promising indicators for screening rice genotypes with enhanced NUE under limited N scenarios. In summary, the study conclusively identifies Varadhan as a genotype demonstrating high efficiency in nitrogen utilization, both in terms of grain yield and GYEI, particularly under reduced N regimes.

Keywords

chlorophyll fluorescence; grain yield efficiency index; grain yield; nitrogen use efficiency; nitrogen; rice

Introduction

Rice is a staple food crop and global rice production must be increased by 2–3 % annually to meet the growing demand while maintaining self-sufficiency with limited resources (1). Nutrient management in agriculture, particularly nitrogen (N) and phosphorus (P), greatly contributes to the maximum of the sustainable development goals (SDGs) (2). N is an essential macronutrient for rice growth and critically influences its production (3). 80 % of anthropogenic N is emitted into the environment (4), damaging the environment and human well-being. Nitrogen directly influences photosynthesis, biomass accumulation, tiller and spikelets formation, grain filling and quality (5). Improving the utilization efficiency of N is essential to achieve sustainable agriculture (6). As N in Indian soils is low, yield is reduced by greater than 50 % under N-deficient conditions (7). External N fertilizer application is necessary to attain higher yields from the modern rice varieties (8). It is evident from the reported studies that higher N application in rice could enhance the total chlorophyll content of leaves, thereby promoting photosynthetic rate and traits associated with improved grain yield. However, the optimum N application rate varied with soil fertility and genotype (9,10). Excessive N application in crop intensification reduces the N recovery rate (<50 %) and leads to significant environmental issues (11). It was assessed that around 60 % of applied N is in excess for rice as it uptakes only 30-50 % of N applied and the rest of N is released into the environment (12). Among cereal crops, rice and wheat have the least nitrogen use efficiency (NUE), < 30 –40 % (12–13). Improving crop NUE can lead to higher yields with limited N application while minimizing environmental impact (14). Excess N is supplied to compensate for the low uptake efficiency and to attain increased grain production (15). Improved N management results in greater absorption and improved harvest index of N (NHI). Meanwhile, optimum N supply increases N uptake during grain filling, thereby increasing grain N content through remobilization (16).

SPAD Chlorophyll Meter Readings (SCMR) can measure the *in-situ* leaf N content based on light transmittance (17). A significant correlation between SCMR values, leaf N content and grain yield was earlier reported (18), demonstrating the possibility of using SCMR values as markers to determine optimum N requirement. Chlorophyll fluorescence traits offer valuable insights into photosynthetic changes, as they are closely linked to several reaction processes in photosynthesis (19). F_v/F_m in rice leaves enhanced with increasing N application and proper N treatment could increase F_v/F_m in rice leaves (20). Our earlier studies found that flag leaf chlorophyll fluorescence traits, i.e., F_v/F_m, ΦPSII, ETR and qP, can be deployed as physiological markers for screening of N use efficient rice cultivars under limited N conditions (21). The aim was to characterize variations in flag leaf traits, chlorophyll fluorescence, biomass accumulation and grain yield under different nitrogen treatments. The better-performing lines under reduced N application were identified. They can be used as donors in breeding programmes for NUE and will be further explored for NUE components and their surrogate traits.

Materials and Methods

In this study, 95 rice genotypes were evaluated at four graded levels of N fertilizer application viz., N0 (control-0 kg N/ha), N50 (50 % of recommended N-50 kg N/ha), N100 (recommended N-100 kg N/ha) and N150 (50 % above recommended N-150 kg N/ha) at ICAR- IIRR farm during Kharif-2022 (Table 1). Flag leaf traits, SCMR values and leaf chlorophyll fluorescence traits were measured during the 50 % flowering stage. Plant height and tiller number per plant were recorded at physiological maturity. Sowing took place in June, followed by transplanting in July. N

Table 1. List of genotypes included in the study

S.No.	Genotype name	Description
1	Aditya	Variety
2	Ajaya	Variety
3	Akshaydhan	Variety
4	Anjali	Variety
5	ARC 14855	LR_IRGC 41790-1
6	Aus Kushi	LR_IRGC 66688-1
7	Aus12	LR_IRGC 28875-1
8	Aus175	LR_IRGC 29007-1
9	Aus309	LR_IRGC 29097-1
10	Aus328	LR_IRGC 29115-1
11	Bawoi	LR
12	Bowalia 2	LR_IRGC 27538-1
13	Brown Gora SB 92	LR_IRGC 44855-1
14	BV-1704	BL (BPT5204/Varadhan-1704)
15	BV-24	BL (IET31103)
16	BV-31	BL (BPT5204/Varadhan-31)
17	BV-1690	BL (BPT5204/Varadhan-1690)
18	BV-1692	BL (BPT5204/Varadhan-1692)
19	INGR22067	BL (BPT5204/Varadhan-1705)
20	Dhanarasi	Variety
21	DRR Dhan 38	Variety
22	DRR Dhan 39	Variety
23	DRR Dhan 41	Variety
24	DRR Dhan 42	Variety
25	DRR Dhan 43	Variety
26	DRR Dhan 44	Variety
27	DRR Dhan 45	Variety
28	DRR Dhan 46	Variety
29	DRR Dhan 47	Variety
30	DRR Dhan 48	Variety
31	DRR Dhan 49	Variety
32	DRR Dhan 50	Variety
33	DRR Dhan 51	Variety
34	DRR Dhan 52	Variety
35	DRR Dhan 53	Variety
36	DRR Dhan 54	Variety
37	DRR Dhan 55	Variety
38	DRR Dhan 56	Variety
39	DRR Dhan 57	Variety
40	DRR Dhan 58	Variety
41	DRR Dhan 59	Variety
42	DRR Dhan 60	Variety
43	DRR Dhan 62	Variety
44	DRR Dhan 64	Variety
45	DRR Dhan 65	Variety
46	DRR Dhan 66	Variety
47	DRRH2	Hybrid
48	DRRH3	Hybrid
49	DRRH4	Hybrid
50	DV 123	LR
51	Gontrabidhan	Variety
52	GQ25	INGR20001
53	IC463254	LR
54	Idsa 44	Variety
55	IR64	Variety
56	Improved Samba Mahsuri	Variety
57	Jarava	Variety
58	Jati Aus	LR_IRGC 34940-1
59	Jaya	Variety
60	Kali Aus	LR_IRGC 45992-1

61	Kasalath	LR
62	Kasturi	Variety
63	KMR-3R	Variety
64	Kolajoha3	LR
65	Krishnahamsa	Variety
66	Mandya Vijaya	Variety
67	Mima	LR
68	MTU 1010	Variety
69	Nagarjuna	Variety
70	Nidhi	Variety
71	Panvel	Variety
72	Phalguna	Variety
73	Phul Pattas 72	LR_IRGC 6263-1
74	Pokkali	LR
75	Pooja	Variety
76	Prasanna	Variety
77	PTB1	Variety
78	Rasi	Variety
79	Ratnasundari	Variety
80	RHZ-IR-135	BL
81	RNR15048	Variety
82	RV 38-1	BL (Rasi/Vibhava-38-1)
83	RV-14	BL (Rasi/Vibhava-14)
84	Salivahana	Variety
85	Sampada	Variety
86	SJ3	BL (Sampada/Jaya-3-INGR23076)
87	Suraksha	Variety
88	Swarna	Variety
89	Swarnadhan	Variety
90	Triguna	Variety
91	Tulasi	Variety
92	Varadhan	Variety
93	Vasumati	Variety
94	Vikas	Variety
95	Vikramarya	Variety

LR-land race; BL-breeding line

was applied equally in three splits, such as urea at the basal, maximum vegetative and panicle initiation stages. Standard agronomic practices were followed throughout the crop growth period.

Meteorological data

The climate of the experimental site is tropical, wet and dry and its' relatively warm year-round. During the crop growth period, the average duration of bright sunshine was 4.8 hours/day and the average relative humidity was 93.4 %. The mean maximum and minimum temperatures were 30.2°C and 20.6°C and the total rainfall received was 981.1 mm. Maximum temperature was recorded on 6th June (40.3°C) and minimum temperature was recorded on 30th November (10.5 °C). The highest rainfall was received on 23rd July (84.8 mm), with 57 rainy days (Fig. 1).

Experimental site and design

The experimental site had clay soil, which was non-saline (EC 0.73 dS/m), slightly alkaline (pH 8.21) and contained medium organic carbon levels (0.51 %). Available N was low (201 kg/ha) with high phosphorus (95 kg/ha) and high potassium (644 kg/ha) available in soil. The experiment followed a factorial randomized block design with four nitrogen levels and three replications.

Morpho-physiological traits and grain yield

The duration taken for half of the plants to flower was recorded as days to 50 % flowering (DFF). Flag leaf traits, specific leaf weight (SLW) and specific leaf area (SLA) were measured at 50 % flowering in five random leaves per genotype (22). Flag leaf length was noted from bottom to leaf tip and width was pointed out at the widest part of the leaf with a ruler. Flag leaf area was derived per the standard method as given in Equation 1 (23).

Flag leaf area = Length of leaf × Width of leaf × 0.75

(Eqn. 1)

Flag leaf dry weight was measured with an electronic balance after drying to constant weight in the oven for three days at 80 °C. SLA was determined by dividing the leaf area by the dry weight of the leaf (24) and SLW was derived by dividing the dry weight of the leaf by the leaf area (24-25). At physiological maturity, one square meter plots were harvested, threshed and dried to a moisture content of 14 % to determine grain yield and express it in t/ha (22). The sum of the dry weights of all plant components was expressed as total dry matter in t/ha (26).

Chlorophyll fluorescence traits

Chlorophyll fluorescence traits were recorded for flag leaf at 50 % flowering stage with Photosynthesis Yield Analyzer (MINI PAM-II) using WinControl-3 (Heinz Walz GmbH, Germany) software. Flag leaves were initially adapted in the dark for half an hour. After that, the subsequent fluorescence traits were determined: actual quantum yield of PSII (ΦPSII), maximum quantum yield of PSII (Fv/Fm), electron transport rate (ETR), coefficient of photochemical quenching (qP) and coefficient of non-photochemical quenching (qN) (27).

Grain yield efficiency index (GYEI)

The grain yield efficiency index (GYEI) is a valuable metric for evaluating nitrogen-efficient genotypes under field screening, as given in Equation 2 (28).

$$\text{GYEI} = \frac{\text{Grain yield of genotype at low N input} \times \text{grain yield of genotype at high N input}}{\text{Mean grain yield of all genotypes at low N input} \times \text{mean grain yield of all genotypes at high N input}} \quad \text{(Eqn. 2)}$$

Results

ANOVA revealed that all 17 morpho-physiological traits and grain yield exhibited significant variation with N application and among the genotypes (Table 2). Interaction between N levels and genotypes was also substantial for the measured traits. Range and cumulative mean values of morpho-physiological traits and grain yield under graded N levels are given in Table 3.

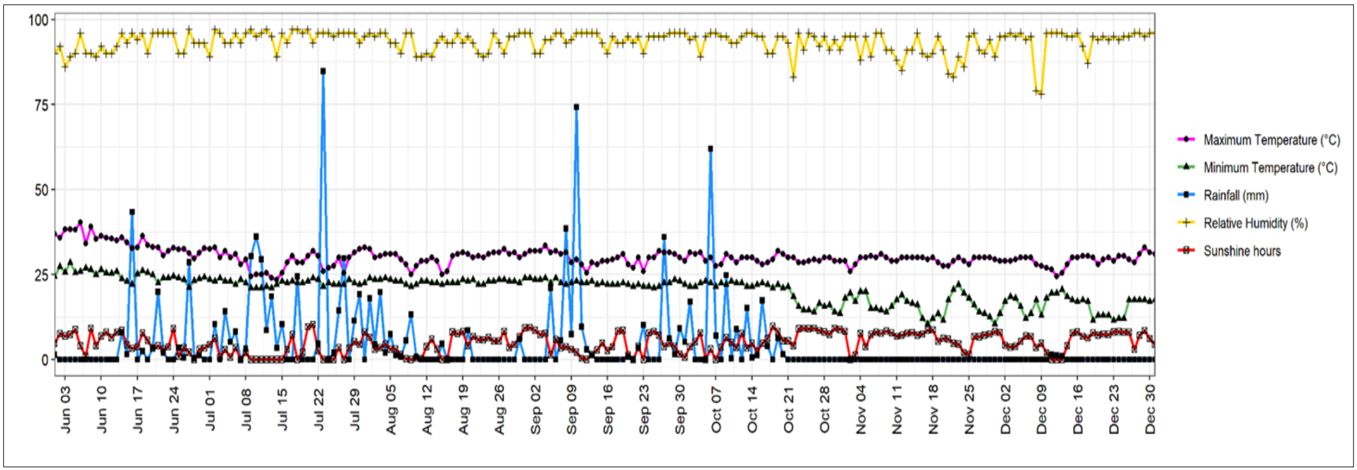

Fig. 1. Important weather parameters recorded during crop growing period at ICAR-IIRR, Hyderabad.

Table 2. ANOVA for morpho-physiological parameters and grain yield

		Days to 50 % flowering				SPAD chlorophyll meter reading				Flag leaf length			
Effect	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Treatment (T)	3	6335	2111.68	751.60	<0.0001	2864	954.55	310.41	<0.0001	8591	2863.59	319.89	<0.0001
Genotype (G)	94	4917	52.31	18.62	<0.0001	6921	73.63	23.94	<0.0001	22970	244.36	27.30	<0.0001
T × G	282	2186	7.75	2.76	<0.0001	4105	14.56	4.73	<0.0001	11639	41.27	4.61	<0.0001
Residual	758	2130	2.81			2331	3.08			6785	8.95		
Total	1139	15616	13.71			16555	14.53			50650	44.47		
CV (%)		1.49				4.39				8.19			
		Flag leaf width				**Flag leaf area**				**Flag leaf dry weight**			
Effect	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Treatment (T)	3	7.32	2.440	182.33	<0.0001	33420	11140	418.60	<0.0001	0.65	0.2162	662.09	<0.0001
Genotype (G)	94	24.16	0.257	19.21	<0.0001	60372	642	24.13	<0.0001	1.67	0.0178	54.42	<0.0001
T × G	282	8.76	0.031	2.32	<0.0001	26519	94	3.53	<0.0001	0.91	0.0032	9.83	<0.0001
Residual	758	10.14	0.013			20172	27			0.25	0.0003		
Total	1139	50.56	0.044			142340	125			3.49	0.0031		
CV (%)		7.27				11.75				8.03			
		Specific leaf area				**Specific leaf weight**				**Maximum quantum yield of PSII**			
Effect	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Treatment (T)	3	14851	4950	8.44	<0.0001	11.7	3.90	8.60	<0.0001	0.184	0.06141	1136.08	<0.0001
Genotype (G)	94	336892	3584	6.11	<0.0001	244.2	2.60	5.74	<0.0001	0.069	0.00073	13.49	<0.0001
T × G	282	779961	2766	4.72	<0.0001	543.6	1.93	4.26	<0.0001	0.029	0.00010	1.91	<0.0001
Residual	758	444458	586			343.2	0.45			0.041	0.00005		
Total	1139	1581554	1389			1149.5	1.01			0.326	0.00029		
CV (%)		12.21				12.88				0.91			
		Actual quantum yield of PSII				**Electron transport rate**				**Coefficient of photochemical quenching**			
Effect	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Treatment (T)	3	0.78	0.2597	228.06	<0.0001	3508	1169.45	203.93	<0.0001	1.31	0.4381	138.45	<0.0001
Genotype (G)	94	0.42	0.0044	3.91	<0.0001	2146	22.83	3.98	<0.0001	1.30	0.0138	4.36	<0.0001
T × G	282	0.99	0.0035	3.08	<0.0001	4907	17.40	3.03	<0.0001	2.73	0.0097	3.06	<0.0001
Residual	758	0.86	0.0011			4347	5.73			2.40	0.0032		
Total	1139	3.24	0.0028			15867	13.93			8.14	0.0071		
CV (%)		9.26				9.23				10.28			
		Coefficient of non-photochemical quenching				**Plant height**				**Tiller number/plant**			
Effect	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Treatment (T)	3	1.12	0.3726	271.22	<0.0001	32376	10792	228.44	<0.0001	1037	345.64	489.79	<0.0001
Genotype (G)	94	0.83	0.0088	6.44	<0.0001	236002	2511	53.15	<0.0001	1116	11.87	16.82	<0.0001
T × G	282	1.27	0.0045	3.29	<0.0001	42246	150	3.17	<0.0001	1656	5.87	8.32	<0.0001
Residual	758	1.04	0.0014			35809	47			535	0.71		
Total	1139	4.26	0.0037			349739	307			4478	3.93		
CV (%)		10.47				5.99				11.12			
		Grain yield				**Total dry matter**				**Harvest index**			
Effect	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Treatment (T)	3	1293	431.15	2324.89	<0.0001	5426	1808.67	2495.73	<0.0001	9488	3162.6	190.49	<0.0001
Genotype (G)	94	549	5.84	31.47	<0.0001	1473	15.67	21.62	<0.0001	38465	409.2	24.65	<0.0001
T × G	282	223	0.79	4.26	<0.0001	1128	4.00	5.52	<0.0001	17501	62.1	3.74	<0.0001
Residual	758	141	0.19			549	0.72			12584	16.6		
Total	1139	2256	1.98			9024	7.92			78103	68.6		
CV (%)		11.38				8.69				10.67			

Table 3. Range and cumulative mean values of morpho-physiological traits and grain yield under graded N levels.

	N0			N50			N100			N150		
Traits	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**	**Mean**	**Minimum**	**Maximum**	**Mean**
PH (cm)	78.2	156.3	107.0	81.7	172.3	113.0	90.3	175.5	117.9	93.0	181.5	121.1
TN	3.0	9.7	6.2	3.3	11.0	7.2	3.7	14.0	7.9	4.7	15.3	8.8
DFF	100	114	109	109	119	112	111	121	114	112	124	115
FLL (cm)	24.4	47.3	32.5	22.7	56.2	36.0	23.0	60.8	37.5	25.4	65.7	40.1
FLW (cm)	0.90	1.90	1.48	1.13	2.00	1.57	1.20	2.13	1.62	1.10	2.05	1.70
FLA (cm^2)	17.2	64.7	36.2	23.0	64.6	42.5	24.0	77.8	45.7	22.0	85.0	51.2
FLDW (g)	0.084	0.267	0.192	0.119	0.335	0.217	0.126	0.393	0.235	0.131	0.408	0.256
SLA (cm^2/g)	128.6	380.9	193.2	98.0	268.1	198.7	143.8	286.2	197.4	140.5	311.4	203.3
SLW (mg/cm^2)	2.82	8.63	5.39	3.79	10.20	5.19	3.52	7.02	5.20	3.32	7.31	5.11
SCMR	29.3	43.9	37.8	27.9	46.9	39.3	30.3	47.9	40.7	35.5	49.7	42.1
Fv/Fm	0.740	0.811	0.789	0.780	0.823	0.804	0.795	0.830	0.814	0.802	0.837	0.823
φPSII	0.222	0.435	0.327	0.253	0.448	0.355	0.310	0.448	0.379	0.331	0.459	0.397
ETR	15.8	30.6	23.3	18.2	31.9	25.4	22.4	32.0	27.3	23.1	32.3	27.8
qP	0.305	0.644	0.497	0.384	0.698	0.536	0.430	0.686	0.568	0.472	0.702	0.586
qN	0.272	0.580	0.401	0.259	0.474	0.362	0.217	0.443	0.335	0.208	0.449	0.318
TDM (t/ha)	3.73	11.53	6.89	6.11	12.24	8.84	7.75	14.79	10.69	9.36	18.32	12.77
GY (t/ha)	0.67	3.88	2.33	1.59	4.88	3.34	1.94	5.92	4.26	2.97	7.68	5.20
HI (%)	15.0	54.4	33.5	19.9	53.6	38.0	17.0	55.3	40.1	23.5	57.9	41.0

PH-plant height; TN-tiller number per plant; DFF-days to 50 % flowering; FLL-flag leaf length; FLW-flag leaf width; FLA-flag leaf area; FLDW-flag leaf dry weight; SCMR-SPAD chlorophyll meter reading; Fv/Fm-maximum quantum yield of PSII; φPSII-actual quantum yield of PSII; ETR-electron transport rate; qP-coefficient of photochemical quenching; qN-coefficient of non-photochemical quenching; TDM-total dry matter; GY-grain yield; HI-harvest index.

Increased N fertilizer application noted significant enhancement in grain yield and total dry matter. Mean grain yield enhanced from 2.33 t/ha at N0 to 3.34 t/ha at N50, 4.26 t/ha at N100 and 5.20 t/ha at N150. Mean total dry matter increased from 6.89 t/ha at N0 to 8.84 t/ha at N50, 10.69 t/ha at N100 and 12.77 t/ha at N150. Compared with N100, mean grain yield and total dry matter reduced by 45.2 % and 35.6 % with N0, 21.4 % and 17.3 % with N50, while increasing by 22.3 % and 19.5 % with N150. Compared to N100, MTU-1010 showed the least reduction in grain yield at N50 (10.0 %), followed by Vasumati (10.5 %), DRR Dhan 58 (10.6 %), Varadhan (10.7 %), Phalguna (10.8 %), DRR Dhan-56 (10.95 %), Brown Gora SB 92 (11.03 %) and RV 38-1 (11.26 %). While maximum reduction was noticed in RNR15048 (60.4 %) followed by DRR Dhan-38 (54.0 %), DRR Dhan-66 (45.1 %), DRR Dhan-49 (44.8 %), DRR Dhan-51 (42.1 %) and DRR Dhan-39 (37.9 %) (Fig. 2). In comparison with grain yield at N0 over N100, Brown Gora SB 92 (23.0 %) exhibited minimum reduction followed by Tulasi (23.4 %), MTU 1010 (23.4 %), Vasumati (23.8 %), DRR Dhan 52 (24.2 %), DRR Dhan 41 (24.5 %), BV-1692 (24.7 %) and Varadhan (25.4 %). In contrast, maximum reduction was observed in DRR Dhan 38 (87.3 %), followed by DRR Dhan 54 (82.7 %), AUS 12 (79.5 %), DRR Dhan 57 (78.3 %), DRR Dhan 48 (76.5 %), INGR22067 (76.1 %), RNR 15048 (75.8 %) and DRR Dhan 45 (73.0 %) (Fig. 3). Total dry matter reduced by 35.55 % with N0 and 17.31 % with N50 while enhanced by 19.46 % with N150 over N100. Compared with total dry matter at N100, Brown Gora SB92 (1.5 %) at N50 and DRRH3 (8.5 %) at N0 exhibited the least reduction. While DRR Dhan 66 (43.4 %) at N50 and DRR Dhan 38 (67.4 %) at N0 exhibited the highest reduction.

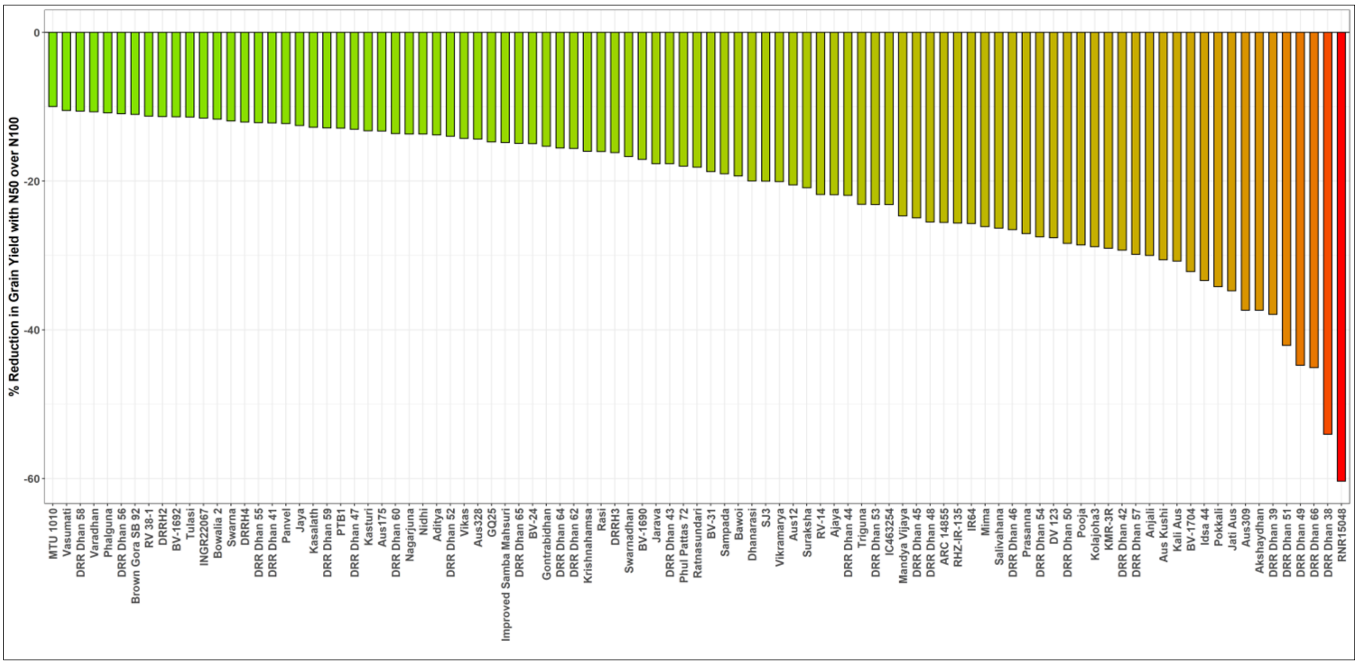

Fig. 2. Percent reduction in grain yield of genotypes with N50 over N100.

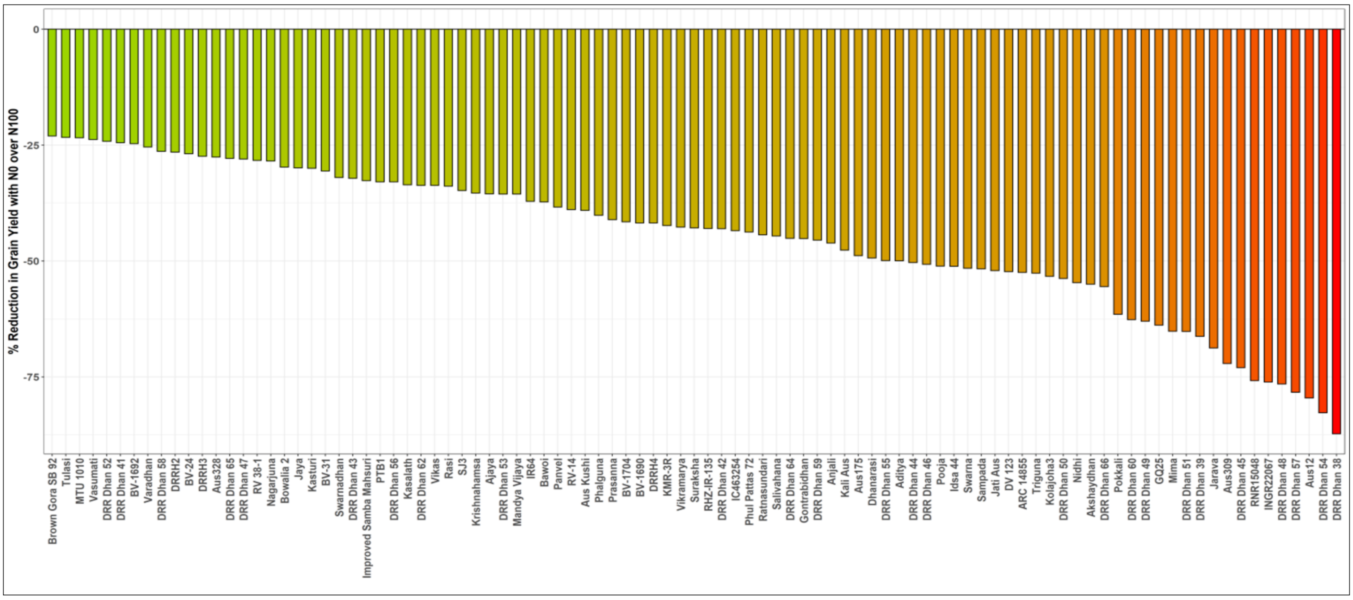

Fig. 3. Percent reduction in grain yield of genotypes with N0 over N100.

The mean harvest index enhanced significantly from 33.5 % to 41.0 % with an increase in N fertilizer application from N0 to N150. Compared with N100, the mean harvest index declined by 16.3 % with N0 and 5.1 % with N50, while it increased by 2.5 % with N150. Among the genotypes, the maximum harvest index was noted by Kali Aus at N100 (55.3 %) and DRR Dhan-56 (53.6 %) at N50, whereas Ratnasundari (17.0 %) at N100 and Jati Aus (19.9 %) at N50 recorded minimum harvest index. Increased application of N fertilizer significantly delayed days to 50 % flowering and improved SCMR values. Mean days to 50 % flowering increased from 109 at N0 to 112 at N50, 114 at N100 and 115 days at N150 and mean SCMR values increased from 37.8 at N0 to 39.3 at N50, 40.7 at N100 and 42.1 at N150. Mean days to 50 % flowering and SCMR values reduced by 4.39 % and 7.10 % with N0, 1.65 % and 3.31 % with N50 and increased by 1.13 % and 3.41 % with N150. Among the genotypes, Tulasi recorded the shortest days to 50 % flowering (109 days), while Dhanarasi had the longest mean days to flowering (119 days). Vikas at N100 (47.9) and RV 38-1 at N50 (46.9) recorded the highest SCMR values, whereas Kasalath at N100 (30.3) and N50 (27.9) recorded the lowest values. Flag leaf length, width, area and dry weight significantly increased with N fertilizer application. From N0 to N150, flag leaf length increased from 32.5 cm to 40.1 cm and width increased from 1.48 cm to 1.70 cm, the area increased from 36.2 cm^2 to 51.2 cm^2 and dry weight increased from 0.192 g to 0.256 g. In comparison with N100, flag leaf length was reduced by 13.48 % and 4.10 %, width was reduced by 8.65 % and 3.22 %, area was decreased by 20.78 % and 6.97 % and dry weight was reduced by 18.54 % and 7.86 % at N0 and N50. Flag leaf length was enhanced by 6.70 %, width by 4.98 %, area by 11.98 % and dry weight by 9.02 % at N150, compared with N100.

With the increase in N application from N0 to N150, SLA increased from 193.2 cm^2/g^1 to 203.3 cm^2 /g and SLW reduced from 5.39 mg/cm^2 to 5.11 mg/cm^2. Compared with N100, SLA decreased by 2.14 %, SLW increased by 3.50 % at N0 and SLA increased by 2.99 %, while SLW decreased by 1.83 % at N150. SLA and SLW were found to be nearly identical at both N50 and N100.

Fv/Fm, ΦPSII, ETR and qP increased significantly with increased application of N fertilizer, while qN noted a significant reduction. Mean Fv/Fm, ΦPSII, ETR and qP were 0.814, 0.379, 27.3 and 0.568 at N100, respectively. However, they reduced to 0.804 (1.27 %), 0.355 (6.26 %), 25.4 (6.66 %) and 0.536 (5.73 %) at N50 and 0.789 (3.11 %), 0.327 (13.72 %), 23.3 (14.56 %) and 0.497 (12.53 %) at N0 whereas they increased to 0.823 (1.08 %), 0.397 (4.64 %), 27.8 (1.83 %) and 0.586 (3.15 %) at N150 respectively. The mean qN decreased from 0.401 at N0 to 0.362 at N50, 0.335 at N100 and 0.318 at N150. qN increased by 19.70 % with N0, 8.06 % with N50 while reduced by 5.07 % with N150 over N100. Compared with N100, Phul Pattas 72, Nidhi, MIMA, Tulasi and DRR Dhan 65 showed the least reduction in Fv/Fm. In contrast, DRR Dhan 60, Bowalia 2, Jati Aus, Anjali and DRRH2 showed the highest reduction under N50. The highest ΦPSII was recorded in DRR Dhan-48 at N100 (0.448) and Anjali at N50 (0.448), while the lowest values were observed in BV-1690 at N100 (0.310) and Aus12 at N50 (0.253). BV-1690 at N100 (22.4) and Aus12 at N50 (18.2) recorded the lowest ETR, while IC463254 at N100 (32.0) and Anjali at N50 (31.9) recorded the highest ETR. BV-1690 at N100 (0.430) and DRR Dhan-53 at N50 (0.384) exhibited the least qP, while Aus Kushi at N100 (0.686) and DRR Dhan-47 at N50 (0.698) exhibited the highest qP. IDSA44 at N100 (0.443) and DRR Dhan 65 (0.474) at N50 exhibited the highest qN, while the lowest qN was noted in Aus12 at N100 (0.217) and DRR Dhan 51 at N50 (0.259).

Plant height and tiller number per plant increased significantly with increased N application. Mean plant height increased from 107.0 cm at N0 to 113.0 cm at N50, 117.9 cm at N100 and 121.1 cm at N150. The mean tiller number per plant increased from 6.2 at N0 to 7.2 at N50, 7.9 at N100 and 8.8 at N150. The mean reduction in plant height and tiller number per plant was 9.25 % and 21.2 % with N0, 4.19 % and 8.91 % with N50 and increased by 2.72 % and 11.7 % with N150, compared to N100. Significant variation was observed among the genotypes for plant height and tiller number per plant and interaction between treatment and genotypes was found to be substantial. Swarna at N100 (90.3 cm) and Tulasi at N50 (81.7 cm) recorded the lowest plant height, whereas Pokkali at N100 (175.5 cm) and N50 (172.3 cm) recorded the highest. DRR Dhan-60 at N100 (14.0) and Swarna at N50 (11.0) exhibited the maximum tiller number per plant, while MIMA exhibited the minimum number at N100 (3.7) and N50 (3.3).

GYEI values between N50 and N100 demarcated DRRH4, DRR Dhan 64, Gontrabidhan, Triguna, DRR Dhan 56, Phalguna, IC463254, Varadhan, DRR Dhan 47, RV 38-1, DRR Dhan 45, RHZ-IR-135, DRR Dhan 65 and BV31 as highly efficient genotypes. In contrast, Ratnasundari, Pokkali, Jati Aus, Mima, DRR Dhan 39, Kasalath, Aus Kushi, Nagarjuna, DRR Dhan 50 and ISM were identified as highly inefficient genotypes (Fig. 4). Likewise, GYEI values between N0 and N100 identified Varadhan, DRR Dhan 47, RV 38-1, DRR Dhan 56, DRR Dhan 65, DRRH4, IC463254, DRR Dhan 64, Gontrabidhan, BV31, RHZ-IR-135, Phalguna, Mandya Vijaya, Triguna and MTU 1010 as highly efficient genotypes. In contrast, Ratnasundari, AUS12, Mima, Pokkali, DRR Dhan 48, DRR Dhan 38, DRR Dhan 39, Jati Aus, DRR Dhan 54 and INGR22067 were identified as highly inefficient (Fig. 5).

Correlation

Multiple correlation analysis showed a strong positive relationship between tiller number per plant, total dry matter and harvest index with grain yield at all the four graded N levels (Fig. 6-9). Interestingly, ΦPSII, ETR and qP showed a strong positive association with grain yield under reduced N conditions (N0 and N50) compared with recommended (N100) and high N application (N150). Therefore, leaf chlorophyll fluorescence traits (ΦPSII, ETR and qP) can be utilized in screening rice genotypes for NUE under reduced N application. Plant height was positively correlated with total dry matter and this association strengthened increased N application. SLA showed a significant positive association with flag leaf length, width and area but a significant negative association with flag leaf dry weight. SLW showed a significant negative association with flag leaf length, width and area, but a significant positive association with flag leaf dry weight was observed. At N100, the grain yield and harvest index showed a strong negative correlation with SLA and a positive correlation with SLW.

Discussion

Nitrogen (N) is a crucial macronutrient and N fertilizer application is vital for rice growth, development and productivity (29). As the optimum N fertilizer application is mandatory, the grain yield of rice can be significantly reduced by insufficient or without N fertilizer application, while excess N fertilizer application leads to increased agronomic and economic losses and simultaneously negatively affects the environment (30-31). As the N cascades are intricately linked with many SDGs' (32), reducing reactive N waste by half before 2030 is crucial to achieving sustainable N management and SDGs (33). The amount of N applied considerably affects the physiological processes and photosynthesis (16,21), which eventually influences the rice yield potential. The current study shows that all the recorded morpho-physiological traits and grain

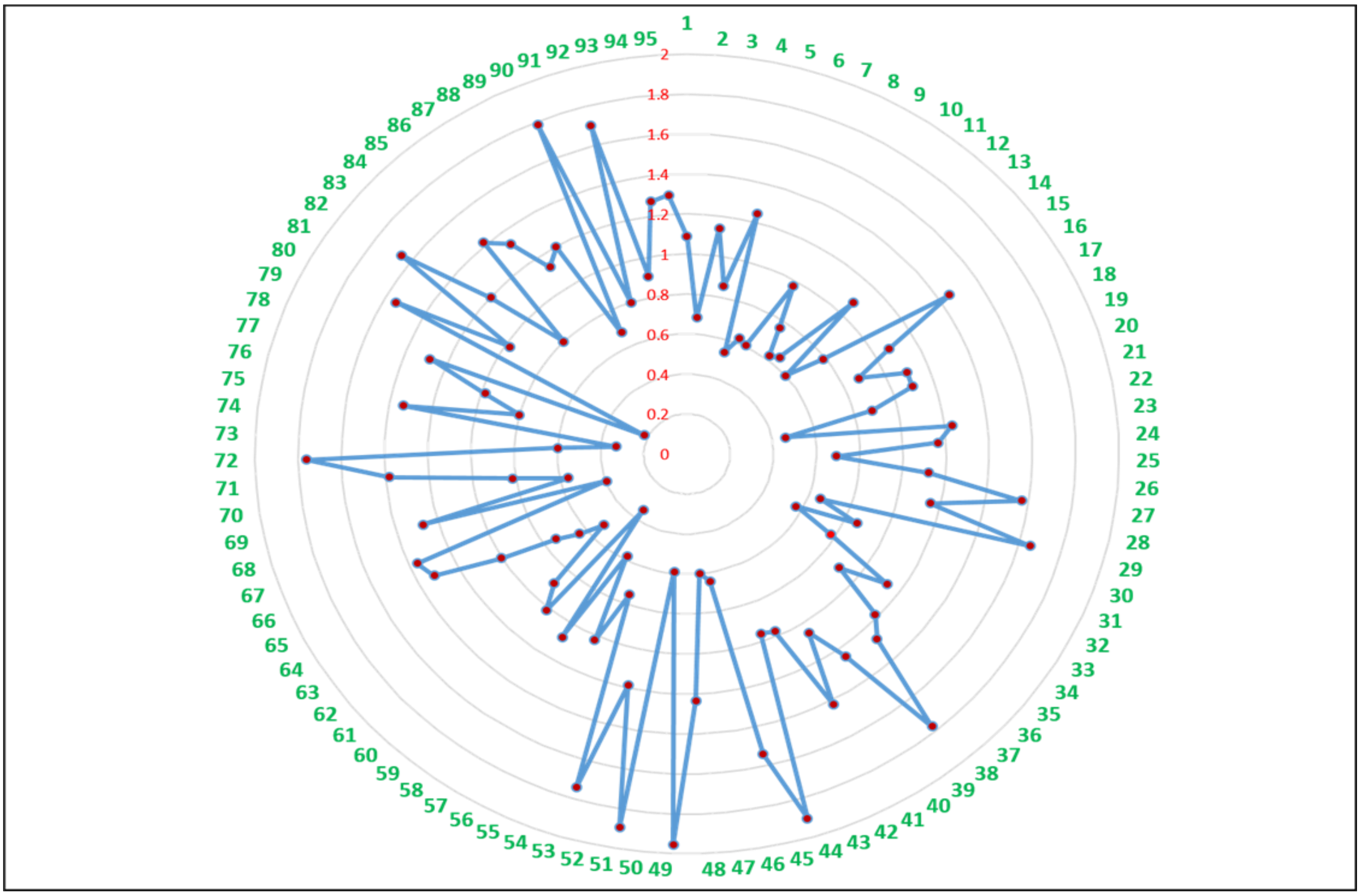

Fig. 4. Grain yield efficiency index (GYEI) of genotypes between N50 and N100 (numbered according to the list of genotypes in Table 1).

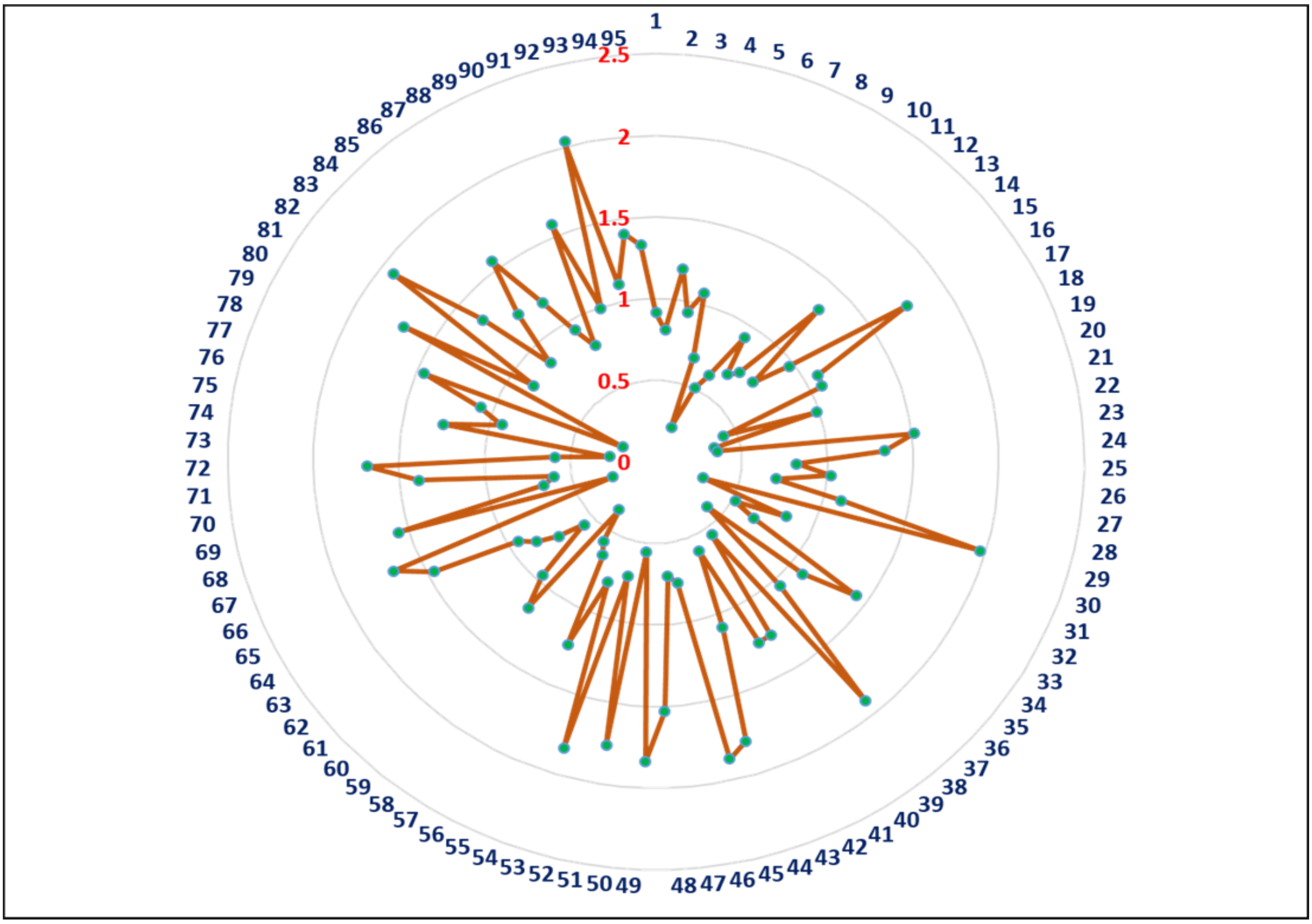

Fig. 5. Grain yield efficiency index (GYEI) of genotypes between N0 and N100 (numbered according to the list of genotypes in Table 1).

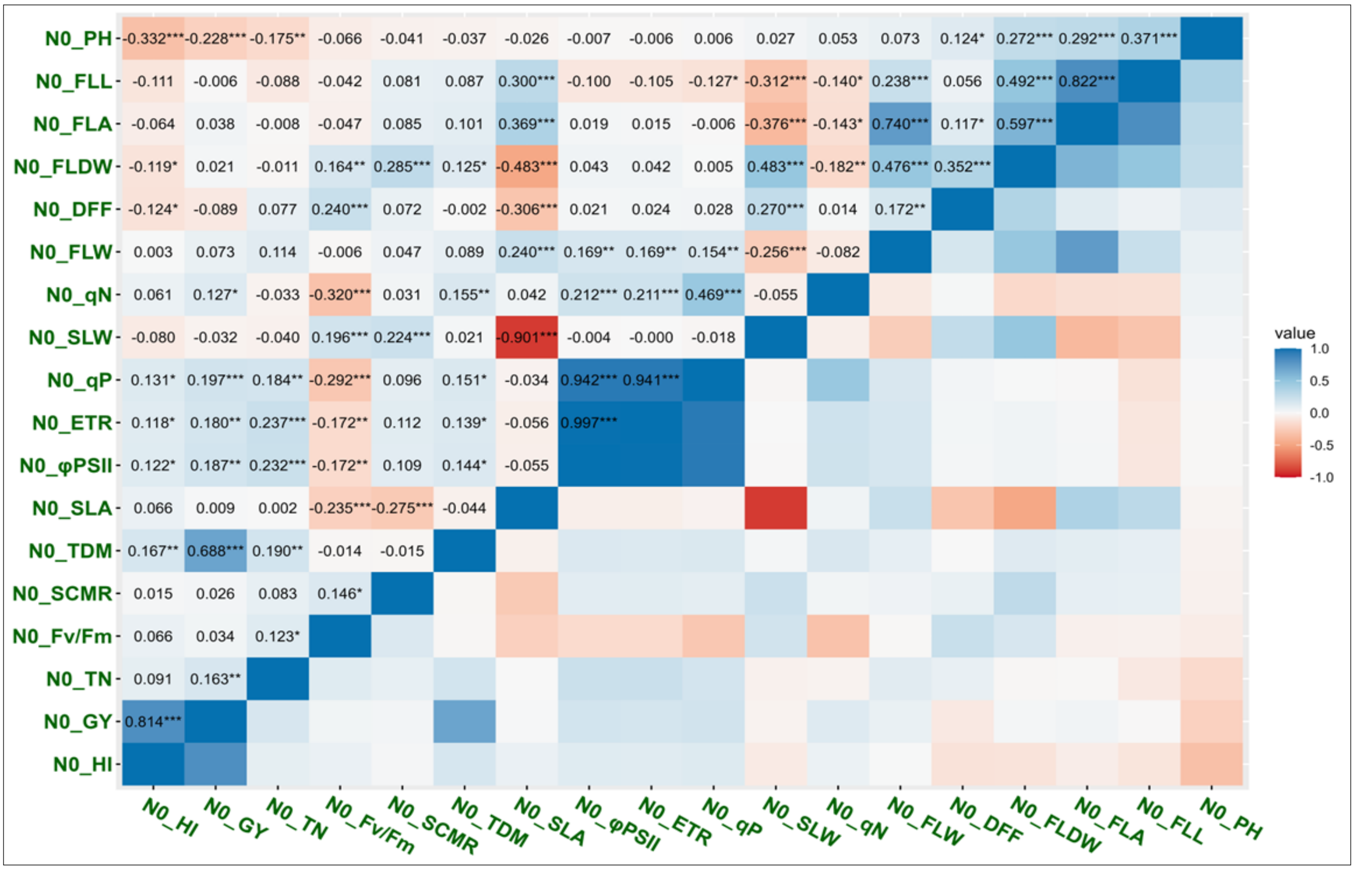

Fig. 6. Correlation among the morpho-physiological parameters along with grain yield at N0. PH-plant height; TN-tiller number per plant; DFF-days to 50 % flowering; FLL-flag leaf length; FLW-flag leaf width; FLA-flag leaf area; FLDW-flag leaf dry weight; SLA-specific leaf area; SLW-specific leaf weight; SCMR-SPAD Chlorophyll meter reading; Fv/Fm-maximum quantum yield of PSII; ϕPSII-actual quantum yield of PSII; ETR-electron transport rate; qP-coefficient of photochemical quenching; qN-coefficient of non-photochemical quenching; TDM-total dry matter; GY-grain yield; HI-harvest index. *** - p≤ 0.001, **- p≤ 0.01, *- p≤ 0.05, • - p≤ 0.1.

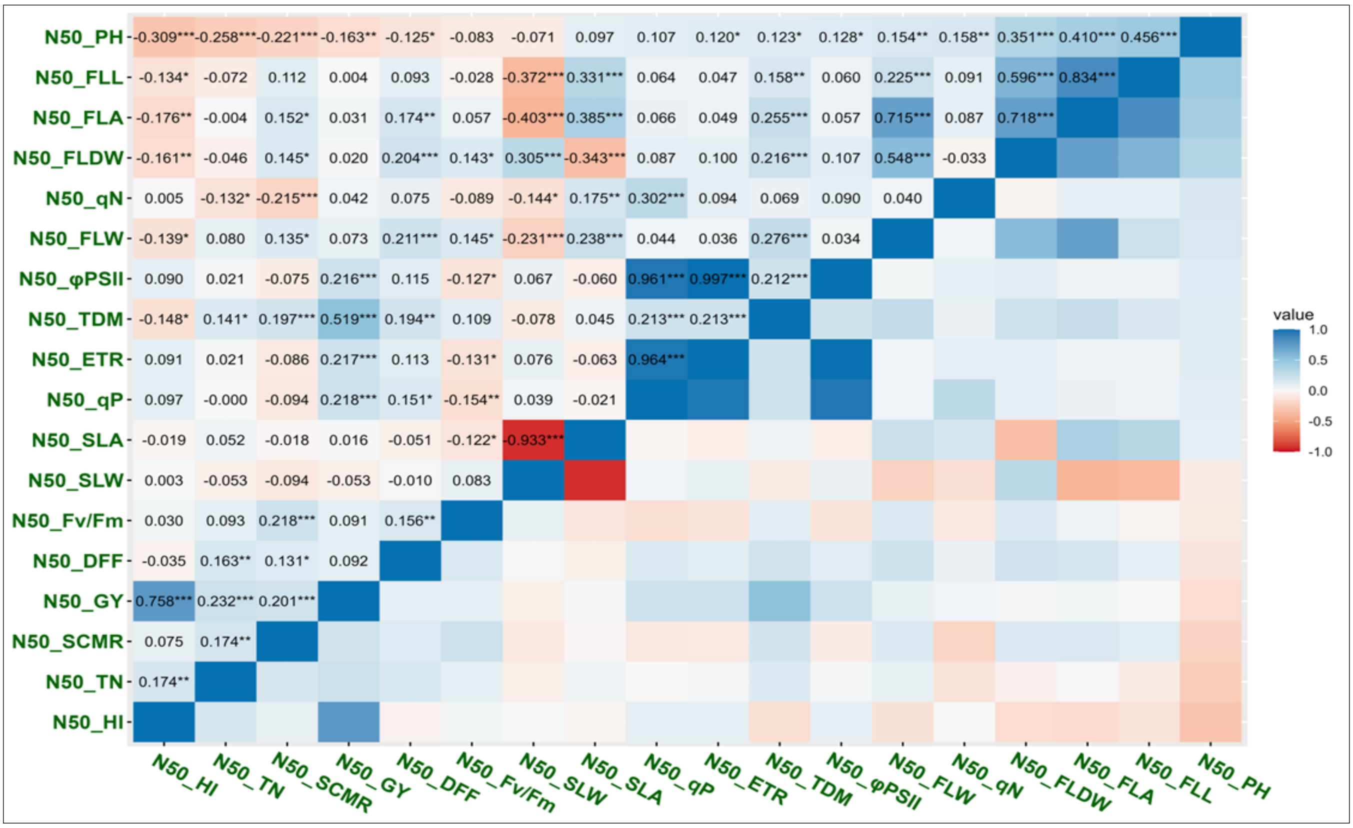

Fig. 7. Correlation among the morpho-physiological parameters along with grain yield at N50. PH-plant height; TN-tiller number per plant; DFF-days to 50 % flowering; FLL-flag leaf length; FLW-flag leaf width; FLA-flag leaf area; FLDW-flag leaf dry weight; SLA-specific leaf area; SLW-specific leaf weight; SCMR-SPAD Chlorophyll meter reading; Fv/Fm-maximum quantum yield of PSII; ϕPSII-actual quantum yield of PSII; ETR-electron transport rate; qP-coefficient of photochemical quenching; qN-coefficient of non-photochemical quenching; TDM-total dry matter; GY-grain yield; HI-harvest index. *** - p≤ 0.001, **- p≤ 0.01, *- p≤ 0.05, - p≤ 0.1.

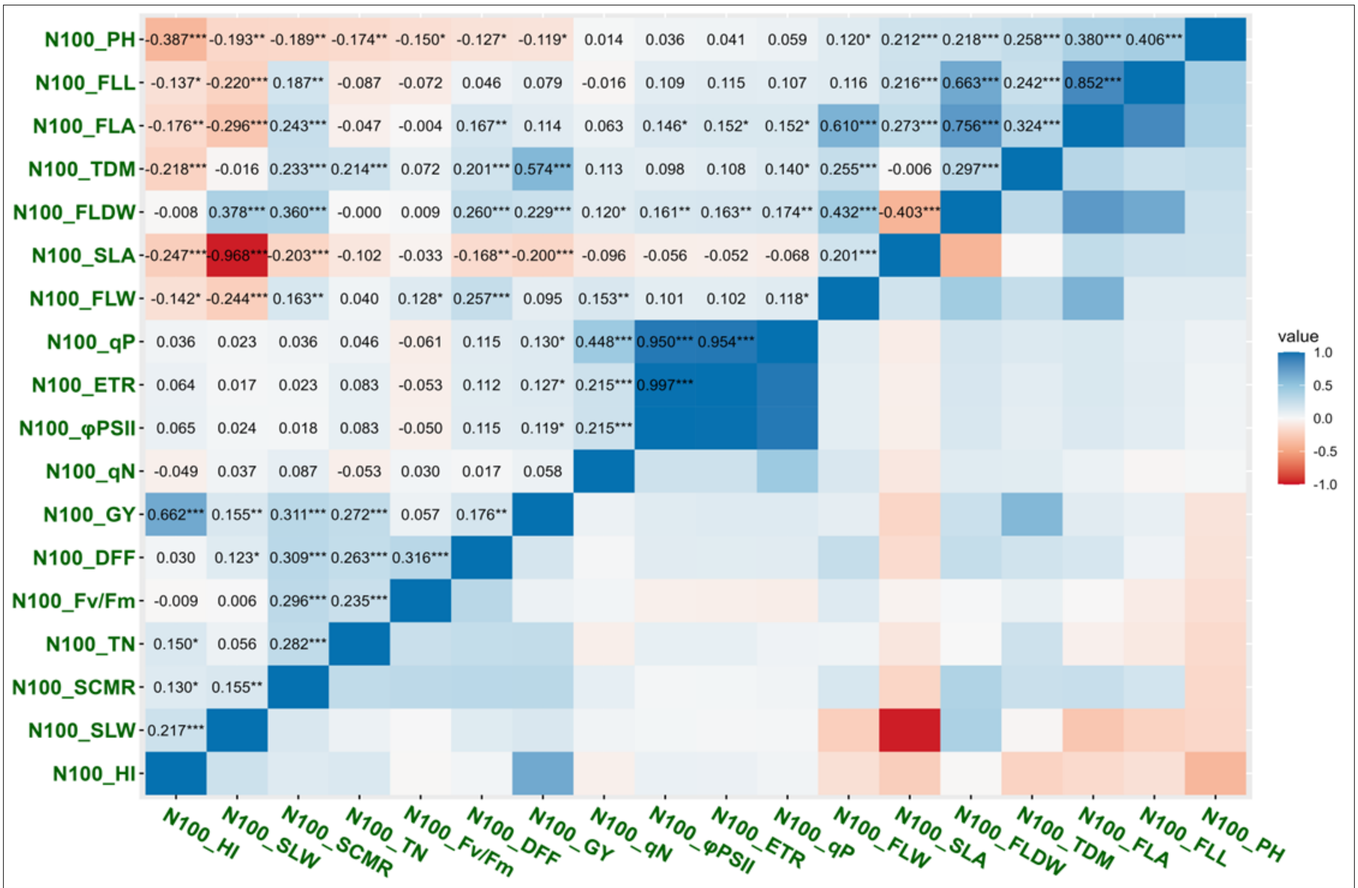

Fig. 8. Correlation among the morpho-physiological parameters along with grain yield at N100. PH-Plant height; TN-Tiller number per plant; DFF-Days to 50 % flowering; FLL-Flag leaf length; FLW-Flag leaf width; FLA-Flag leaf area; FLDW-Flag leaf dry weight; SLA-Specific leaf area; SLW-Specific leaf weight; SCMR-SPAD Chlorophyll Meter Reading; Fv/Fm-Maximum quantum yield of PSII; ϕPSII-Actual quantum yield of PSII; ETR-Electron transport rate; qP-Coefficient of photo-chemical quenching; qN-Coefficient of non-photochemical quenching; TDM-Total dry matter; GY-Grain yield; HI-Harvest index.. *** - p≤ 0.001, **- p≤ 0.01, *- p≤ 0.05, - p≤ 0.1.

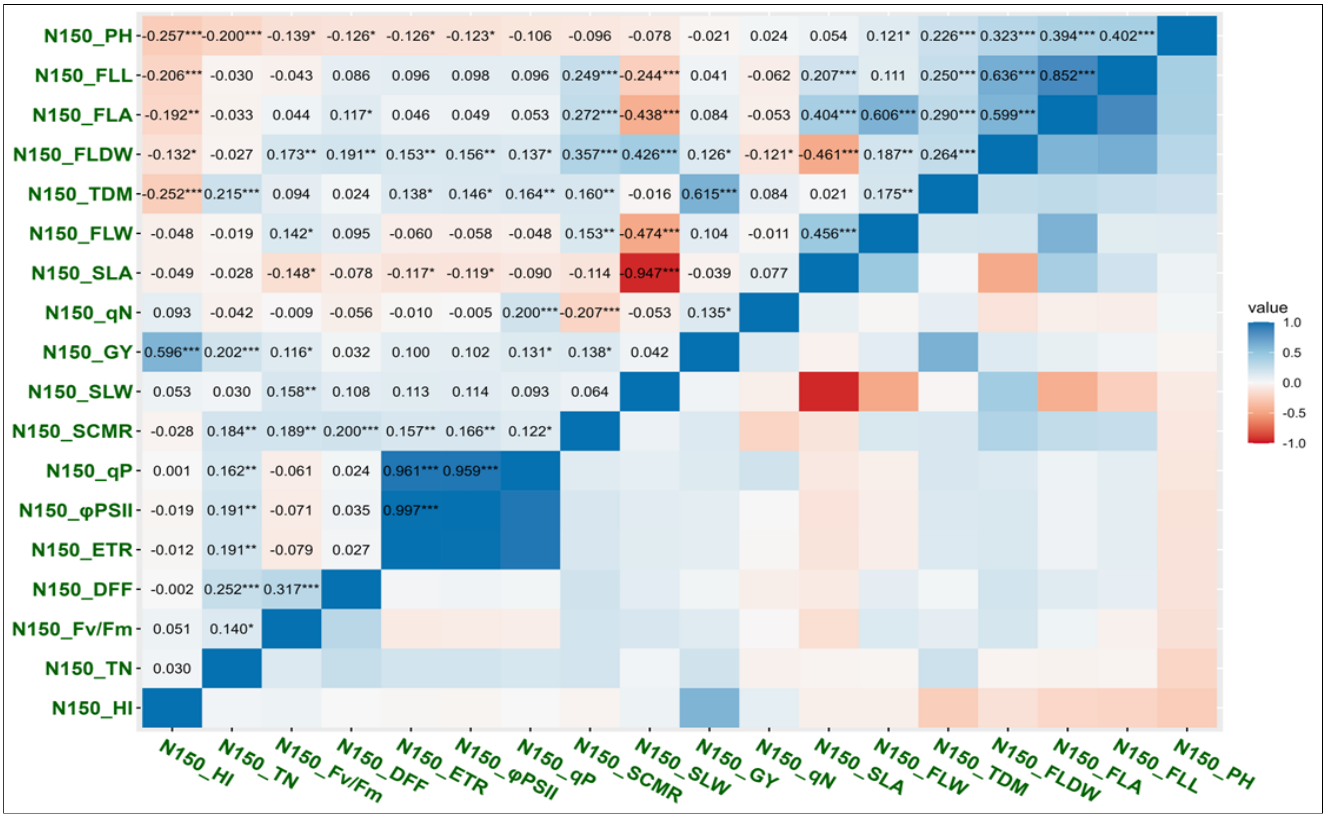

Fig. 9. Correlation among the morpho-physiological parameters along with grain yield at N150. PH-Plant height; TN-Tiller number per plant; DFF-Days to 50 % flowering; FLL-Flag leaf length; FLW-Flag leaf width; FLA-Flag leaf area; FLDW-Flag leaf dry weight; SLA-Specific leaf area; SLW-Specific leaf weight; SCMR-SPAD Chlorophyll Meter Reading; Fv/Fm-Maximum quantum yield of PSII; ϕPSII-Actual quantum yield of PSII; ETR-Electron transport rate; qP-Coefficient of photochemical quenching; qN-Coefficient of non-photochemical quenching; TDM-Total dry matter; GY-Grain yield; HI-Harvest index.. *** - p≤ 0.001, **- p≤ 0.01, *- p≤ 0.05, - p≤ 0.1.

yield of rice genotypes varied significantly under graded N levels and N application improved the overall performance. Increased N application has enhanced the plant height and results from adequate N in improving the growth, length of internodes and overall metabolism. Increased N supply leads to cell expansion, stimulating stem elongation (34). Increased N application improved tiller number per plant and is congruent (35). N availability in adequate amounts at the tillering plays a role in cell division, resulting in enhanced tiller bud outgrowth and ultimately leading to higher tiller numbers per plant (36).

The increase in N application rates resulted in a higher accumulation of photosynthetic pigment contents, which is reflected through SCMR values in the current investigation (19). The chlorophyll content of plant leaves is enhanced with optimum N application, resulting in improved photosynthetic efficiency (37). Similar results of increased SCMR values with increased N application (38). Enhanced flag leaf length, width, area and dry weight observed in this study could be attributed to accelerated cell division and expansion with increased N application, resulting in more significant biomass accumulation (39). Earlier researchers reported increased flag leaf length, width, area and dry weight in rice with increase in N application (38,40). In agreement with the present study, maximum specific leaf area (SLA) and minimum specific leaf weight (SLW) were observed at the highest N application rate. In contrast, minimum SLA and maximum SLW were noted at the lowest N application rate (21,41).

Evaluating chlorophyll fluorescence traits in flag leaf at 50 % flowering under limited N scenarios can distinguish genotypes varying in grain yield and NUE (21). Nitrogen is the major determinant in photosynthetic apparatus allocation that regulates plant photosynthetic rate (Pn) and Fv/Fm. Fv/Fm is one of the best parameters to determine the inhibited degree of rice photosynthesis. Rubisco regulation and regulating photochemical and non -photochemical quenching may improve plant growth under reduced N conditions (42). The present study showcased that the Fv/Fm of rice varieties showed a downtrend under reduced N application. Limited amounts of stored leaf N under reduced N application have led to depletion in the amount of Rubisco and its activity, resulting in the diminished Fv/Fm of the plants. Compared with N100, with an increase in nitrogen application, the Fv/ Fm of the rice varieties leaves increased significantly. Under optimum N availability, improved electron transfer centre in rice leaves, facilitating better solar energy conversion (43).

Under limited N conditions, the performance of reaction centers and PSII photochemical efficiency was reduced and conversely, there was an escalation in PSII photo-inhibition (44). Present results indicate the reduction of ϕPSII and Fv/Fm under reduced N application and escalation of qN. qP and qN showed inverse behaviour under reduced N conditions, as the qP reduced with reduced N treatment while qN increased, compared with recommended N. The decline in electron transport rate and increased non-photochemical quenching of PSII negatively affected carbon fixation and photorespiration

(44). In our study, ETR was highly decreased with reduced N conditions and other researchers also noted a similar reduction in photochemical efficiency in plants under stress (19,45). Minimal reduction of photochemical efficiency of the genotypes with better grain yield under reduced N application may be attributed to the accelerated leaf chlorophyll synthesis, which enhanced the efficiency of absorption, conversion and transmission of light energy. In comparison with recommended (N100) and high N application (N150), ϕPSII, ETR and qP noted a strong positive correlation with grain yield under reduced N conditions (N0 and N50). This implies that PSII photochemistry is crucial in determining grain yield under limited N availability.

Biomass and grain yield of rice were enhanced by increased N application (16,46) and could be accredited to the improved performance of yield attributes and better contribution of biomass-enhancing traits, including plant height and tiller number. Significant genetic variation was noticed for grain yield, which can be utilized to improve NUE in rice (47–48). Reduced biomass and grain yield under limited N application is due to decreased translocation of N from culms to leaves, reducing photosynthates further limiting nutrient translocation to developing panicles (49). Enhanced biomass accumulation with N fertilization has also been reported (36). About 42.4 to 799.6 % increase in grain yield and 33.1 to 352.7 % increase in total dry matter was noticed among the genotypes in the current study with increasing N application from N0 to N150. It indicates the existence of promising genetic variability among the genotypes with graded N application for grain yield, which could be exploited to develop N-use efficient varieties. Large-scale field-level demonstrations (FLDs) could be conducted with widely adopted varieties such as MTU-1010, which exhibit the least reduction in grain yield with limited N application to adopt N50 conditions among the farming community to achieve SDG targets.

Conclusion

This study showcases the feasibility of cultivating rice with reduced nitrogen (N) application by leveraging the extensive genetic diversity among genotypes subjected to graded N application. Among the tested varieties, MTU-1010, Vasumati, DRR Dhan-58, Varadhan, Brown Gora SB 92, Tulasi, BV-1692 and DRRH2 exhibited minimal reductions in grain yield under reduced N application levels (N0 and N50) and thus hold potential as valuable contributors to breeding programs aimed at enhancing nitrogen use efficiency (NUE). These genotypes warrant further investigation to delve into various components of NUE and identify surrogate traits. Leaf chlorophyll fluorescence traits such as ϕPSII, ETR and qP can effectively screen rice genotypes for NUE under reduced N application conditions. In conclusion, Varadhan emerges as a standout genotype in terms of grain yield and GYEI under reduced N application, highlighting its efficiency in nitrogen utilization.

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Authors' contributions

CNN has conceptualized and designed the study. BS, KS and VJ carried out material preparation and data collection. BS and VJ have performed data analysis. BS has prepared the first draft of the manuscript. DSR and DS critically edited the manuscript and all authors commented on previous versions. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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