RESEARCH ARTICLE https://doi.org/10.58297/USZC7294

Genotypic Variation in Photosynthetic Traits, Grain Yield and Nitrogen Use Efficiency in Rice (*Oryza sativa* L.) Under Differential Nitrogen Levels

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Received: 10th April, 2023; Accepted: 23rd May, 2023

Abstract

Nitrogen (N) is one of the yield limiting nutrients for rice. Unwarranted usage of N fertilizer to achieve higher crop returns is affecting environment and increasing the cost of cultivation. A field experiment was conducted under two differential N experimental plots (N-Low and N-Rec) to evaluate the effect of N on photosynthesis, grain yield and nitrogen use efficiency (NUE) of six rice genotypes belonging to three diverse groups. At N-Rec, Kolajoha3 exhibited highest mean SCMR value (43.2), flag leaf length (39.0 cm), flag leaf width (1.77 cm), flag leaf area (53.8 cm^2), photosynthetic rate (19.50 µmol CO_2 m^{-2} s^{-1}), stomatal conductance (0.38 mol [H_2O] m^{-2} s^{-1}), transpiration rate (10.72 mmol [H_2O] m^{-2} s^{-1}). IC463254 recorded highest mean grain yield (621.5 g m^{-2}), total dry matter (1302.5 g m^{-2}), harvest index (47.7%), grain N uptake (84.4 kg ha^{-1}) and nitrogen use efficiency (18.2). Significant reduction in growth, photosynthetic rate and yield of rice occurred under N-Low compared with N-Rec. In comparison N-Rec, Kolajoha3 exhibited least mean reduction in plant height (10.68%), photosynthetic rate (14.96%), productive tiller number (35.40%), grain yield (50.63%), straw yield (24.83%), total dry matter (36.03%), agronomic efficiency (14.6%) and NUE (26.21%) under N-Low, while IC463254 exhibited least mean reduction in SCMR value (14.11%) and flag leaf width (23.66%).

Keywords: Rice, nitrogen, photosynthetic rate, grain yield, NUE.

Introduction

Rice is a staple cereal crop, cultivated in ~167 Mha with a production of 770 million tons and productivity of 4.10 t ha^{-1}. An increase of 2% - 3% in annual rice production has to be promised to ensure the self-sufficiency with the available resources (Haque and Haque 2016). In the scenario of global climate change, agriculture segment witnesses the snag to attain marked crop returns. Influence of high-temperature episodes, unexpected weather events, increased CO_2 concentrations and non-CO_2 Green House Gas (CH_4 and N_2O) emissions on the global food security is being realized world over. Reduction of greenhouse gases based on IPCC recommendations is crucial to emphasize the future food security, planning and its implementation. Applying excessive nitrogen (N) fertilizer doses to the agricultural lands increases the concentration of NO_3-N and NH_4-N in ground water and has negative impact on environment and soil health (Savci 2012). High quantity of N fertilizer usage rises the input cost to the farmers and effects the environment adversely. In particular, N fertilizer import, and subsidy outgo has an impact on nation's economy as well. To achieve two-fold raise in the aggregate agricultural yield since 1960 to 1995, seven folds of higher N fertilizer applied to farmlands (Tilman *et al*., 2002). Considering this perspective, Climate Resilient Agriculture (CRA) is an innovative to precisely solve the problem through potent mitigation practices like increasing NUE in crop plants besides sustainably increasing the crop yields.

Nitrogen plays a significant part in photosynthetic activity of rice leaves as it is the primary constituent of proteins which are in turn constituents of protoplasm, chloroplasts, phyto-hormones, and enzymes (Murata and Osada, 1959). Increased N fertilizer usage contributes to enhanced yield. Nitrogen deficiency in rice fields leads to increase in stomatal resistance which is the critical step in carbon dioxide diffusion during leaf photosynthesis (Oritani *et al*., 1979; Makino *et al*., 1983; Weng and Chen, 1987). More than 90% of crop biomass is derived from assimilates of photosynthesis. According to Long *et al*., (2006) there is a close relationship between enhanced photosynthesis, biomass, and yield; this suggests that increasing photosynthesis increases yield, if other genetic factors are not altered.

Although, N is essential to rice plant metabolism, all the rice genotypes do not need same N fertilizer doses. Every genotype has possessed best N application rate which depends on its Nitrogen Use Efficiency (NUE). NUE can be defined as the capability of a genotype to absorb and mount up the adequate nitrogen to raise and make over to better grain yield per unit of available N (native N + supplied N) in the soil (Mi *et al*., 2007 and Hirel *et al.,* 2021). Only 33% of NUE was noticed globally for cereal crops and the rest 67% is lost into environment worth INR 72,000 crore per annum (Abrol *et al*., 2007). According to Mahajan *et al*., (2010), Basmati varieties do not bear high N fertilizer doses to enhance yields; high N application may cause for susceptibility to lodging, disease prone conditions, and insect pest attacks. Vijayalakshmi *et al*., (2015a and 2015b) was also observed that an aromatic rice genotype, Basmati 370 has shown tolerance and performed well under ‘N’ stress conditions. Thus, there is a necessity to enhance NUE in rice, that future rice breeding programmes should be in such a way that rice genotypes exhibit high NUE as a natural instinct. There is a prerequisite to be aware of the photosynthetic activity in developing cultivars at limiting soil-N levels (Abrol *et al*., 2008). Thus, rice genotypes with high NUE, optimum photosynthetic rate and sustainable grain yield under N stress conditions were considered in this study.

Materials and Methods

Six rice genotypes *viz.,* Basmati370 and Kolajoha3 (aromatic), IC463222 and IC463254 (germplasm) and Giza178 and Zardrome (ACC32379) (IRHTN) were selected based on earlier studies (Rao *et al.,* 2018). Field experiments were conducted during *kharif 2020*, *rabi 2021* and *kharif 2021* at Indian Institute of Rice Research, Hyderabad, India (17.530 19’ N, 78.270 29’ E and 542.7 MSL). Two separate plots were maintained with zero N fertilizer application (N-Low) and recommended N (N-Rec) since 2010 (Vijayalakshmi *et al.,* 2015a; Swamy *et al.,* 2016; Vishnukiran *et al.,* 2020). Nitrogen fertilizer @ 100 kg ha^{-1} was supplied in the form of urea in three equal split applications to the N-Rec treatment (at basal, active tillering and panicle initiation stages). Phosphorus (@ 40 kg ha^{-1}), potassium (@ 40 kg ha^{-1}) and zinc (@ 25 kg ha^{-1}) were applied to both plots.

Soil samples were collected from experimental plots prior to experiment to determine the preliminary soil properties. Samples were collected from four different areas of experimental plot and the mixed composite was used to determine N content by following semi micro Kjeldahl method (Kjeldahl, 1883). The soil characteristics during three successive seasons for N-Rec treatment plot: soil pH 7.29, 7.39, 7.42; electrical conductivity (EC) 0.24, 0.28, 0.27 dS m^{-1}; organic carbon content 0.69%, 0.72%, 0.72%; available nitrogen (N) 220, 255, 252 kg ha^{-1}; available phosphorus (P_2O_5) 60, 63, 61 kg ha^{-1}; available potassium (K_2O) 682, 705, 789 kg ha^{-1}; and for N-Low treatment plot: soil pH 7.20, 7.42, 7.46; electrical conductivity (EC) 0.22, 0.26, 0.29 dS m^{-1}; organic carbon content 0.58%, 0.66%, 0.66%; available nitrogen (N) 202, 243, 233 kg ha^{-1}; available phosphorus (P_2O_5) 56, 52, 50 kg ha^{-1}; available potassium (K_2O) 628, 692, 721 kg ha^{-1}.

The experiment was laid out in split plot design with two N levels (N-Rec and N-Low) as main plots and six genotypes as subplots and replicated thrice. Rice seeds were sown as nursery and 30 days old seedlings were transplanted in square meter area with one seedling per hill at a spacing of 10 × 20 cm. Recommended package of rice crop production and protection practices were followed (www.rkmp.co.in).

Agro-morphological parameters, grain yield and NUE were studied for two consecutive seasons and during third season in addition to above mentioned traits, flag leaf photosynthetic traits were also studied. Flag leaf traits and SCMR values were measured at reproductive stage. Agro-morphological, yield and yield related attributes were recorded at physiological maturity. In situ leaf chlorophyll content (SCMR values) was noted down by using Konica Minolta Corporation's Chlorophyll SPAD-502 plus, (USA). A portable photosynthesis measuring system, LI6400XT (LI-COR Environmental, USA) connected to leaf chamber fluorimeter (6400-40, LI-COR, USA) was used to assess the leaf photosynthetic characteristics of flag leaf between 10.00-13.00 h. At this moment leaf temperature was maintained at 30 °C, which is equal to the ambient temperature prevailing at the time of measurements and PAR was maintained at 1200 μ mol (photon) $m^{-2}s^{-1}$. Measurements were made at ambient CO_2 levels (400 ± 6 ppm). Plants from each replicate were cut at ground level and were threshed manually. Moisture content of grain was adjusted to 14% before determining the total grain weight. Straw samples were dried at 70 °C to take straw weight. Nitrogen content in grain and straw samples was analysed in Kjeldahl method (Kjeldahl, 1883). Agronomic Efficiency (AE) and Physiological efficiency (PE) was calculated as per formula given by Dobermann (2007). Nitrogen use efficiency (NUE) was calculated as described in Huang *et al*., (2018). Data analysis was done using open software R language with *agricolae* package (Mendiburu and Yaseen 2021).

Results and Discussion

Plant height

Nitrogen application has significantly influenced the plant height **(Table 1)**. Maximum mean plant height (100.8 cm) was recorded with N-Rec whereas N-Low recorded minimum (79.9 cm). Significant differences were noticed among the genotypes for plant height. At N-Rec, Basmati370 (155.3 cm and 135.6 cm) during *kharif-2020* and *kharif-2021*, Zardrome (125.7 cm) during *rabi-2021* has showed significantly higher plant height compared to other genotypes, while lowest plant height was recorded in IC463222 (74.7 cm and 77.9 cm) during *kharif-2020* and *kharif-2021*, Kolajoha3 (71.0 cm) during *rabi-2021*. In comparison with N-Rec, plant height has decreased significantly under N-Low in all three seasons **(Figure 1)**. Kolajoha3 has showed the least reduction in plant height (10.65%, 6.57% and 14.13%) while highest reduction was noticed in Basmati370 (30.04%), Zardrome (27.32%) and IC463254 (35.57%) during *kharif-2020*, *rabi-2021* and *kharif-2021*. Results for plant height were supported by the findings of Zhang *et al*., (2020), who reported remarkable improvements in plant height with increased N application. Similar results have also been demonstrated by Jahan *et al*., (2022), who described that an increase in N supply to rice genotypes caused a significant increase in the height of rice plants.

SPAD Chlorophyll Meter Readings (SCMR)

SCMR value has increased significantly with nitrogen application **(Table 1).** N-Low has showed lowest mean SCMR value (31.7) whereas highest value was recorded with N-Rec (38.1). SCMR value has differed significantly among the genotypes. At N-Rec, Kolajoha3 (44.4, 44.2 and 41.0) has showed significantly higher SCMR value compared to other genotypes, while lowest value was recorded in Zardrome (32.9, 36.0 and 34.4) during *kharif-2020*, *rabi-2021* and *kharif-2021*. In comparison with N-Rec, SCMR value has decreased significantly under N-Low in all three seasons **(Figure 1).** Basmati370 (11.26% and 10.24%) during *kharif-2020* and *rabi-2021*, IC463254 (12.05%) during *kharif-2021* has showed the least reduction in SCMR value while highest reduction was noticed in Giza178 (27.43%), IC463222 (19.79%) and Basmati370 (25.10%) during *kharif-2020*, *rabi-2021* and *kharif-2021*. The linear relationships for chlorophyll content and N application rate have been explained by Abunyewa *et al.,* (2016).

Flag leaf traits

Flag leaf length was significantly increased by nitrogen application **(Table 1).** Maximum mean flag leaf length was recorded with N-Rec (31.1 cm) while N-Low (21.8 cm) has exhibited the minimum length. Significant differences were noticed among the genotypes for flag leaf length. Kolajoha3 has showed higher flag leaf length (42.6 cm, 30 cm and 44.3 cm), while lowest length was noticed in IC463254 (28 cm), Giza178 (19.5 cm) and Zardrome (29.6 cm) in *kharif-2020*, *rabi-2021* and *kharif-2021* at N-Rec. Significant reduction in flag leaf length was observed with N-Low compared to N-Rec in all three seasons. In *kharif-2020* and *kharif-2021*, Kolajoha3 (50.19% and 49.62%) has showed higher reduction in flag leaf length while least reduction was noticed in Zardrome (11.56% and 9.59%). In *rabi-2021*, Basmati370 (46.56%) has recorded higher reduction while IC463254 (15.36%) and Giza178 (15.37%) has exhibited least reduction.

Nitrogen application has significantly increased the flag leaf width **(Table 1).** N-Rec (1.37 cm) has exhibited highest mean flag leaf width while lowest width was recorded with N-Low (0.95 cm). The tested genotypes differed significantly for flag leaf width. Kolajoha3 has showed higher flag leaf width (2.13 cm, 1.21 cm and 1.99 cm), while lowest width was noticed in Giza178 (1.32 cm), Zardrome (0.86 cm) and Basmati370 (1.26 cm) in *kharif-2020*, *rabi-2021* and *kharif-2021* at N-Rec. In comparison with N-Rec, flag leaf width has decreased significantly under N-Low in all three seasons. Giza178 (25.51%), IC463254 (8.55%) and Basmati370 (21.96%) has exhibited least reduction in flag leaf width while highest reduction was noticed in Kolajoha3 (44.53%, 50.41% and 45.30%) in *kharif-2020*, *rabi-2021* and *kharif-2021*.

Significantly highest mean flag leaf area was recorded with N-Rec (33.2 cm^2) while N-Low (15.7 cm^2) has exhibited the least area. Flag leaf area has differed significantly among the genotypes. At N-Rec, Kolajoha3 has showed higher flag leaf area (68.4 cm^2, 27.2 cm^2 and 66.1 cm^2), while lowest area was noticed in IC463254 (29.4 cm^2), Zardrome (15 cm^2 and 30.2 cm^2) in *kharif-2020*, *rabi-2021* and *kharif-2021*. Significant reduction in flag leaf area was observed with N-Low compared to N-Rec in all three seasons. Kolajoha3 (72.44%, 62.63% and 72.46%) has showed highest reduction in flag leaf area while least reduction was noticed in Zardrome (36.85%), IC463254 (22.6%) and Zardrome (30.16%) in *kharif-2020*, *rabi-2021* and *kharif-2021*.

Table 1: Effect of nitrogen application on agro-morphological traits, grain yield and NUE in six rice genotypes

Treat	Entry	Plant height (cm)				SCMR value				Flag leaf length (cm)				Flag leaf width (cm)				Flag leaf area (cm²)			
		S1	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**
N - Low	BASMATI 370	108.7	87.7	104.7	100.3	36.1	32.4	28.0	32.2	22.6	15.5	23.3	20.5	0.91	0.83	0.98	0.91	15.4	9.7	17.1	14.1
	GIZA 178	80.7	73.0	83.2	79.0	27.2	34.5	29.1	30.2	24.9	16.5	27.2	22.9	0.98	0.81	1.02	0.94	18.4	10.0	20.7	16.3
	IC-463222	58.0	69.3	56.7	61.3	27.8	30.6	30.7	29.7	20.5	18.0	21.2	19.9	0.93	1.00	0.97	0.97	14.4	13.5	15.4	14.4
	IC-463254	79.8	71.0	68.0	72.9	34.1	32.9	34.5	33.8	22.9	19.9	23.3	22.1	0.95	1.03	1.00	1.00	16.4	15.4	17.6	16.5
	KOLA JOHA 3	72.7	66.3	72.9	70.6	37.5	36.5	31.3	35.1	21.2	22.7	22.3	22.1	1.18	0.60	1.09	0.96	18.9	10.2	18.2	15.7
	ZARDROME	99.0	91.3	95.3	95.2	27.8	29.4	29.8	29.0	25.2	18.1	26.7	23.3	1.11	0.70	1.05	0.95	20.9	9.5	21.1	17.2
N-Low - Mean		**83.1**	**76.4**	**80.1**	**79.9**	**31.7**	**32.7**	**30.6**	**31.7**	**22.9**	**18.5**	**24.0**	**21.8**	**1.01**	**0.83**	**1.02**	**0.95**	**17.4**	**11.4**	**18.4**	**15.7**
N - Rec	BASMATI 370	155.3	100.3	135.6	130.4	40.7	36.1	37.4	38.1	31.8	29.0	32.7	31.1	1.32	1.02	1.26	1.20	31.5	22.3	30.9	28.2
	GIZA 178	97.7	80.3	105.9	94.6	37.5	40.6	35.0	37.7	31.8	19.5	33.3	28.2	1.32	1.11	1.33	1.25	31.5	16.2	33.3	27.0
	IC-463222	74.7	75.3	77.9	76.0	33.6	38.1	36.1	35.9	35.9	29.9	36.2	34.0	1.50	1.20	1.64	1.45	40.4	27.1	44.4	37.3
	IC-463254	99.7	89.0	105.6	98.1	40.2	38.7	39.2	39.4	28.0	23.5	30.0	27.2	1.40	1.13	1.39	1.31	29.4	19.9	31.1	26.8
	KOLA JOHA 3	81.3	71.0	84.9	79.1	44.4	44.2	41.0	43.2	42.6	30.0	44.3	39.0	2.13	1.20	1.99	1.77	68.4	26.9	66.1	53.8
	ZARDROME	129.7	125.7	124.2	126.5	32.9	36.0	34.4	34.4	28.5	23.3	29.6	27.1	1.55	0.86	1.36	1.26	33.1	15.0	30.2	26.1
N-Rec - Mean		**106.4**	**90.3**	**105.7**	**100.8**	**38.2**	**39.0**	**37.2**	**38.1**	**33.1**	**25.9**	**34.3**	**31.1**	**1.54**	**1.09**	**1.49**	**1.37**	**39.0**	**21.2**	**39.3**	**33.2**
LSD (T)		1.80*				1.51**				0.58*				0.056*				1.98*			
LSD (S × T)		4.74**				ns				1.52**				0.14**				5.21**			
LSD (E)		5.07**				1.04*				0.95**				ns				1.58*			
LSD (S × E)		8.78**				2.41**				1.66**				0.11**				3.66**			
LSD (T × E)		5.39*				ns				1.35**				0.093**				2.98**			
LSD (S × T × E)		12.41**				2.57*				2.34**				0.12*				5.17**			
CV (T)%		4.25				6.09				4.66				10.22				17.24			
CV (R)%		6.32				4.51				4.08				6.41				9.74			

Treat	Entry	Productive tiller number m^{-2}				Grain yield (g m^{-2})				TDM (g m^{-2})				HI (%)				Nitrogen Use Efficiency			
		S1	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**
N - Low	Basmati370	176.7	283.3	196.1	218.7	220.5	117.3	214.5	184.1	549.7	399.7	569.4	506.3	40.0	29.2	37.5	35.6	10.9	9.2	4.8	8.3
	Giza178	223.3	250.0	248.9	240.7	247.8	196.0	254.2	232.6	723.2	523.0	716.6	654.2	34.2	37.5	35.5	35.8	12.3	10.9	8.1	10.4
	IC-463222	210.0	316.7	233.3	253.3	238.2	221.7	186.7	215.5	566.6	611.3	532.2	570.0	42.0	36.2	35.1	37.8	11.8	8.0	9.1	9.6
	IC-463254	360.0	266.7	300.6	309.1	305.8	280.7	289.0	291.8	780.0	693.2	690.9	721.4	39.2	40.5	41.9	40.5	15.1	12.4	11.6	13.0
	KolaJoha3	173.3	183.3	227.2	194.6	88.5	244.7	94.0	142.4	285.2	648.0	341.5	424.9	30.9	37.8	27.7	32.1	4.4	4.0	10.1	6.2
	Zardrome	193.3	220.0	165.3	192.9	183.8	242.0	251.7	225.8	396.2	575.7	692.5	554.8	46.2	42.1	36.3	41.5	9.1	10.8	10.0	10.0
N-Low - Mean		**222.8**	**253.3**	**228.6**	**234.9**	**214.1**	**217.1**	**215.0**	**215.4**	**550.1**	**575.1**	**590.5**	**571.9**	**38.8**	**37.2**	**35.7**	**37.2**	**10.6**	**9.2**	**8.9**	**9.6**
N - Rec	Basmati370	286.7	416.7	396.7	366.7	601.8	413.2	578.7	531.2	1383.0	885.7	1276.8	1181.8	43.5	46.6	45.3	45.2	18.8	16.4	11.6	15.6
	Giza178	416.7	500.0	418.1	444.9	581.7	423.9	478.3	494.6	1350.0	986.5	1082.6	1139.7	43.1	43.0	44.2	43.4	18.2	13.6	11.9	14.6
	IC-463222	480.0	536.7	472.2	496.3	645.1	415.2	461.7	507.3	1327.7	993.2	961.0	1094.0	48.5	41.8	48.0	46.1	20.2	13.1	11.7	15.0
	IC-463254	503.3	466.7	513.9	494.6	671.6	656.2	536.7	621.5	1435.7	1338.5	1133.4	1302.5	46.8	49.0	47.4	47.7	21.0	15.2	18.5	18.2
	KolaJoha3	246.7	296.7	360.6	301.3	201.0	420.8	243.3	288.4	540.5	843.0	609.2	664.2	37.2	49.9	39.9	42.3	6.3	6.9	11.9	8.3
	Zardrome	266.7	366.7	311.4	314.9	548.6	429.9	531.3	503.3	1186.9	951.3	1178.0	1105.4	46.3	45.3	45.2	45.6	17.1	15.1	12.1	14.8
N-Rec - Mean		**366.7**	**430.6**	**412.1**	**403.1**	**541.6**	**459.9**	**471.7**	**491.1**	**1204.0**	**999.7**	**1040.1**	**1081.3**	**44.2**	**45.9**	**45.0**	**45.1**	**16.9**	**13.4**	**13.0**	**14.4**
LSD (T)		44.23**				27.68**				7.40*				1.64*							
LSD (S × T)		ns				47.94**				19.42**				ns							
LSD (E)		62.46**				19.69*				34.68*				ns							
LSD (S × E)		ns				45.35**				79.90**				3.93**							
LSD (T × E)		88.34**				27.84*				49.05*				ns							
LSD (S × T × E)		ns				64.14**				113.0**				5.56**							
CV (T)%		19.43				10.98				1.9				8.51							
CV (R)%		22.08				8.36				6.29				6.22							

Treat	Entry	Grain N uptake (Kg N ha^{-1})				Straw N uptake (Kg N ha^{-1})				Agronomic efficiency (kg grain kg^{-1} N)				Physiological efficiency (kg kg^{-1})			
		S1	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**
N - Low	Basmati370	20.0	10.7	20.3	17.0	13.3	11.0	16.9	13.7	-	-	-	-	-	-	-	-
	Giza178	26.8	21.6	29.4	25.9	18.2	13.5	18.4	16.7	-	-	-	-	-	-	-	-
	IC-463222	26.5	24.6	19.7	23.6	10.4	13.6	11.1	11.7	-	-	-	-	-	-	-	-
	IC-463254	32.4	29.8	31.1	31.1	18.7	17.5	16.6	17.6	-	-	-	-	-	-	-	-
	KolaJoha3	8.3	22.9	8.6	13.3	9.1	17.3	12.6	13.0	-	-	-	-	-	-	-	-
	Zardrome	19.0	25.1	26.6	23.6	8.6	14.4	18.6	13.9	-	-	-	-	-	-	-	-
N-Low - Mean		**22.2**	**22.5**	**22.6**	**22.4**	**13.0**	**14.5**	**15.7**	**14.4**	-	-	-	-	-	-	-	-
N - Rec	Basmati370	58.9	40.5	70.4	56.6	41.2	24.9	41.7	35.9	38.1	29.6	36.4	34.7	56.9	49.3	67.8	58.0
	Giza178	70.7	53.1	61.3	61.7	33.1	30.2	31.1	31.5	33.4	22.8	22.4	26.2	56.8	51.0	47.6	51.8
	IC-463222	85.3	54.8	58.4	66.1	27.9	27.6	21.6	25.7	40.7	19.3	27.5	29.2	53.3	56.4	43.9	51.2
	IC-463254	91.4	89.2	72.5	84.4	32.8	33.1	31.8	32.6	36.6	37.5	24.8	33.0	49.9	43.4	50.2	47.8
	KolaJoha3	23.2	47.9	27.0	32.7	19.7	21.8	22.2	21.2	11.2	17.6	14.9	14.6	44.1	53.4	59.3	52.2
	Zardrome	68.4	50.5	61.4	60.1	32.3	28.7	36.2	32.4	36.5	18.8	28.0	27.7	49.9	53.4	47.4	50.2
N-Rec - Mean		**66.3**	**56.0**	**58.5**	**60.3**	**31.2**	**27.7**	**30.8**	**29.9**	**32.8**	**24.3**	**25.7**	**27.6**	**51.8**	**51.1**	**52.7**	**51.9**
LSD (T)		3.49**				1.80**				-				-			
LSD (S × T)		6.05**				2.06*				-				-			
LSD (E)		2.37*				ns				3.68*				ns			
LSD (S × E)		5.46**				4.64**				8.59**				13.73**			
LSD (T × E)		3.35*				2.85*				-				-			
LSD (S × T × E)		7.72**				4.94*				-				-			
CV (T)%		11.85				11.44				-				-			
CV (R) %		8.59				13.65				13.89				11.78			

S1 - Kharif 2020; S2 – Rabi 2021; S3 – Kharif 2021; T – Treatment; S – Season; E – Entry; R - Residual

Increase in length and width of flag leaf with increased application of nitrogen was observed in both field and pot experiments and is attributed to enhanced photosynthetic activity with increased nitrogen content of plant (Manzoor *et al*., 2006; Bahmaniar *et al*., 2007; Kumar *et al*., 2008). The results obtained in present study were in accordance with Wang *et al*., (2006), who reported that increased nitrogen application has significantly increased the leaf area in rice and stated that nitrogen plays a very significant role in cell division which in turn is very essential for the increase in leaf area.

Flag leaf gas exchange traits

Data on flag leaf gas exchange traits is provided in **Table 2.** Significantly higher mean photosynthetic rate was recorded at N-Rec (15.55 µ mol CO_2 m^{-2} s^{-1}) compared with N-Low (12.11 µ mol CO_2 m^{-2} s^{-1}). Photosynthetic rate has differed significantly among the genotypes. Kolajoha3 (18.04 µ mol CO_2 m^{-2} s^{-1}) has exhibited significantly highest mean photosynthetic rate whereas Zardrome (11.57 µ mol CO_2 m^{-2} s^{-1}) showed least value. Photosynthetic rate has decreased significantly under N-Low compared to N-Rec in all the genotypes. Kolajoha3 (14.96%) has exhibited least reduction in photosynthetic rate while highest reduction was noticed in Basmati370 (32.82%). Stomatal conductance has increased significantly with N-Rec (0.30 mol [H_2O] m^{-2} s^{-1}) compared to N-Low (0.23 mol [H_2O] m^{-2} s^{-1}). Significant differences were observed among the genotypes for stomatal conductance. Kolajoha3 (0.32 mol [H_2O] m^{-2} s^{-1}) has recorded maximum stomatal conductance while minimum value was noticed in Zardrome (0.18 mol [H_2O] m^{-2} s^{-1}). Significant reduction in stomatal conductance of all the genotypes was observed with N-Low compared to N-Rec. Basmati 370 (15.89%) has exhibited least reduction in stomatal conductance whereas Kolajoha3 (32.23%) has showed highest reduction. Significant interaction was noticed between N treatments and genotypes. Maximum stomatal conductance was observed in Kolajoha3 at N-Rec (0.38 mol [H_2O] m^{-2} s^{-1}) whereas Zardrome at N-Low (0.16 mol [H_2O] m^{-2} s^{-1}) has recorded minimum value.

N-Rec (275 ppm) has showed significantly higher mean internal CO_2 concentration compared to N-Low (262 ppm). Genotypes has differed significantly for internal CO_2 concentration. Kolajoha3 (258 ppm) has recorded lowest mean internal CO_2 concentration while highest value was noticed in Giza178 (277 ppm). Mean transpiration rate has increased significantly with N-Rec (8.07 m mol [H_2O] m^{-2} s^{-1}) compared to N-Low (6.00 m mol [H_2O] m^{-2} s^{-1}). Significant differences were noticed among the genotypes for transpiration rate. Mean maximum transpiration rate was observed in Kolajoha3 (8.77 m mol [H_2O] m^{-2} s^{-1}) whereas Zardrome (5.51 m mol [H_2O] m^{-2} s^{-1}) has exhibited minimum value. Transpiration rate of all the genotypes has reduced significantly with N-Low compared to N-Rec. Giza178 (14.41%) has showed least reduction in transpiration rate while highest reduction was noticed in Kolajoha3 (36.52%). Significant interaction was observed between treatments and genotypes for transpiration rate. Maximum transpiration rate was observed in Kolajoha3 with N-Rec (10.72 m mol [H_2O] m^{-2} s^{-1}) whereas minimum value was noticed in Zardrome with N-Low (4.70 m mol [H_2O] m^{-2} s^{-1}).

Several studies have reported that N deficiency decreased photosynthetic rate in crop leaves, while sufficient N supply extended the longevity of functional leaves, hence, the photosynthetic capacity and grain yield improved. Similarly, in this study, photosynthetic rate increased with the nitrogen application. The results obtained in current study correspond to the reports for rice by Huang *et al*., (2004), who reported a significant reduction in photosynthetic rate and stomatal conductance (g_s) under low N supply.

Table 2: Effect of nitrogen application on leaf photosynthetic traits in six rice genotypes at 50% anthesis stage

Entry/Treat	Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1})			Stomatal conductance (mol [H_2O] m^{-2} s^{-1})			Internal CO_2 concentration (ppm)			Transpiration rate (m mol [H_2O] m^{-2} s^{-1})		
	N-Low	N-Rec	Mean	N-Low	N-Rec	Mean	N-Low	N-Rec	Mean	N-Low	N-Rec	Mean
Basmati370	10.55	15.70	13.13	0.23	0.28	0.25	273	271	272	5.45	7.45	6.45
Giza178	10.18	14.42	12.30	0.27	0.36	0.31	286	268	277	6.78	7.92	7.35
IC-463222	13.35	15.80	14.57	0.23	0.30	0.27	253	285	269	5.75	8.18	6.97
IC-463254	12.03	14.76	13.39	0.23	0.31	0.27	263	281	272	6.53	7.80	7.16
KolaJoha3	16.58	19.50	18.04	0.26	0.38	0.32	240	275	258	6.81	10.72	8.77
Zardrome	10.00	13.14	11.57	0.16	0.20	0.18	256	271	264	4.70	6.32	5.51
Mean	**12.11**	**15.55**	**13.83**	**0.23**	**0.30**	**0.27**	**262**	**275**	**269**	**6.00**	**8.07**	**7.03**
LSD (T)	2.03*			0.049**			11.92*			1.21**		
LSD (E)	1.76**			0.030**			9.41**			0.73**		
LSD (T × E)	ns			0.042**			13.30**			1.04**		
CV (T) %	10.23			5.54			3.09			5.22		
CV (R) %	7.77			6.83			2.13			6.38		

T-Treatment; E-Entry; R-Residual

Yield and Yield attributes

Significant increase in number of productive tillers per m^2 was observed with nitrogen application **(Table 1).** N-Rec has recorded highest mean productive tillers (403.1) while lowest number (234.9) was observed in N-Low. Genotypes has differed significantly for productive tiller number. At N-Rec, IC463254 (503.3 and 513.9) during *kharif-2020* and *kharif-2021*, IC463222 (536.7) during *rabi-2021* has exhibited higher number of productive tillers while lowest number was noticed in Kolajoha3 (246.7 and 296.7) during *kharif-2020* and *rabi-2021*, Zardrome (311.4) during *kharif-2021*. In comparison with N-Rec, productive tiller number has decreased significantly under N-Low in all three seasons. Zardrome (27.50%), Basmati370 (32.00%) and Kolajoha3 (36.98%) has exhibited least reduction in productive tiller number while highest reduction was noticed in IC463222 (56.25%), Giza178 (50.00%) and Basmati370 (50.81%) in *kharif-2020*, *rabi-2021* and *kharif-2021*.

Grain yield was significantly influenced by nitrogen application. Highest mean grain yield was recorded with N-Rec (491.1 g m^{-2}) whereas N-Low (215.4 g m^{-2}) has recorded the least. Significant differences were noticed among the genotypes for grain yield. At N-Rec, IC463254 (671.6 and 656.2 g m^{-2}) during *kharif-2020* and *rabi-2021*, Basmati370 (578.7 g m^{-2}) during *kharif-2021* has recorded higher grain yield, while lowest grain yield was noticed in Kolajoha3 (201.0 and 243.3 g m^{-2}) during *kharif-2020* and *kharif-2021*, Basmati370 (413.2 g m^{-2}) during *rabi-2021*. Significant reduction in grain yield was observed with N-Low compared with N-Rec in all three seasons **(Figure 1).** Zardrome (66.50%) during *kharif-2020*, Basmati370 (71.61% and 62.93%) during *rabi-2021* and *kharif-2021* has exhibited highest reduction in grain yield while least reduction was noticed in IC463254 (54.46% and 46.15%) during *kharif-2020* and *kharif-2021*, Kolajoha3 (41.86%) during *rabi-2021*.

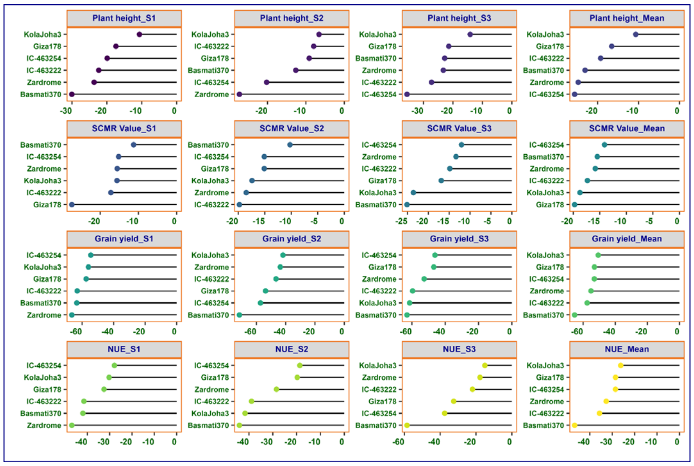

Figure 1: Per cent reduction in plant height, SCMR Value, grain yield and NUE of rice genotypes under N-Low compared with N-Rec

Nitrogen application has significantly influenced the total dry matter. N-Rec (1081.3 g m^{-2}) has exhibited the highest mean total dry matter while least value was recorded with N-Low (571.9 g m^{-2}). Genotypes has differed significantly for total dry matter. IC463254 (1435.7 and 1338.5 g m^{-2}) during *kharif-2020* and *rabi-2021*, Basmati370 (1276.8 g m^{-2}) during *kharif-2021* has recorded significantly higher total dry matter compared to other genotypes, while lowest was recorded in Kolajoha3 (540.5, 843.0 and 609.2 g m^{-2}) during *kharif-2020*, *rabi-2021* and *kharif-2021* at N-Rec. In comparison with N-Rec, total dry matter has decreased significantly under N-Low in all three seasons. Zardrome (66.62%) during *kharif-2020*, Basmati370 (54.87% and 55.40%) during *rabi-2021* and *kharif-2021* has exhibited the highest reduction in total dry matter while least reduction was noticed in IC463254 (45.67%), Kolajoha3 (23.13%) and Giza178 (33.81%) during *kharif-2020*, *rabi-2021* and *kharif-2021*.

Harvest index has increased significantly with Nitrogen application. Highest mean harvest index was observed with N-Rec (45.1%) whereas N-Low (37.2%) has recorded the least. At N-Rec, IC463222 (48.5 and 48.0%) during *kharif-2020* and *kharif-2021*, Kolajoha3 (49.9%) during *rabi-2021* has recorded highest harvest index while least harvest index was observed in Kolajoha3 (37.2 and 39.9%) during *kharif-2020* and *kharif-2021*, IC463222 (41.8%) during *rabi-2021*. Harvest index has decreased significantly with N-Low compared to N-Rec in all three seasons. Least reduction in harvest index was noticed in Zardrome (0.16% and 7.03%) during *kharif-2020* and *rabi-2021*, IC463254 (11.62%) during *kharif-2021* while highest reduction was observed in Giza178 (20.56%), Basmati370 (37.36%) and Kolajoha3 (30.70%) during *kharif-2020*, *rabi-2021* and *kharif-2021*.

Jahan *et al*., (2022) observed that N fertilization increased the number of tillers m^{-2}, which resulted due to the increased N availability for cell division which supports results obtained in present investigation. Srikanth *et al*., (2022) also indicated that suitable dosage of N fertilizer is a key factor to increase rice grain yield. The dry matter production of rice plants is a vitally important factor that determines the formation of rice grain yield (Ye *et al*., 2013). Dry matter production is the result of the accumulation and translocation of photosynthates in different plant organs, which is significantly affected by nitrogen management (Deng *et al*., 2015; Peng *et al*., 2007; Qiao *et al*., 2013). Optimal nitrogen application amounts and rational N application timing would be beneficial to improve rice population quality, and increasing dry matter accumulation and grain yield.

Nitrogen uptake and Nitrogen Use Efficiency

Grain N uptake has increased significantly with N application. N-Rec (60.3 kg ha^{-1}) has recorded highest mean grain N uptake whereas N-Low (22.4 kg ha^{-1}) has recorded the least. Significant differences were noticed among the genotypes for grain N uptake. At N-Rec, IC463254 (91.4, 89.2 and 72.5 kg ha^{-1}) has recorded highest grain N uptake during *kharif-2020*, *rabi-2021* and *kharif-2021* while least uptake was observed in

Kolajoha3 (23.2 and 27.0 kg ha^{-1}) during *kharif-2020* and *kharif-2021*, Basmati370 (40.5 kg ha^{-1}) during *rabi-2021*. In comparison with N-Rec, grain N uptake has decreased significantly under N-Low in all three seasons. Zardrome (72.15%) during *kharif-2020*, Basmati 370 (73.61% and 71.12%) during *rabi-2021* and *kharif-2021* has exhibited the highest reduction in grain N uptake while least reduction was noticed in Giza178 (62.04% and 52.04%) during *kharif-2020* and *kharif-2021*, Zardrome (50.20%) during *rabi-2021*.

Nitrogen application has significantly increased the straw N uptake. Highest mean straw N uptake was observed with N-Rec (29.9 kg ha^{-1}) whereas N-Low (14.4 kg ha^{-1}) has recorded the least. At N-Rec, Basmati370 (41.2 and 41.7 kg ha^{-1}) during *kharif-2020* and *kharif-2021*, IC463254 (33.1 kg ha^{-1}) during *rabi-2021* has showed highest straw N uptake while lowest uptake was recorded in Kolajoha3 (19.7 and 21.8 kg ha^{-1}) during *kharif-2020* and *rabi-2021*, IC463222 (21.6 kg ha^{-1}) during *kharif-2021*. Straw N uptake has decreased significantly with N-Low compared to N-Rec in all three seasons. Highest reduction was observed in Zardrome (73.45%) during *kharif-2020*, Basmati370 (55.88% and 59.41%) during *rabi-2021* and *kharif-2021* while IC463254 (43.13%) during *kharif-2020*, Kolajoha3 (20.77%) during *rabi-2021* and Giza178 (40.95%) during *kharif-2021* has exhibited least reduction in straw N uptake.

Agronomic efficiency has differed significantly among the genotypes **(Table 1).** IC463222 (40.7 kg grain kg^{-1} N), IC463254 (37.5 kg grain kg^{-1} N) and Basmati370 (36.4 kg grain kg^{-1} N) has exhibited highest agronomic efficiency, whereas Kolajoha3 (11.2 kg grain kg^{-1} N, 17.6 kg grain kg^{-1} N and 14.9 kg grain kg^{-1} N) has exhibited least agronomic efficiency during *kharif-2020*, *rabi-2021* and *kharif-2021*. No significant differences were noticed among the genotypes for physiological efficiency. Kolajoha3 (44.1 kg kg^{-1}), IC463254 (43.4 kg kg^{-1}) and IC463222 (43.9 kg kg^{-1}) has recorded least physiological efficiency during *kharif-2020*, *rabi-2021* and *kharif-2021*, while highest physiological efficiency was noticed in Basmati370 (56.9 kg kg^{-1}) and Giza178 (56.8 kg kg^{-1}) during *kharif-2020*, IC463222 (56.4 kg kg^{-1}) during *rabi-2021* and Basmati370 (67.8 kg kg^{-1}) during *kharif-2021*.

NUE has increased significantly with nitrogen application. N-Rec has showed significantly higher mean NUE (14.4) whereas N-Low has recorded the lowest (9.6). Significant differences were noticed among the genotypes for NUE. At N-Rec, IC463254 (21.0 and 18.5) during *kharif-2020* and *kharif-2021*, Basmati 370 (16.4) during *rabi-2021* has recorded higher NUE while lowest value was noticed in Kolajoha3 (6.3 and 6.9) during *kharif-2020* and *rabi-2021*, Basmati370 (11.6) during *kharif-2021*. NUE was reduced significantly under N-Low compared to N-Rec in all three seasons **(Figure 1).** IC463254 (27.86% and 18.65%) during *kharif-2020* and *rabi-2021*, Kolajoha3 (15.06%) during *kharif-2021* has showed least reduction while highest reduction was observed in Zardrome (46.93%) during *kharif-2020*, Basmati 370 (44.00% and 58.52%) during *rabi-2021* and *kharif-2021*.

NUE is largely influenced by grain yield, N fertilizer input and N uptake by the plant (Qiao *et al*., 2012). As to the present experiment, the increase in N supply has been reported to increase grain and straw N concentration, and grain and straw N uptake for rice (Yesuf and Balcha 2014). Under optimum management practices agronomic efficiency should be above 25 kg grain kg^{-1} N (Dobermann 2005). In the present study, all the genotypes except Kolajoha3 (14.6 kg grain kg^{-1} N) have showed mean agronomic efficiency above the value suggested by Dobermann. Many scientists have reported variations in NUE of different rice genotypes. These variations may be attributed due to genetic factors, biochemical and physiological processes such as translocation, assimilation, and N remobilization (Fageria and Baligar 2003).

Conclusion

In conclusion, at N-Rec, Kolajoha3 has exhibited highest mean SCMR value (43.2), flag leaf length (39.0 cm), flag leaf width (1.77 cm), flag leaf area (53.8 cm^2), photosynthetic rate (19.50 µ mol CO_2 m^{-2} $s^{-1)}$, stomatal conductance (0.38 mol [H_2O] m^{-2} $s^{-1)}$, transpiration rate (10.72 m mol [H_2O] m^{-2} $s^{-1)}$, and IC463254 has recorded highest mean grain yield (621.5 g m^{-2}), total dry matter (1302.5 g m^{-2}), harvest index (47.7%), grain N uptake (84.4 kg $ha^{-1)}$ and NUE (18.2). Kolajoha3 has exhibited least mean reduction in plant height (10.68%), photosynthetic rate (14.96%), productive tiller number (35.40%), grain yield (50.63%), straw yield (24.83%), total dry matter (36.03%), agronomic efficiency (14.6%) and NUE (26.21%), and IC463254 has exhibited least mean reduction in SCMR value (14.11%) and flag leaf width (23.66%). Hence, Kolajoha3 and IC463254 can be further utilized in breeding programmes for developing nitrogen use efficient rice cultivars.

Acknowledgments

Authors were grateful to Indian Council of Agriculture Research (ICAR), Indian Institute of Rice Research (ICAR-IIRR) and Global Challenges Research Fund - South Asian Nitrogen Hub (GCRF-SANH) for their financial support and facilities for research.

Competing interests

Authors have declared that no competing interests exist.

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