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Aus rice: a source of promising donor genotypes for low N tolerance under field conditions

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Excess nitrogen (N) fertilizer application is posing a serious concern on sustainable development of the earth's environment. Although, several studies aim reduction of N fertilizer input rate in rice, the target is yet to be achieved. Exploration of genetic diversity and identification of tolerant genotypes to low N is one of the promising strategies to achieve nitrogen use efficiency (NUE) in rice. Aus sub population is reported to possess unique stress tolerance traits, an underutilized resource for NUE. Hence, in present study, we screened the Bengal and Assam Aus Panel (BAAP) under three graded N levels (0, 50 and 100 kg N ha^{-1}) for two wet seasons (2021 and 2022) and one dry season (2022). Data was recorded for seven traits viz., single plant grain yield (SPGY), single plant biomass yield (SPBY), single plant straw yield (SPSY), plant height (PH), tiller number (TN), SPAD chlorophyll meter readings (SCMR) and days to 50% flowering (DFF). Existence of wide genotypic variability was noticed not only for SPGY, but also for SPBY, SPSY, PH, TN, SCMR and DFF. Heat map clustering categorized the low N tolerant genotypes and grouped SPGY, SPBY and SPSY as sensitive traits to low N. M202 noted as highly tolerant to low N in wet season but was intermediate in dry season, indicating variable response of the genotype with season. Improved varieties viz., IR64-21, M202, BINA Dhan 5, BRRI Dhan 47 found to be tolerant to low N and can be immediately used in NUE breeding programmes.

Keywords Rice, Nitrogen (N), Nitrogen use efficiency (NUE), Single plant grain yield (SPGY), Genotypic variation

Enhanced crop productivity is often attributed to increased global nitrogen (N) fertilizer application[1–4]. Excess N fertilizer application is reported to contribute not only to the climate change, air and water pollution and but also to the decline of the nitrogen use efficiency (NUE) in agriculture[5–7]. Around 50% of global N fertilizer is applied to major cereals: wheat-18%, maize-17% and rice-16%[8,9]. Only 33% N of the total N fertilizer applied translates into grain in global cereal production[10–12]. Rice is a staple food crop more than half of the world's population and around 16% of global N fertilizer is applied to rice[8,9]. However, only 30 to 50% N of the total applied N fertilizer is being utilized by rice and the remaining applied fertilizer N is lost through surface runoff, leaching, volatilization, and denitrification[12–15]. Nitrous oxide (N_2O) is about 270 times potent green house gas than carbondioxide and N_2O emissions from rice fields are suggested to be linked to environmental pollution (www.unep.org). It is estimated that improving crop NUE just by 1% globally will save about $1.1 billion annually in fertilizer cost[16]. While 4R principles of nutrient management as right source, right rate of quantity, right time and right place of application for improved NUE in rice are being studied, genetic solutions for enhancing NUE in rice are also being researched[17–21]. With the crucial role of N manangement in cereal-based systems, a new sustainable development goal (SDG) "halve nitrogen waste" is also being proposed[22,23].

NUE is a complex trait, influenced by various morphological, physiological, biochemical, and molecular processes[24,25]. It is defined as the grain yield produced per unit of soil available N and depends on uptake and remobilization of N in the plant[26,27]. Strong association of grain yield with other agro -physiological traits is critical to identify low N tolerant and high yielding genotypes at graded N levels[28,29]. Greater N recovery from

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the soil under reduced N fertilizer input without compromising the yield can be achieved by breeding genotypes with high NUE traits[30]. Since late 1990's, the genotypic variation of NUE was investigated in various sets of rice genotypes[17,18,31–33]. Several studies reported the evaluation of NUE in a single to 180 genotypes (mostly released varieties/breeding lines) of rice under graded levels of N for understanding the association of agro -physiological traits with grain yield/NUE[28,34–36]. NUE varies with season and noted to be high in dry season compared to wet season which could be attributed to more sunshine hours leading to efficient utilization of absorbed N[37,38]. Evaluation of the vast and unutilized rice germplasm for their possible genetic potential could be a promising approach for identifying genotypes with NUE[39]. Nevertheless, efforts for screening of large-scale germplasm accessions in the field for NUE were scarce because there were no conscious breeding programs aimed at NUE in rice. With the advent of genome wide association studies, hundreds of rice genotypes are evaluated for their NUE through either hydroponics or graded N levels at field conditions and promising donors are being identified[38,40,41].

Aus sub population, one of the five rice sub populations, is photoperiod sensitive and believed to own unique stress tolerance traits such as drought, cold, heat, submergence tolerance, low P tolerance and other traits[42–45]. Several important private alleles for grain quality, domestication and plant height in aus sub population of the 3,000 Rice Genome Project (3 K-RGP) accessions were also reported[46]. Aus population was shown to possess positive allele for tillering in natural habitat with low N content[47]. Based on the genetic diversity and special geographic coverage, a rice association-mapping panel mostly comprising aus accessions was developed known as the Bengal and Assam Aus Panel (BAAP)[44,48]. The BAAP has been evaluated for salt tolerance, performance under alternate and wet drying conditions, grain mineral/cation content and mineral deficiency conditions[48–54]. Promising alleles for early root vigour traits under dry direct seeded low nitrogen condition were also identified in BAAP[55].

Though several researchers reported wide genotypic variations for NUE under reduced levels of N application, understanding of grain yield and its allied NUE traits is yet to be elucidated in rice[56]. Low N tolerant genotypes are defined as genotypes with equivalent grain yield under reduced N input compared with recommended N[57]. Of the various traits reported for NUE of rice, we selected seven viz., days to 50% flowering (DFF) (a phenology trait), plant height (PH) and tiller number (TN) (morphological or architectural traits), SPAD chlorophyll meter readings (SCMR) (physiological trait), single plant grain yield (SPGY), single plant biomass yield (SPBY) and single plant straw yield (SPSY) to characterize BAAP genotypes for their NUE.

Flower development and booting was significantly affected with N fertilizer application[58]. Flowering time and NUE is mediated by N via floral regulators in rice[59]. N fertilizer application lead to the elongation of various internodes, particularly the basal internodes, resulting in increased plant height[60]. The productive tiller number is mainly influenced by soil N availability during the early phases of tiller bud formation[61] and is crucial for the grain formation and the increase in filled grains percentage[62]. SCMR serve for quick in situ estimation of leaf chlorophyll content and N is critical element to chlorophyll, SCMR value indicates the N content or its availability. Chlorophyll content is always linked with plant metabolism and directly reflects the leaf N content[63]. Chlorophyll in leaf which ultimately influences the photochemistry is the marker to identify genotypes with high NUE under low N field conditions[64–66]. In the past four decades, SPAD was widely used to monitor rice N status at various growth stages to judge crop N demand and thereby to enhance grain yield and NUE[67–69]. Grain yield increases with N fertilizer application and is strongly correlated with total plant N content[17]. Grain yield and biomass accumulation enhanced with increased N application[56,70,71].

Several studies have identified genes that play key role in low N tolerance and NUE in rice[72–74]. Many transporter genes associated with uptake and remobilization viz., *NRT1.1B*, *OsNR2*, *OsNRT2.3b*, *OsNPF3.1* were reported in rice[75–77]. In addition, regulatory genes such as *DNR1* (auxin balance), *GRF4* (coordination of nitrogen–carbon metabolism) and *NGR5* (N-responsive tillering) play crucial role in maintaining yield under low N conditions[78–80]. Association mapping and gene editing approaches are also deployed to study the genes associated with NUE in rice[81,82].

The present study targeted to identify low N tolerant genotypes by exploring genotypic variation in NUE across Bengal and Assam Aus Panel (BAAP) genotypes under two major rice growing seasons viz., wet and dry, and to characterise seven traits under reduced N levels. Genotypes with high relative values of measured traits under reduced N application over recommended N can be utilized in rice NUE breeding programmes.

Materials and methods

Experimental site description

Field evaluation was carried out at the ICAR-Indian Institute of Rice Research (IIRR), Hyderabad, India, for three consecutive seasons viz., wet season-2021, dry season-2022 and wet season-2022. A set of Bengal and Assam Aus Panel (BAAP) genotypes were screened under three graded levels of N fertilizer application viz., 100% recommended dose of N fertilizer (RDN) (100 kg N ha^{-1} - will be referred as N100), 50% RDN (50 kg N ha^{-1} - will be referred as N50) and 0% RDN (0 kg N ha^{-1} - will be referred as N0). The experimental field plots were maintained as RDN, 50% RDN and 0% RDN treatments from the past ten years[64]. Soil samples were collected from the three field plots before initiating the experiments in wet season 2021, dry season 2022 and wet season 2022 to determine physico-chemical properties. The details of soil properties of the three N field plots (N0, N50 and N100) were given in Table 1. Across three N field plots and three seasons, the experimental field has clay soil and is moderately alkaline (pH ranged from 8.15 to 8.46) and non-saline (electronic conductivity ranged from 0.72 dS/M to 0.79 dS/M). The organic carbon in soil was medium (ranged from 0.55% to 0.72%), available nitrogen was low (ranged from 174 kg N ha^{-1} to 251 kg N ha^{-1}) and available phosphorus (ranged from 82 kg P_2O_5 ha^{-1} to 92 kg P_2O_5 ha^{-1}) as well as available potassium (ranged from 606 kg K_2O ha^{-1} to 681 kg K_2O ha^{-1}) were high.

Season	Soil property	N0	N50	N100
Wet season	pH	8.18	8.36	8.34
2021	Electronic conductivity (dS/M)	0.75	0.78	0.78
	Organic carbon (%)	0.55	0.58	0.66
	Available N (kg/ha)	181	220	246
	Available P_2O_5 (kg/ha)	85	86	91
	Available K_2O (kg/ha)	625	646	678
Dry season	pH	8.22	8.31	8.38
2022	Electronic conductivity (dS/M)	0.72	0.75	0.77
	Organic carbon (%)	0.59	0.66	0.69
	Available N (kg/ha)	176	215	251
	Available P_2O_5 (kg/ha)	82	85	88
	Available K_2O (kg/ha)	606	658	669
Wet season	pH	8.15	8.33	8.46
2022	Electronic conductivity (dS/M)	0.76	0.78	0.79
	Organic carbon (%)	0.61	0.68	0.72
	Available N (kg/ha)	174	211	242
	Available P_2O_5 (kg/ha)	84	92	89
	Available K_2O (kg/ha)	633	669	681

Table 1. Soil properties of three N level fields before beginning of experiment in each season.

Meteorological data

The weather parameters noted throughout the crop growth are presented in Fig. S1. In wet season 2021, mean maximum and mean minimum temperatures were 30.3 °C and 22.1 °C. The mean maximum relative humidity is 92.1% and mean minimum relative humidity is 67.3%. A sum of 842.0 mm rainfall was received with an average bright sunshine of 4.9 h day^{-1}. In dry season 2022, mean maximum and mean minimum temperature were 33.4 °C and 18.7 °C. The mean maximum and mean minimum relative humidity were 83.4% and 45.1%. A total of 17.6 mm rainfall was received with an average bright sunshine of 7.6 h day^{-1}. In wet season 2022, mean maximum and mean minimum temperature were 30.5 °C and 21.4 °C. The mean maximum and mean minimum relative humidity were 88.1% and 60.0%. A sum of 948.6 mm rainfall was received with an average bright sunshine of 5.4 h day^{-1}.

Plant material, design and data recorded

The plant material of the present study consisted Bengal and Assam Aus Panel (BAAP) comprising of 266 aus rice accessions, 19 of the OryzaSNP set and a few breeding lines and released varieties from Bangladesh. The ~2 million SNP dataset was generated from the panel[44,48]. The SNP dataset provides the genetic resolution necessary to link observed phenotypic variation in NUE traits to underlying allelic diversity, ensuring biological relevance, statistical robustness, and translational value for rice improvement under low N conditions. A set of 205 genotypes during wet season 2021, 233 genotypes during dry season 2022 and 238 genotypes during wet season 2022 from BAAP were evaluated under the present study. The list of 201 common genotypes screened in the study across three seasons were given in Table S1.

Sowing of seeds on nursery beds was done in June and 25 days old seedlings were transplanted in the main field in July during wet seasons and sowing was done in December and transplanted in January during dry season. Experiments followed split-plot alpha-lattice design with four replications in N50 and N100 and two replications in N0 (because of the limited field space in N0 plot). Each genotype was grown with a row spacing of 20 cm and intra-row spacing of 15 cm in one m^2 plot for each replication. N was supplied as urea to N100 (100 kg N ha^{-1}) and N50 plots (50 kg N ha^{-1}) in three equal doses at basal, maximum vegetative and panicle initiation stages. No external N was applied for N0 plot. Phosphorus (P) and potassium (K) fertilizers were applied as per recommendation (@ 60 kg P_2O_5 ha^{-1} and 40 kg K_2O ha^{-1} as basal dose). Standard package of practices were followed to raise healthy crop.

Plant height (PH) was noted from the stem base to the topmost leaf or panicle with a ruler (marked for every 0.1 cm up to 100 cm) for five random plants in each genotype at maturity. The tiller number in each genotype was counted manually at maturity for five random plants and the average was expressed as tiller number per plant (TN). The duration taken by each genotype to attain flowering in half of plants at each N level was recorded as days to 50% flowering (DFF). The SPAD chlorophyll meter readings (SCMR) indicating relative chlorophyll content of leaves were measured at 50% flowering with SPAD 502 (Minolta Company Ltd, Japan). SCMR was measured for ten leaves in each genotype from midway between the leaf base and tip of the third leaf from top. At physiological maturity, ten plants were harvested manually at 5 cm above the soil from the center of the plot. Grain weight was recorded using electronic balance (Sartorius, Germany) after drying to moisture content of 14% and converted to single plant grain yield (SPGY) and straw weight was also noted for the same, expressed as single plant straw yield (SPSY)[83]. Single plant biomass yield (SPBY) was determined as sum of SPGY and SPSY[84].

Statistical analysis

Three-way analysis of variance (ANOVA) was conducted with R software using Agricolae package. The graphs were prepared in R software using various packages. Relative values of seven traits viz., SPGY, SPBY, SPSY, PH, TN, SCMR and DFF at N0 over N100 and at N50 over N100 were used to develop heat maps for wet and dry seasons based on the Euclidean squared distance metric and the variations were reflected by a color change gradient. Heatmaps were developed using the gplots, dendextend and ComplexHeatmap packages. The genotypes in the heat map were categorized into three major groups with descending relative values and categorized as tolerant to low N, intermediate and sensitive to low N. Figures 2A and 3D and Fig. S1 were drawn using ggplot2, scales and ggrepel packages. Based on relative reduction in SPGY under N0 compared with N100 and under N50 compared with N100, the top 25 low N tolerant and bottom 25 low N sensitive grain yielding genotypes were selected[57]. To determine the N efficiency of genotypes under available soil N and responsiveness of genotypes to applied N, mean values of SPGY were used to group the genotypes into four quadrants viz., (i) N efficient and responsive, (ii) N efficient and non-responsive, (iii) N inefficient and responsive, and (iv) N inefficient and non-responsive[85]. At N0, genotypes above mean were categorized as efficient and below mean were categorized as inefficient. Likewise, at N50 and N100, genotypes above mean were categorized as responders and below mean were categorized as non-responders. PCA was performed using the "factoextra" and "FactoMineR" R packages[86], which generate uncorrelated principal components from the set of correlated original variables. The R packages "agricolae," "lme4," "lmerTest," and "ggplot2" were employed for estimating genetic parameters, including the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, and genetic advance as per cent of mean (GAM)[87,88].

The variations for the seven traits of the study were also analysed as per the five genetic groups of BAAP as described by Norton et al.[48] and geographical adaptation viz., India and Bangladesh. Mean vs. Stability views of GGE biplot were performed using the metan package.

Results

The observations of the evaluation of BAAP genotypes across two wet and one dry seasons under three graded N levels (N100, N50 and N0) were presented as (i) analysis of variance, (ii) descriptive statistics along with the percentage reduction of seven traits under N0 and N50 in comparison to N100, (iii) Heat map of relative values of seven traits, (iv) identification of tolerant and sensitive genotypes to low N tolerance based on relative reduction of SPGY, (v) identification of N efficient/in-efficient/responsive/non-responsive genotypes and (vi) Mean vs. Stability views of GGE biplot to identify stable genotypes across graded N levels.

Analysis of variance

Pooled analysis of variance (Table 2) was performed for 201 genotypes, which were common across the three seasons and for seven agro-physiological and grain yield traits. Three way ANOVA clearly indicated significant differences among the seasons (S), treatments (T), genotypes (G) and their interactions (S × T; S × G; T × G; S × T × G) for SPGY, SPBY, SPSY and TN. Significant differences were not observed for PH, DFF and SCMR

		Single plant grain yield				Single plant biomass yield				Single plant straw yield				Plant height			
Effect	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Sign**	**Sum Sq**	**Mean Sq**	**F value**	**Sign**	**Sum Sq**	**Mean Sq**	**F value**	**Sign**	**Sum Sq**	**Mean Sq**	**F value**	**Sign**
Season (S)	2	40,939	20,469	2272	***	49,740	24,870	626.7	***	9411	4705	282.0	***	314,629	157,315	1750	***
Treatment (T)	2	151,301	75,651	8398	***	648,908	324,454	8176	***	173,983	86,992	5213	***	689,134	344,567	3834	***
Genotype (G)	200	45,406	227	25.2	***	294,459	1472	37.1	***	119,274	596	35.74	***	584,580	2923	32.5	***
S × T	4	10,742	2685	298.1	***	13,000	3250	81.9	***	435	109	6.51	***	4812	1203	13.3	***
S × G	400	30,055	75	8.34	***	131,782	329	8.30	***	47,943	120	7.18	***	162,313	406	4.51	***
T × G	400	10,123	25	2.80	***	60,186	150	3.79	***	25,664	64	3.84	***	36,863	92	1.02	
S × T × G	800	10,395	13	1.44	***	52,150	65	1.64	***	25,014	31	1.87	***	62,972	79	0.87	
Residuals	4221	38,021	9			167,494	40			70,428	17			379,318	90		
		Tiller number per plant				Days to 50% flowering				SPAD chlorophyll meter reading							
Effect	Df	Sum Sq	Mean Sq	F value	Sign	Sum Sq	Mean Sq	F value	Sign	Sum Sq	Mean Sq	F value	Sign				
Season (S)	2	15,000	7500	2221	***	849,486	424,743	14,766	***	62	31	4.46	*				
Treatment (T)	2	30,176	15,088	4469	***	36,536	18,268	635.1	***	56,576	28,288	4046	***				
Genotype (G)	200	21,541	108	31.9	***	39,606	198	6.88	***	9090	45	6.50	***				
S × T	4	2724	681	201.6	***	483	121	4.19	**	651	163	23.2	***				
S × G	400	7019	18	5.19	***	31,308	78	2.72	***	2849	7	1.01					
T × G	400	2380	6	1.76	***	2736	7	0.23		4769	12	1.70	***				
S × T × G	800	3212	4	1.18	***	6445	8	0.28		3147	4	0.56					
Residuals	4221	14,250	3			121,410	29			29,506	7						

Table 2. Analysis of variance for agro-physiological traits and grain yield of 201 BAAP genotypes for three seasons (wet season-2021, dry season-2022 and wet season-2022). Sign. codes: ***$p \leq 0.001$, **-$p \leq 0.01$, *-$p \leq 0.05$.

among S × T × G. For PH and DFF, T × G interaction was not significant and for SCMR, S × G interaction was not significant.

Agro-physiological traits and grain yield and their relative reduction

Data of two wet seasons (2021 and 2022) was averaged for seven traits (SPGY, SPBY, SPSY, PH, TN, SCMR and DFF) in the three graded N levels. Descriptive statistics of the data (Table 3) showed that SPGY ranged from 9.4 g (AUS 89) to 29.9 g (FR13A) with mean of 17.2 g at N100, 6.2 g (Boraya) to 22.0 g (FR13A) with mean of 12.5 g at N50 and 1.1 g (AUS 366) to 12.5 g (FR13A) with mean of 5.4 g at N0. FR13A recorded highest SPGY, SPBY and SPSY at three graded N levels. Pokkali noted highest PH and DA24 noted highest TN at three graded N levels. Lali Boro at N0, AUS 366 at N50 and BRRI dhan47 at N100 showed maximum SCMR. Aswina at N0, FR13A at N50 as well as at N100 noted maximum DFF. Mean reduction percentage of the seven recorded traits at N0 and N50, compared with N100 was given in Table S2.

Descriptive data analysis of dry season (Table 4) showed that SPGY ranged from 13.2 g (DV 123) to 36.2 g (Jhona 349) with mean of 23.4 g at N100, 6.4 g (Azucena) to 29.7 g (Saita Boro) with mean of 17.5 g at N50 and 1.0 g (AUS 385) to 13.0 g (Bina Dhan5) with mean of 5.9 g at N0. Lali Boro at N0, Lafai at N50 as well as at N100 recorded highest SPBY. Lali Boro at N0, Dhaliboro 111-3 at N50 and Lafai at N100 noted highest SPSY. Pokkali noted highest PH at three graded N levels. Lali Boro at N0, Lafai at N50 and Shada Boro at N100 noted maximum TN. Azucena at N0, BRRI dhan47 at N50 and BR 3 at N100 recorded maximum SCMR. FR13A at N0, Chhola boro (2) at N50 as well as at N100 exhibited maximum DFF.

Heat map clustering analysis

In N0 over N100 heat map of wet season (mean of two wet seasons) with seven traits (Fig. 1A), the first group included only one genotype viz., M202, categorized as highly tolerant. The 25 genotypes were clustered into the intermediate group. The third group included 175 low N sensitive genotypes. In N50 over N100 heat map of wet season (mean of two wet seasons) with seven traits (Fig. 1B), the first group included 142 low N tolerant genotypes among which Chhola Boro, AUS 151, Chhola Boro (2), Kal Buri, DV 118, Kalo Bora, M202, Kanchanoni and Azucena were in top sub cluster and can be considered as highly tolerant. The second group included five genotypes which can be categorized as intermediate. The third group included 54 low N sensitive genotypes. Based on clustering of seven traits, two distinct groups were noted in N0 over N100 heat map indicating that SPGY, TN, SPBY and SPSY are highly sensitive to reduced N application. DFF, PH and SCMR were clustered together and observed to be less sensitive to reduced N application. But, in N50 over N100 heat map, TN is clustered along with DFF, PH and SCMR and noted to be less sensitive to reduced N application, while SPGY, SPBY and SPSY were clustered together and noted to be highly sensitive to reduced N application.

In N0 over N100 heat map of dry season across seven traits (Fig. 1C), the first group included 40 low N tolerant genotypes among which Dubhi Gora, AUS 93, W418, Lali Boro, CN2-175-5-31, Boro and AUS 411 were in top sub cluster and highly tolerant to low N. 100 genotypes were clustered into the second group which can be considered as intermediate. The third group included 61 low N sensitive genotypes. In N50 over N100 heat map of dry season across seven traits (Fig. 1D), the first group included 149 low N tolerant genotypes in which

Trait	Mean	Minimum	Maximum	Range	Median	Mode	Standard error	Standard deviation	Sample variance	Kurtosis	Skewness
SPGY_N0	5.4	1.1	12.5	11.4	5.1	NA	0.13	1.9	3.5	1.23	0.78
SPGY_N50	12.5	6.2	22.0	15.8	12.5	NA	0.21	2.9	8.5	− 0.22	0.26
SPGY_N100	17.2	9.4	29.9	20.5	16.6	16.0	0.27	3.8	14.7	− 0.24	0.44
SPBY_N0	13.6	4.8	34.4	29.6	13.2	14.4	0.31	4.4	19.6	2.19	0.95
SPBY_N50	30.4	14.6	58.8	44.2	29.2	26.8	0.52	7.4	55.0	0.41	0.64
SPBY_N100	40.1	22.1	74.4	52.3	37.8	31.5	0.70	10.0	99.8	− 0.14	0.64
SPSY_N0	8.2	3.7	21.9	18.2	7.7	6.8	0.20	2.8	7.9	3.38	1.23
SPSY_N50	17.9	8.1	36.8	28.8	17.1	NA	0.35	5.0	24.8	0.94	0.96
SPSY_N100	22.9	12.2	44.5	32.3	21.3	NA	0.46	6.5	41.9	0.03	0.79
PH_N0	98.3	69.3	128.8	59.5	98.1	94.8	0.68	9.6	91.7	0.38	0.07
PH_N50	117.1	80.8	159.6	78.9	116.9	110.1	0.74	10.5	110.6	1.76	0.19
PH_N100	126.1	88.4	167.6	79.2	125.3	124.4	0.76	10.8	116.4	1.50	0.16
TN_N0	6.0	3.1	10.5	7.4	5.9	5.5	0.10	1.4	1.8	0.37	0.66
TN_N50	9.3	6.1	15.7	9.6	9.0	9.0	0.13	1.8	3.3	0.28	0.72
TN_N100	10.9	7.4	18.3	10.8	10.5	9.8	0.15	2.1	4.5	0.09	0.69
SCMR_N0	37.4	31.5	41.8	10.3	37.3	36.8	0.16	2.2	4.9	− 0.33	− 0.35
SCMR_N50	42.7	39.0	46.3	7.3	42.7	43.4	0.09	1.2	1.6	0.43	− 0.07
SCMR_N100	46.0	42.8	49.7	6.9	45.9	48.3	0.09	1.2	1.5	0.28	0.25
DFF_N0	100.1	89.8	108.5	18.8	100.0	101.5	0.21	3.0	9.0	0.80	− 0.22
DFF_N50	104.6	98.5	114.1	15.6	104.4	103.9	0.17	2.3	5.5	1.33	0.63
DFF_N100	106.7	99.5	121.5	22.0	106.4	106.3	0.18	2.6	6.5	5.43	1.22

Table 3. Descriptive analysis for wet season data (mean of two wet seasons).

Trait	Mean	Minimum	Maximum	Range	Median	Mode	Standard Error	Standard Deviation	Sample Variance	Kurtosis	Skewness
SPGY_N0	5.9	1.0	13.0	12.0	5.6	4.0	0.17	2.3	5.5	− 0.10	0.40
SPGY_N50	17.5	6.4	29.7	23.3	17.1	13.8	0.28	4.0	16.3	0.01	0.45
SPGY_N100	23.4	13.2	36.2	23.0	22.6	26.7	0.37	5.2	27.2	− 0.53	0.50
SPBY_N0	15.2	5.1	31.8	26.7	15.1	13.4	0.32	4.5	20.6	0.43	0.34
SPBY_N50	37.5	21.0	60.6	39.5	36.1	35.6	0.64	9.1	83.4	− 0.26	0.67
SPBY_N100	47.3	25.7	85.1	59.4	45.4	40.9	0.84	11.9	141.3	0.05	0.73
SPSY_N0	9.2	2.8	22.3	19.5	9.0	7.2	0.21	3.0	9.0	1.26	0.68
SPSY_N50	20.0	10.7	43.8	33.1	18.8	17.2	0.41	5.9	34.3	1.34	1.09
SPSY_N100	24.0	12.3	49.8	37.5	22.6	23.4	0.49	7.0	48.3	1.00	0.99
PH_N0	85.9	63.0	128.8	65.8	85.5	85.5	0.68	9.6	91.8	1.42	0.57
PH_N50	106.7	83.0	159.5	76.5	104.8	121.9	0.85	12.1	145.5	0.87	0.65
PH_N100	117.9	86.4	172.8	86.4	116.5	117.8	0.97	13.8	190.4	0.39	0.46
TN_N0	6.7	3.8	11.8	8.0	6.5	6.3	0.11	1.6	2.6	− 0.25	0.43
TN_N50	12.6	7.9	20.5	12.6	12.1	10.9	0.20	2.8	7.9	0.03	0.74
TN_N100	15.4	9.4	26.5	17.1	14.8	18.5	0.23	3.3	11.1	0.30	0.73
SCMR_N0	37.6	31.9	42.8	10.9	37.6	37.2	0.15	2.1	4.4	− 0.11	− 0.33
SCMR_N50	42.7	38.1	47.0	9.0	42.7	42.5	0.12	1.6	2.7	0.11	− 0.10
SCMR_N100	45.4	40.6	50.8	10.3	45.4	45.0	0.13	1.8	3.4	0.11	0.09
DFF_N0	111.2	103.5	124.0	20.5	111.0	109.0	0.24	3.5	12.0	0.92	0.67
DFF_N50	116.0	108.0	129.0	21.0	115.5	115.3	0.30	4.2	17.9	0.09	0.64
DFF_N100	118.2	110.3	130.8	20.5	117.8	117.8	0.32	4.5	20.0	− 0.06	0.65

Table 4. Descriptive analysis for dry season data.

Brown Gora S.B. 92, Dula Aus, Dubhi Gora, Kasalath, AUS 362 and BRRI Dhan47 were in top sub cluster and highly tolerant to low N. The second group included two genotypes with intermediate response and the third group included 50 low N sensitive genotypes. In N0 over N100 and N50 over N100 heat maps, SPGY, TN, SPBY and SPSY were clustered together and found to be highly sensitive to reduced N application. DFF, PH and SCMR were clustered together and observed to be less sensitive to reduced N application.

Only common genotype viz., M202 was observed in N0 over N100, N50 over N100 in wet seasons and N50 over N100 dry season (Table S3).

Low N tolerant and low N sensitive genotypes

As SPGY is the most critical trait, based on relative reduction in SPGY under N0 and N50 compared to N100, top 25 low N tolerant and bottom 25 low N sensitive genotypes were identified in wet and dry seasons[57]. In wet season (mean of two wet seasons), among the 25 low N tolerant genotypes at N0, six genotypes (AUS 382, Azucena, BRRI Dhan47, IR 64-21, M202 and Padma Sail) were common with 25 low N tolerant genotypes at N50 (Fig. 2A). Among the 25 low N sensitive genotypes at N0, nine genotypes were common with 25 low N sensitive genotypes at N50 (Fig. 2B). In dry season, among the 25 low N tolerant genotypes at N0, five genotypes (Deshi Boro, DV 123, FR13A, IR 64-21 and Padma Sail) were common with 25 low N tolerant genotypes at N50 (Fig. 2C). Among the 25 low N sensitive genotypes at N0, six genotypes were common with 25 low N sensitive genotypes at N50 (Fig. 2D). IR 64-21 and Padma Sail were the common low N tolerant genotypes and AUS 169 as common low N sensitive genotype across wet and dry seasons under N0 and N50. Azucena was found to be the low N tolerant genotype in wet season but low N sensitive in dry season across N0 and N50.

Under N0, Deshi Boro and FR13A were common low N tolerant genotypes and AUS 353, AUS 169, ARC 10376 and MTU18 were common low N sensitive genotypes across wet and dry seasons. Several genotypes were found to be low N tolerant in wet season but were low N sensitive under N0 and N50 (Fig. 2A and D).

N efficiency and responsiveness to applied N

In wet season (mean of two wet seasons), SPGY of 201 genotypes at N0 is 5.4 g, at N50 is 12.5 g and at N100 is 17.2 g. For SPGY at N0 vs. SPGY at N50 (Fig. 3A). FR13A (284) followed by Dula Aus (180) and M202 (287) noted as highly N efficient and responsive to applied N. In SPGY at N0 vs. SPGY at N100 (Fig. 3B), FR13A (284) and Dula Aus (180) were highly N efficient and responsive to applied N. AUS 89 (32) and AUS 366 (75) found to be highly N inefficient and non-responsive to applied N. Kalasu (187) and AUS 309 (62) were observed to be highly N inefficient and responsive to applied N, whereas Padma Sail (157) and Balam Aus (93) found to be highly N efficient and non-responsive to applied N.

In dry season, SPGY of 201 genotypes at N0 is 5.9 g, at N50 is 17.5 g and at N100 is 23.4 g. In SPGY at N0 vs. SPGY at N50 (Fig. 3C). Saita Boro (184) followed by Chhola Boro (2) (238), Chaili Boroi (241) and Bina Dhan5 (260) noted as highly N efficient and responsive to applied N. In SPGY at N0 vs. SPGY at N100 (Fig. 3D), Saita Boro (184) followed by Chaili Boroi (241), Chhola Boro (2) (238) and Bina Dhan5 (260) found to be highly N efficient and responsive to applied N.

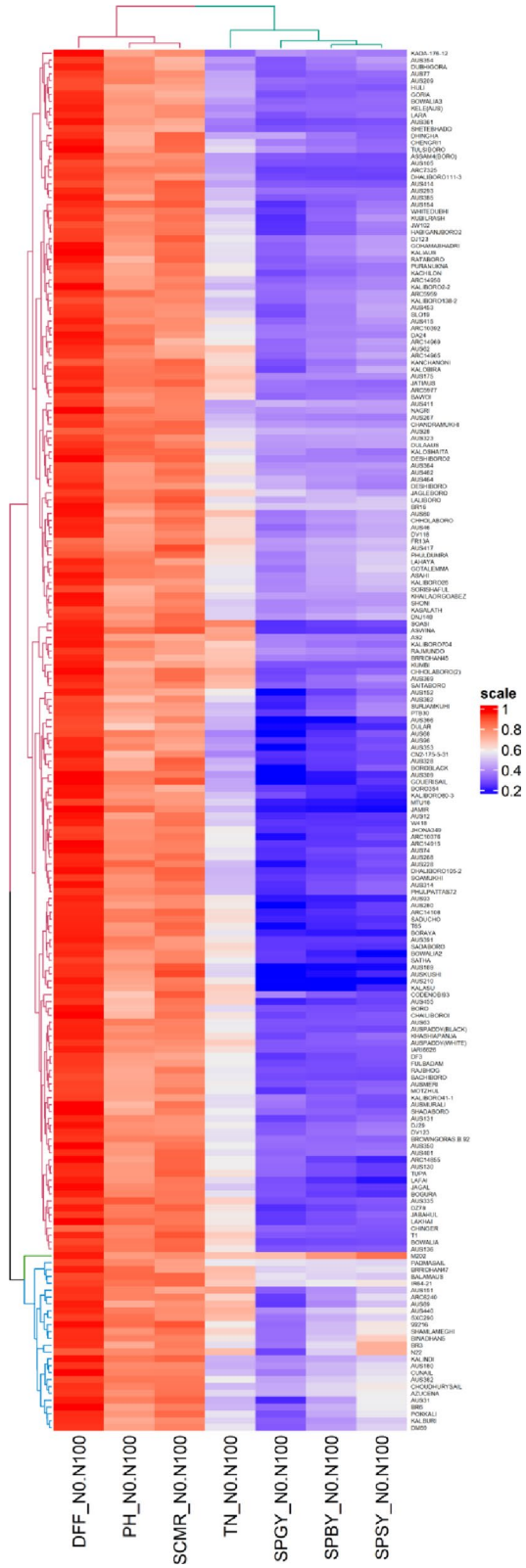

Fig. 1. (**A**) Heatmap clustering for relative values of N0 over N100 in 201 BAAP genotypes for seven traits in wet season (mean of two wet seasons). (**B**) Heatmap clustering for relative values of N50 over N100 in 201 BAAP genotypes for seven traits in wet season (mean of two wet seasons). (**C**) Heatmap clustering for relative values of N0 over N100 in 201 BAAP genotypes for seven traits in dry season. (**D**) Heatmap clustering for relative values of N50 over N100 in 201 BAAP genotypes for seven traits in dry season. DFF, Days to 50% flowering; PH, Plant height; SCMR, SPAD chlorophyll meter reading; TN, Tiller number per plant; SPGY, Single plant grain yield; SPBY, Single plant biomass yield; SPSY, Single plant straw yield.

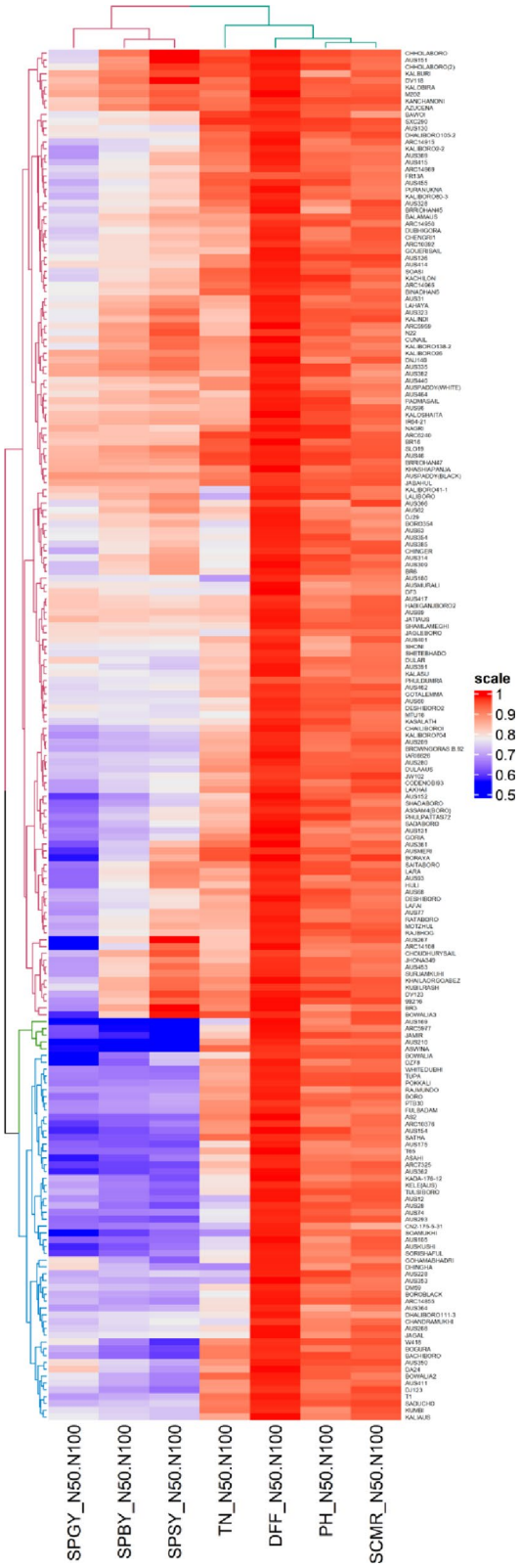

Fig. 1. (continued)

By comparing N efficiency and responsiveness to applied N based on SPGY at N0 vs. N50 and N0 vs. N100 in both wet and dry seasons, 21 N efficient and responsive, 29 N inefficient and non-responsive and five N inefficient and responsive to applied N genotypes were found to be common. None of the N efficient and non-responsive to applied N found to be common (Table S4).

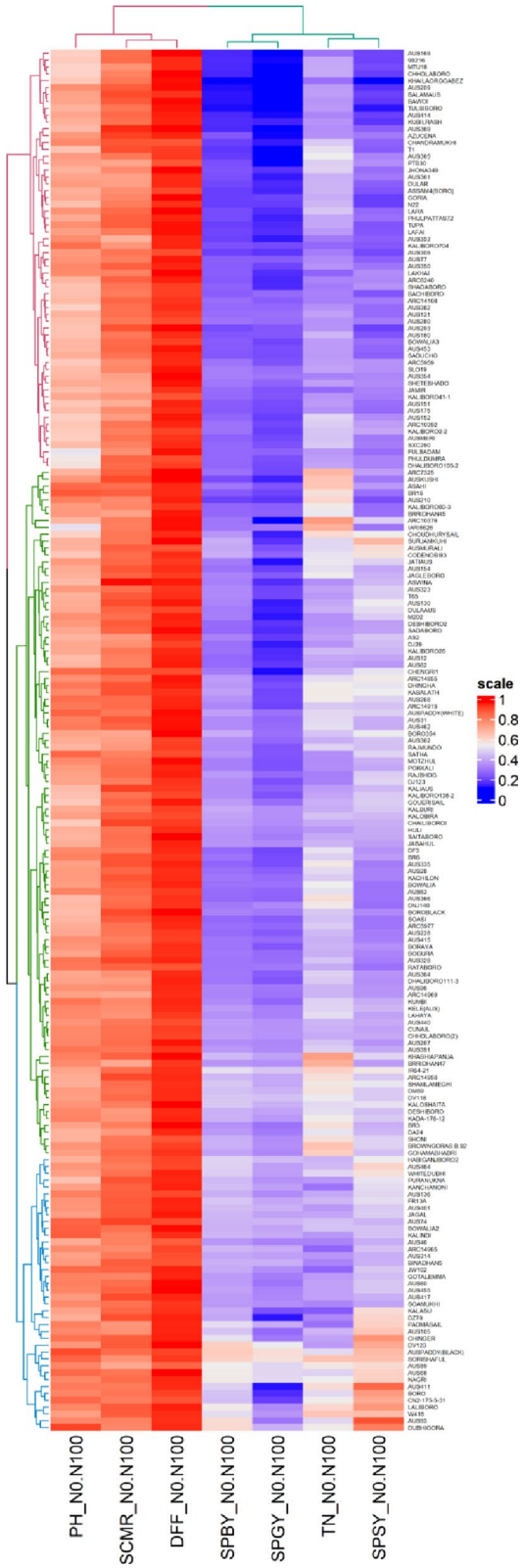

Fig. 1. (continued)

Genetic variability parameters

PCA of wet and dry season's N treatments identified the major axes of variation contributing to trait differentiation. In wet season, for genotypes in N0, the first five PCs (eigenvalues ≥ 1) accounted for 75.3% of the total variance (1st PC- 8.2%, 2nd PC-14.6% and 3rd PC-12.5%). The contributions of specific phenotypic

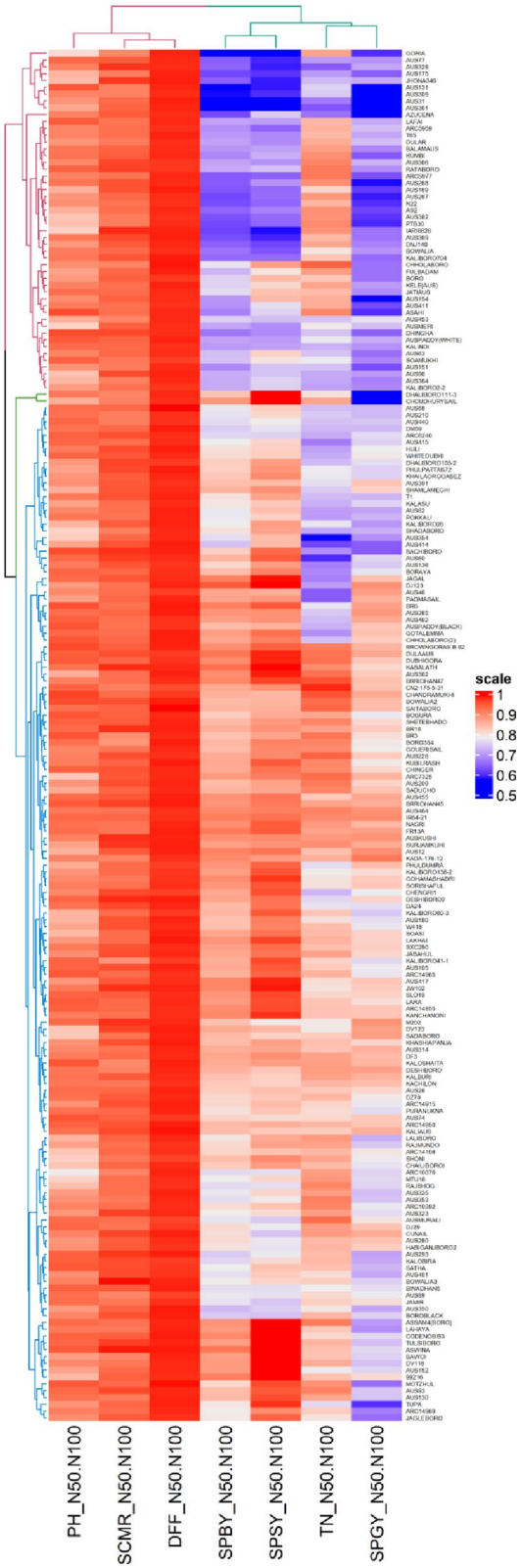

Fig. 1. (continued)

traits to the PCs were noted as SPBY, SPSY and SPGY to PC1 (75.5%), and subsequent PCs dominated by SCMR (PC2 with 59.5%), and SCMR and DFF (PC3 with 83.2%) (Figs. S2, S3 and Table S5). Similarly, for genotypes in N50, the first three PCs explained 82.1% of the variability (1st PC-55.3%, 2nd PC-14.7% and 3rd PC-12.1%) with PC1 (67.8%) strongly influenced by SPBY, SPSY and SPGY, and subsequent PCs dominated by SCMR (PC2

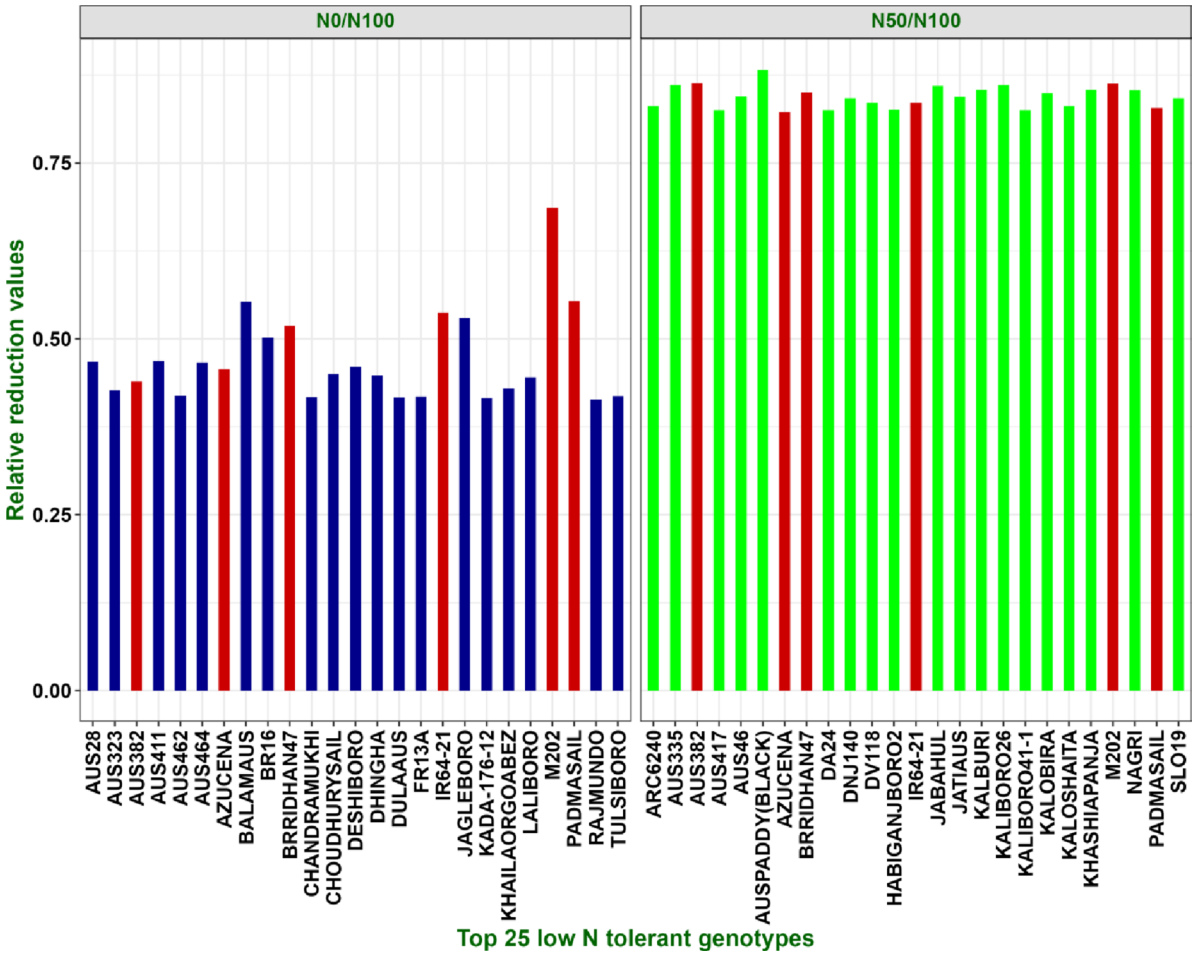

Fig. 2. (**A**) Top 25 low N tolerant genotypes based on relative reduction in SPGY under N0 compared with N100 and N50 compared with N100 in wet season. Dark red colour represents the six common genotypes in N0/N100 and N50/N100. (**B**) Bottom 25 low N sensitive genotypes based on relative reduction in SPGY under N0 compared with N100 and N50 compared with N100 in wet season. Magenta colour represents the nine common genotypes in N0/N100 and N50/N100. (**C**) Top 25 low N tolerant genotypes based on relative reduction in SPGY under N0 compared with N100 and N50 compared with N100 in dry season. Dark red colour represents the five common genotypes in N0/N100 and N50/N100. (**D**) Bottom 25 low N sensitive genotypes based on relative reduction in SPGY under N0 compared with N100 and N50 compared with N100 in dry season. Magenta colour represents the six common genotypes in N0/N100 and N50/N100.

with 85.5%) and DFF (PC3 with 69.9%) (Figs. S4, S5 and Table S6). For genotypes in N100, the first three PCs explained 84.0% of the variability (1st PC-55.8%, 2nd PC-15.7% and 3rd PC-12.5%), with PC1 (70.7%) strongly influenced by SPBY, SPSY and SPGY to PC1; PC2 (93.13%) and PC3 (87.1%) by SCMR and DFF (Figs. S6, S7 and Table S7). PCA across wet season N treatments identified the major contributors to phenotypic variation among genotypes, with three N treatments, the variables of PCA revealed a positive association with SPGY, SPBY, SPSY, PH, TN, SCMR and DFF.

In dry season, for genotypes in N0, the first three PCs (eigenvalues ≥ 1) explained 71.7% of the total variance (1st PC-42.5%, 2nd PC-16.8% and 3rd PC-12.4%), with PC1 and PC2 together accounting for 59.3%, with PC1 mainly driven by SPBY, SPSY and SPGY (73.66%), and subsequent PCs dominated by SCMR and DFF (PC2 with 72.22%) and SCMR and PH (PC3 with 70.93%) (Figs. S8, S9 and Table S8). For genotypes in N50, the first three PCs explained 81.8% of the variation (1st PC-53.4%, 2nd PC-15.9% and 3rd PC-12.5%), with PC1 (68.07%) strongly influenced by SPBY, SPSY and SPGY, whereas DFF and SCMR dominated PC2 (83.77%), and DFF again defined PC3 (77.68%) (Figs. S10, S11 and Table S9). For genotypes in N100, three PCs accounted for 71.2% of the variability (1st PC-55.5%, 2nd PC-16.2% and 3rd PC-12.9%), with PC1 (71.2%) driven by SPBY, SPSY and SPGY, PC2 (95.28%) and PC3 (72.86%) by SCMR and DFF (Figs. S12, S13 and Table S10). The variables of PCA of three N treatments showed a positive association with SPGY, SPBY, SPSY, PH, TN, SCMR and DFF. Overall, yield components consistently dominated the major PCs across N treatments, highlighting their strong contribution to phenotypic variability in dry season.

The genetic variation among the two wet seasons of three N treatments of the seven traits is represented in Table S11. The PCV was found to be higher than the GCV for studied traits. Highest GCV (≥ 20.0) for SPSY

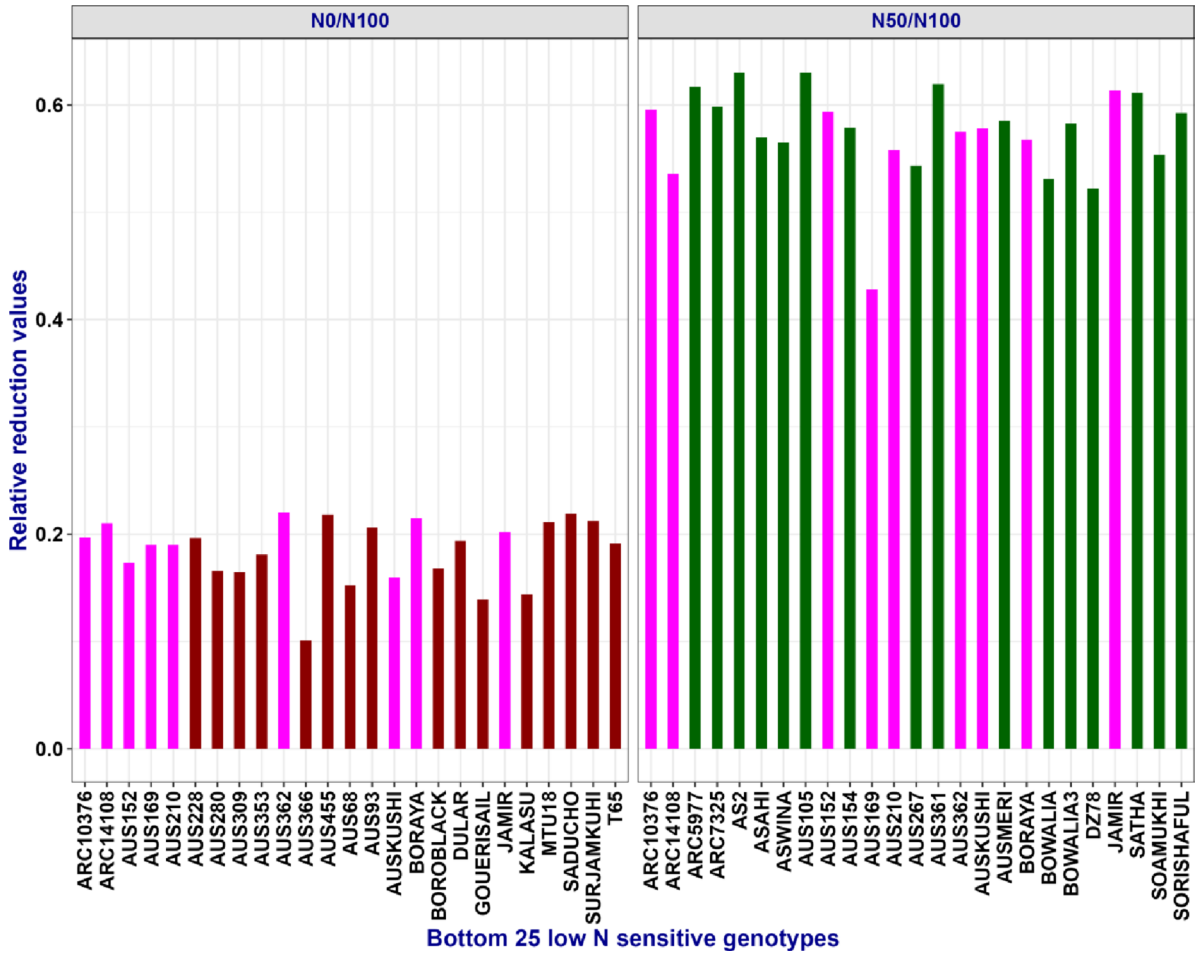

Fig. 2. (continued)

(25.61), SPBY (23.11) and SPGY (21.34), lowest GCV (≤ 3.0) for SCMR (2.26) and DFF (2.18) and the highest PCV (≥ 20.0) for SPSY (32.89), SPBY (29.17), SPGY (28.48) and TN (23.32), lowest PCV (≤ 3.0) for DFF (3.17) and SCMR (5.07) was recorded. The heritability of the wet season treatments of the traits ranged from 19.84 to 68.10 with a mean value of 53.96. Highest heritability (≥ 60.0) values for PH, PN, SPBY and SPSY and the low heritability (≤ 20.0) for the SCMR were noted. The genetic variation among the dry season nitrogen treatments of the traits is represented in Table S12. It was observed that the PCV (Phenotypic Coefficient of Variation) was higher than the GCV (Genotypic Coefficient of Variation) for all the studied traits. Highest GCV (≥ 20.0) for SPSY (25.79), SPBY (22.41), SPGY (20.64) and TN (20.17), lowest GCV (≤ 4.0) for DFF (3.37) and SCMR (3.03) and the highest PCV (≥ 20.0) for SPSY (32.74), SPBY (28.42), SPGY (26.94) and TN (25.33), lowest PCV (≤ 4.0) for SCMR (6.33) and DFF (4.36) were recorded. The heritability of the dry season treatment of the traits ranged from 22.85 to 65.72 with a mean value of 53.71. Highest heritability (≥ 60.0) was observed for PH, NT, SPBY and SPSY and a low heritability (≤ 20.0) observed for the SCMR.

Variations in the genetic groups of BAAP and geographical adaptation

Out of 201 genotypes, 19 genotypes belong to group 1, four genotypes to group 2, 26 genotypes to group 3, 16 genotypes to group 4, 22 genotypes to group 5, 92 genotypes were found to be admixed and 22 genotypes were not grouped[48]. Group 3 performed well for grain yield and other desirable traits among the five groups (Fig. S14). Color coding the PCA for SPGY also revealed group 3 as the most prominent subgroup in wet and dry seasons (Figs. S15 and S16). Relative performance of the desirable traits was promising during the dry season. Significant geographical adaptation patterns were not observed among the genotypes between the two regions (India and Bangladesh) (Fig. S17).

Mean vs. stability views of GGE biplot

In Genotype plus Genotype-vs-Environment interaction (GGE) biplot, position of genotypes were identified in the average environment coordinate (AEC) from the GGE biplot based on their mean and stabilities[89]. The mean vs. stability view of GGE biplot for SPGY in wet season (mean of two wet seasons) explained 95.6% of genotypic and genotype by environment variation (Fig. 4A). As expected, the three N levels (N0, N50 and N100) fell apart from each other in biplot, indicating considerable variation with N application level. FR13A (284) followed by

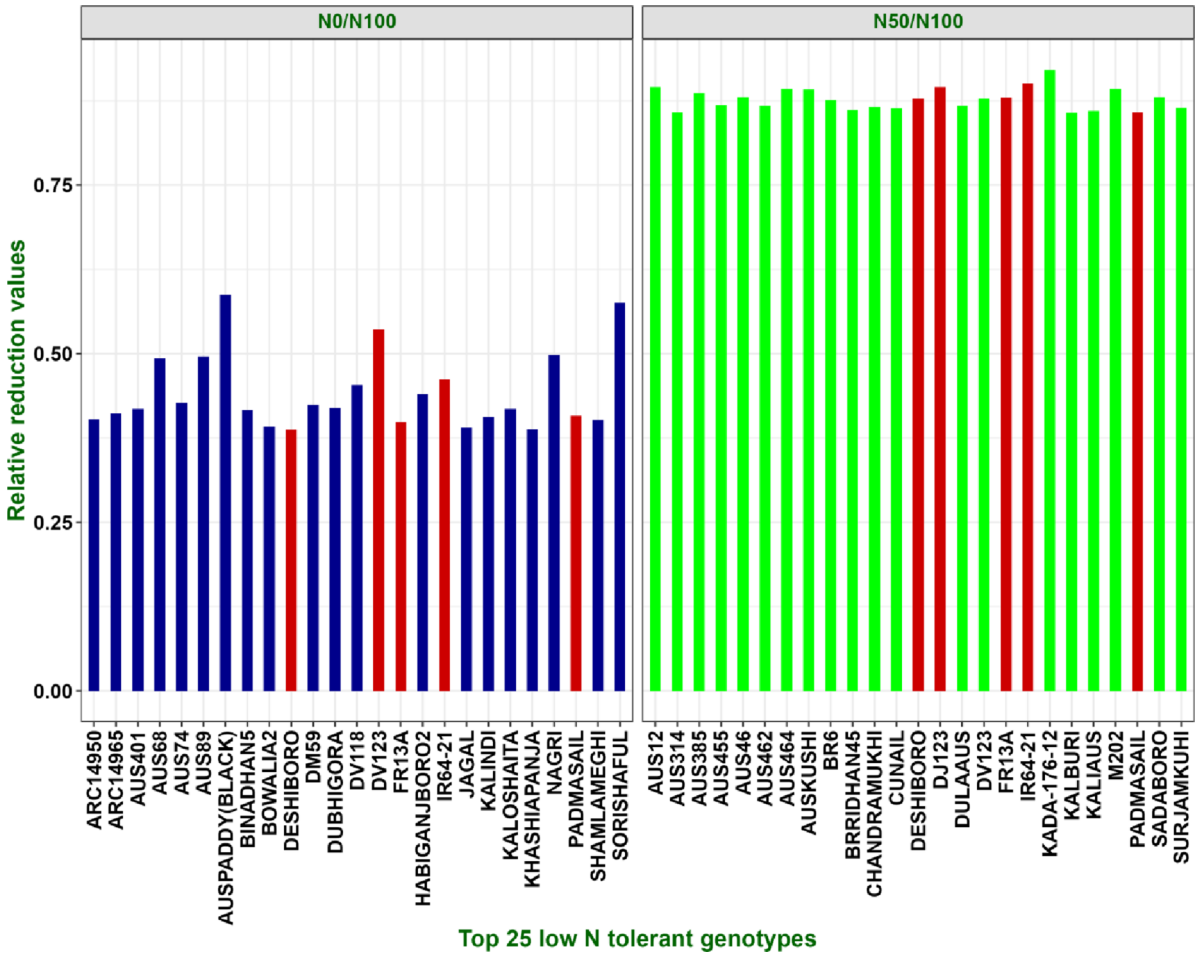

Fig. 2. (continued)

Dula Aus (180), Kasalath (207), Kele (Aus) (122), Chhola Boro (2) (238), Kada-176-12 (168), Dhaliboro 105-2 (133), Pokkali (292), Tupa (236) and Jati Aus (149) noted to be high grain yielders across the graded N levels. The highly stable genotype was placed almost on the AEC ordinate (horizontal axis) and had least projection onto the AEC abscissa (vertical axis). Among the high grain yielders, Kada-176-12 (168) and Jati Aus (149) were stable genotypes and can be considered as ideal genotypes with high grain yield performance and greater stability across the three graded N levels.

The mean vs. stability view of GGE biplot for SPGY in dry season explained 94.1% of genotypic and genotype by environment variation (Fig. 4B). The three N levels (N0, N50 and N100) separated from each other in biplot, indicating considerable variation with N application level. Saita Boro (184), Chhola Boro (2) (238), Chaili Boroi (241), Bogura (233), Jhona 349 (205), Bina Dhan5 (260), Jamir (222), Rata Boro (248), Lafai (239), Bowalia (15), Sada Boro (242) and BRRI Dhan47 (258) noted to be high grain yielders across the graded N levels. Among the high grain yielders, Bina Dhan5 (260) followed by BRRI Dhan47 (258) were most stable genotypes and can be considered as ideal genotypes.

Discussion

The increase in the global food production to ensure the demand of ever-growing population was made possible by extensive N fertilizer application along with N responsive rice varieties[90]. Excess N fertilizer application is leading to adverse effects on environment besides reducing net economic returns to farmers[11,91,92]. The increasing focus on sustainable, green, and climate-smart rice cultivation highlights the urgent need for NUE research. Research in crop management to enhance NUE through effective N source and application methods can be significantly supported by the development of rice varieties with NUE, ultimately lowering N fertilizer requirements. Efforts are underway to integrate NUE into rice breeding programs in several Asian countries. Green Super Rice (GSR) breeding is deploying genomic technology–based selection systems for developing nitrogen use efficient rice varieties. Certification for NUE ranging from 5% to 25% has been made mandatory for GSR varieties in China[93]. In India also, trials on evaluation of breeding material under low nitrogen conditions are being conducted under All India Coordinated Research Project-Rice (AICRP-R) towards development of rice varieties with high NUE (http://www.aicrip-intranet.in). For a successful breeding program, it is essential to explore and deploy the wide genetic variability available for rice germplasm to identify donors for NUE.

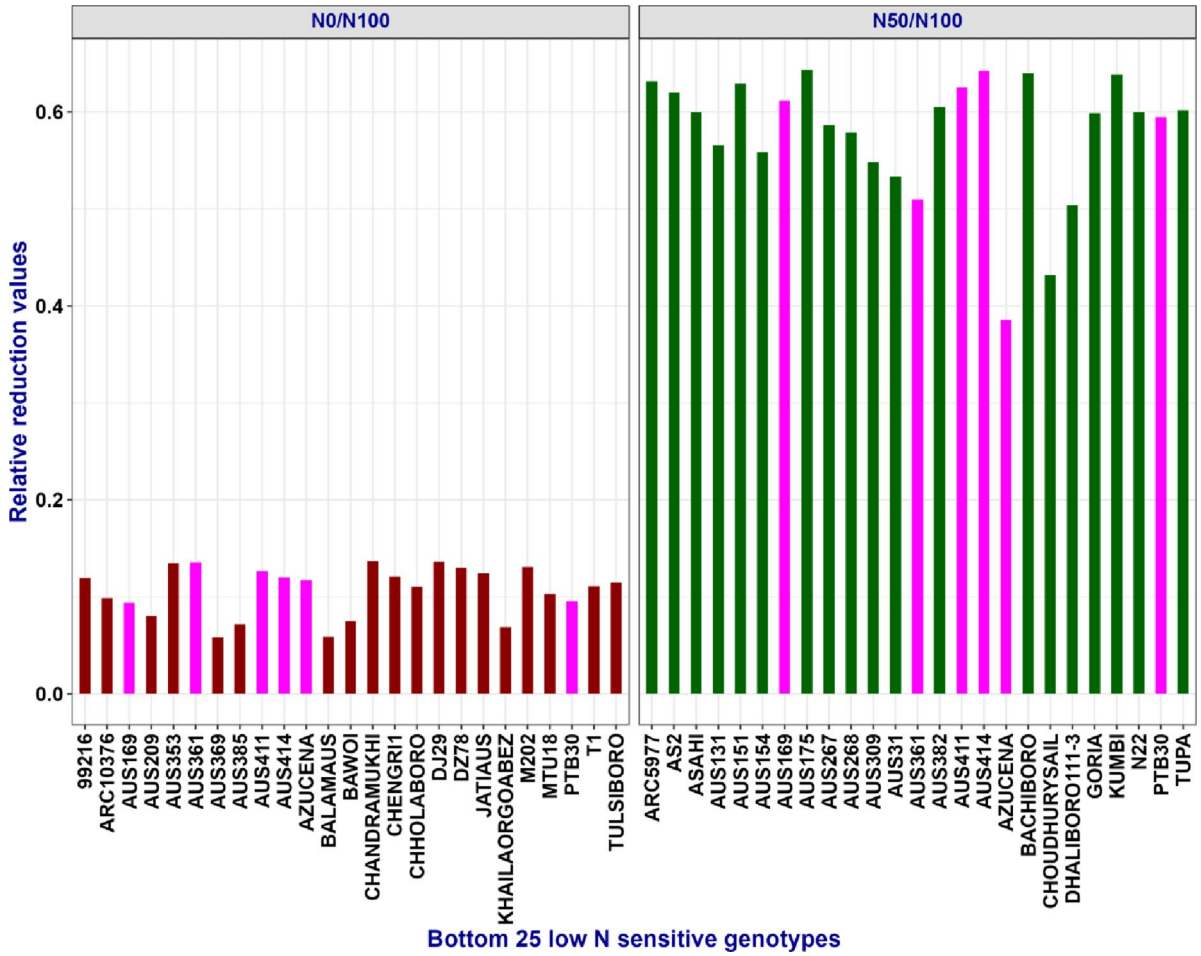

Fig. 2. (continued)

With its unique genetic makeup, aus rice germplasm has provided crucial donor genotypes, contributing significantly to the development of rice varieties with improved tolerance to various abiotic stresses[49,94,95]. The identification of a single donor from aus group for the target trait has proven transformative in rice breeding such as FR13A for submergence tolerance and Kasalath for low phosphorus tolerance[96,97]. With the recent interest in novel genetic resources, several aus genotypes have been characterized for their agro-morphological traits[98], drought tolerance[99], early seedling vigour[100] and NUE[47]. BAAP comprises primarily aus genotypes from environmentally defined and genetically diverse setting, making it ideal for exploring NUE. It is a challenge to perform reliable and precise phenotyping under low N input at field level as it is affected by the genotype (G), environment (E), and G × E interactions[38,64,101]. NUE and related traits were generally evaluated through pot and hydroponic experiments conducted under controlled conditions[102–105]. The assessment of NUE in rice germplasm panels with large number of genotypes generally does not go beyond the seedling stage and a period of 2 years/seasons[106–108]. The BAAP was also characterized for several important traits as described earlier section, but only one study was reported for the evaluation of BAAP under low nitrogen inputs of direct seeded condition[55]. In the present study, we evaluated 201 BAAP genotypes using seven traits viz., SPGY, SPBY, SPSY, PH, TN, SCMR and DFF under three graded N levels in two wet seasons and one dry season at field conditions, to identify genotypes with high NUE.

Variances and interactions in the performance of seven traits under graded N levels

Analysis of variance indicated significant differences among the graded N levels and genotypes for the seven traits of the study. It suggests that environment under graded N levels plays a major role in genotypic variance of traits which are being strongly influenced by the G × E interaction in field corroborating with earlier reports in rice[38,64,109,110], wheat[111] and pearl millet[29]. Previous studies on aus rice also reported significant variation in grain yield, plant height, tiller number, SPAD value and days to flowering with N application[112,113]. Significant interactions of S × T × G along with SPGY, SPSY, SPBY and TN indicate the interdependence and complexity of these four traits. The involvement of tiller number (TN) suggests the possibility of deploying the tiller number also as an indicator for phenotyping of NUE. Based on the highest responsive value of tiller number, it was proposed to be ideal index for phenotyping for genome wide association studies (GWAS) in the study of genomic basis of geographical adaptation to soil nitrogen in rice[47]. For PH and DFF, the effect of N treatments appear to

Fig. 3. (**A**) N efficiency and responsiveness to applied N based on SPGY of 201 BAAP genotypes at N0 and N50 in wet season. The vertical and horizontal lines in the plot represent mean SPGY at N0 and N50, respectively. (**B**) N efficiency and responsiveness to applied N based on SPGY of 201 BAAP genotypes at N0 and N100 in wet season. The vertical and horizontal lines in the plot represent mean SPGY at N0 and N100, respectively. (**C**) N efficiency and responsiveness to applied N based on SPGY of 201 BAAP genotypes at N0 and N50 in dry season. The vertical and horizontal lines in the plot represent mean SPGY at N0 and N50, respectively. (**D**) N efficiency and responsiveness to applied N based on SPGY of 201 BAAP genotypes at N0 and N100 in dry season. The vertical and horizontal lines in the plot represent mean SPGY at N0 and N100, respectively.

be similar regardless of genotypes, so T x G interaction was found to be not significant. Similarly, the differences in SCMR among the genotypes were consistent across seasons, thus the S x G interaction was not significant in the present study.

Descriptive statistics, genetic variabilty and relative reduction of traits under low N

Descriptive analysis of data revealed wide range for SPGY across the three graded N levels in wet and dry seasons, suggesting the wide genotypic variability for grain yield. Earlier studies also revealed genotypic differences for rice grain yield at graded N levels[18,70,114]. Thus, selection of genotypes is crucial to attain the reduction of N fertilizer input for obtaining the yield, due to intrinsic response of genotypes for N supply. More duration of sunshine in dry season resulted in higher mean values of SPGY and SPBY, over wet season.

Fig. 3. (continued)

Increment in SPGY was strongly correlated with rate of N fertilizer application, which could be attributed to increase in plant available N. The results of present study are in accordance with the earlier reports as there was a significant enhancement in grain yield with increase in N fertilizer application and can be attributed to improved tillering, panicle number, and grains[70,114–116]. Earlier studies reported that application of N fertilizer enhanced the biomass and its related traits[117,118]. Likewise, in this study also, TN and SPBY increased with the higher N application rate[119]. Previous studies also reported enhanced accumulation of total dry matter with the increased N application[116,120,121]. Tiller number is dynamic and variable which is majorly affected by cultivating environments, particularly by plant N status[122]. Results of the current study also emphasize that tillering is a major indicator of N response[47]. Tiller number and its quality directly regulate the panicle number and their development, which eventually determines yield of rice[123]. Plant height as well as tiller number increased with increase in N input[124]. This substantial enhancement in growth with N application is supported by the fact that N supply improves the meristematic cell size and number which leads to new shoots development[125]. Also, the level of cytokinin increased with N application which brings cell wall extensibility[126]. Therefore, it is evident that N was directly or indirectly involved in the cell division and enlargement and development of tissues leading to improved growth characteristics particularly plant height and tiller numbers. SCMR is the relative indicator of photosynthetic pigment contents in plants. In the present study, N fertilizer application significantly enhanced SCMR, which implies increase in available leaf N. Likewise, increased SCMR with higher N application was earlier reported[32,127]. Amount of photosynthetic pigments increased with higher N application in japonica rice[128] and indica hybrid rice[129]. Photosynthetic activities of plants enhanced with increase in chlorophyll

Fig. 3. (continued)

content which is associated with appropriate N application[130,131]. Chlorophyll fluorescence has shown to be critical trait for strong adaptability of NUE genotypes in rice[132]. Days to 50% flowering prolonged significantly with higher amounts applied N fertilizer due to prolonged vegetative growth[32,133,134] and improved tillering[135]. Across seasons, TN, SPBY, SPGY and SPSY recorded high genetic parameters suggesting high genetic variability and effectiveness of selection as most of the observed variation is genetic favouring rapid genetic improvement, but may require evaluation over multiple environments to capture genetic gains. DFF for both seasons recorded low GCV, PCV and GAM along with moderate heritability indicating low genetic variability and moderate scope for genetic improvement[136,137]. The selection may be less efficient as a substantial role is played by environment. SCMR also observed to have GCV, PCV, heritability and GAM suggesting a little genetic variability and limited response to selections as environment has a strong influence. For PH, a good potential for improvement through selection appears to be possible during the dry season.

Across the seasons and N treatments, PCA consistently showed that PC1 and PC2 together majorly contributes > 70% of the total phenotypic variation. In this study, yield-related traits particularly SPBY, SPSY, and SPGY were the strongest contributors to PC1, indicating grain and biomass productivity were dominant sources of variation across nitrogen levels, whereas SCMR and DFF predominantly contributed to PC2, indicating the roles of physiological efficiency and phenological development in distinguishing genotypes across nitrogen environments. These patterns indicate that, under both nitrogen-limited and nitrogen-sufficient conditions, yield components remain the dominant sources of variation, while physiological and developmental traits provide additional differentiation among genotypes, consistent with earlier findings in rice PCA and nitrogen-

Fig. 3. (continued)

response studies[138,139]. Under intermediate nitrogen availability (N50), variation in nitrogen use physiology and flowering time became more pronounced, and all the measured traits showed positive associations with significant PCA axes under N50 and N100.

Earlier reports on aus rice also revealed high variability for grain yield, plant height, tiller number and days to flowering[98,140]. Out of two studies reported for NUE in aus populations viz., only yield trait was characterized[47] and root traits were characterized in BAAP[55].

Identification of donors and characterization of traits for low N using heat map clustering

Heat map clustering is a hierarchical representation in graphical form revealing the complex associations among different traits as well as genotypic variations using relative values. In the present study, the clustering of seven traits (SPGY, SPBY, SPSY, PH, TN, SCMR and DFF) from N0 over N100 and N50 over N100 has shown two distinct groups as sensitive traits and tolerant traits under reduced N levels. Sensitive traits viz., SPGY, SPSY and SPBY had more reduction of traits in four comparisons (N0 vs. N100 and N50 vs. N100 in wet and dry seasons). TN was also noted to be sensitive to reduced N, but in N50 over N100 heat map of wet season, TN is clustered in tolerant group. The role of tiller number as a crucial determinant of biomass and grain yield in rice[141,142]. Among three tolerant traits (DFF, PH and SCMR), the relative reduction of DFF was almost similar across four comparisons. Out of three groups of genotypes (tolerant to low N, intermediate and sensitive to low N), genotypes which were tolerant to low N can be genetic resource for the breeding programs and characterization of genes for NUE. In N0 over N100 heat map, M202 was noted as highly tolerant to low N in wet season, but

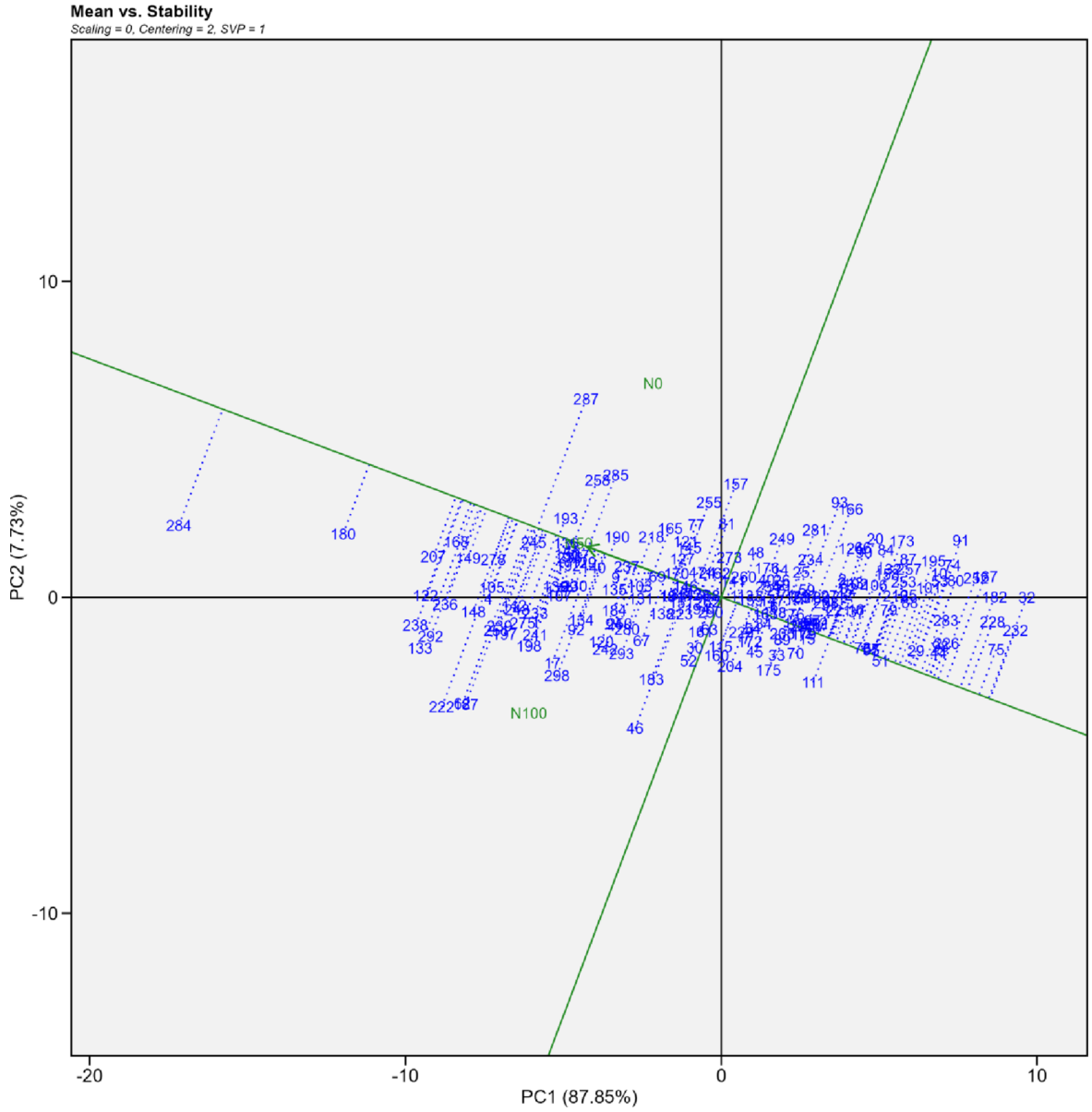

Fig. 4. (**A**) The mean vs. stability view showing the (G + G × E) interaction effect of genotypes at three graded N levels for SPGY in wet season. (**B**) The mean vs. stability view showing the (G + G × E) interaction effect of genotypes at three graded N levels for SPGY in dry season.

was intermediate in dry season. Despite the evaluation of 201 genotypes, no low N tolerant genotype was found to be common across four comparisons suggesting the complexity of NUE and variability of the seven traits of the present study. Out of the 109 landraces and the four improved varieties (IR64-21, M202, BINA Dhan 5, BRRI Dhan 47) identified for their low N tolerance from the heat map clustering, the improved varieties can be immediately utilized in the breeding programs for NUE because of their desirable plant type and high yielding nature.

Identification of low N tolerant and low N sensitive genotypes based on SPGY

Since yield is the major indicator for NUE, based on the relative reduction of SPGY in N0 and N50 in comparison to N100, IR 64-21 and Padma Sail were noted to be the common low N tolerant genotypes and AUS 169 as common low N sensitive genotype across wet and dry seasons among 25 top and bottom genotypes across four comparisons. IR 64-21 and Padma Sail can be used as donors for NUE. Pedigree of IR64 with several landraces and improved varieties must have contributed to its NUE[143]. Mapping populations can be developed with IR 64-21 and Padma Sail with AUS 169 as contrasting parents for NUE. Under N0, Deshi Boro and FR13A were common low N tolerant genotypes across two seasons and are potential donors taken up for superior allele identification for known candidate genes associated with NUE under zero N inputs. Under N50, M202, Kalburi and AUS 46 were common low N tolerant genotypes can also deployed as donors. Especially IR64 and M202,

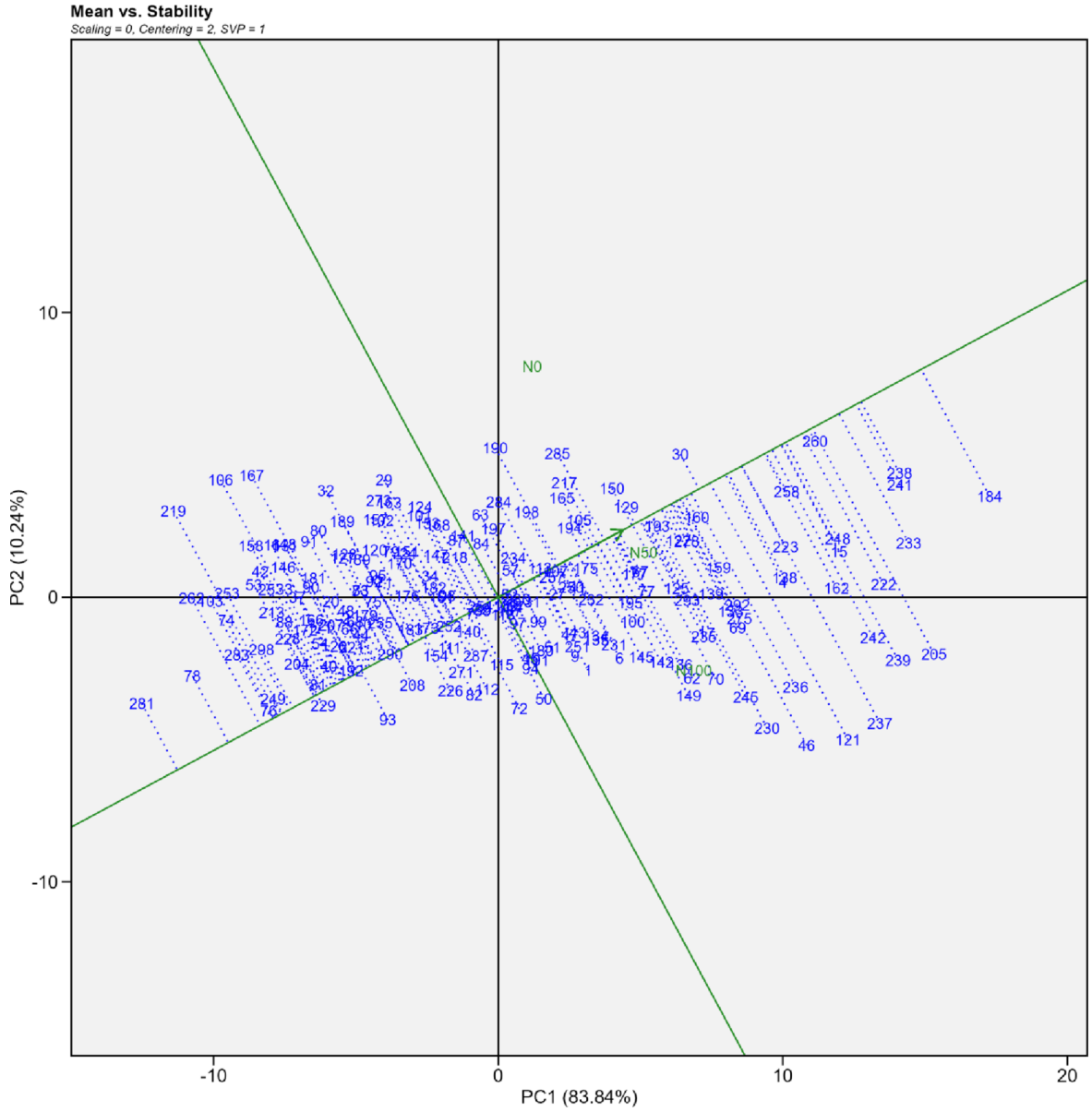

Fig. 4. (continued)

because they are already improved varieties with desirable plant types. The selected low N tolerant genotypes with high NUE and grain yield will definitely help to reduce environmental pollution and will enhance percent profitability to farmers[144,145].

Efficient, responsive and stable rice genotypes for NUE

Based on efficiency of genotypes in terms of grain yield under available soil N and responsiveness of genotypes to applied N, it was clear that the top genotypes from the four quadrants were mostly common for N0 vs. N50 and N0 over N100, but not common for wet and dry seasons. The genotypes belonging to N efficient and N responsive quadrants are of practical utility viz., FR13A, Dula Aus, M202 (wet season) and Saita Boro, Chaili Boro, Chhola Boro, Binadhan5 (dry season) because they can use the available soil N and also use applied N fertilizer, if any. These genotypes will serve as high grain yielders along with high NUE[29]. Outcomes of several studies directed the cultivation of N efficient genotypes along with enhanced grain yield in the farmer fields, resulting in minimized requirement for N fertilizer application thereby leading to increased profits to farmers[64,146,147]. The present study also revealed that the majority of efficient genotypes were responsive to applied N and the inefficient genotypes were non-responsive to applied N indicating the inter-relationship between N use efficiency and responsiveness to applied N. GGE biplot can effectively detect the pattern of G × E interaction and clearly demonstrate the best performing genotypes in multiple environments[148]. Kada-176-12 (168) and Jati Aus (149) were noted to be ideal genotypes in wet season, while in dry season, Bina Dhan5 (260) followed by BRRI Dhan47 (258) were found ideal. Genotypic variation for SPGY with wet and dry seasons can be attributed to change in temperature, relative

humidity, rainfall and sunshine hours[37,149–151]. The stable performing genotypes with higher grain yield across the graded N levels can be adopted for organic cultivation, natural farming as well as in marginal ecologies.

Seasonal variation and genotypic plasticity

Several historical studies have reported seasonal variation as dry season and wet season[37,38,152–154], however the conditions of wet and dry season may vary across the countries. The interesting observation of the present study was the genotypic plasticity of the some varieties/landraces adapting themselves to wet or dry or both seasons and to N0 to N50 to N100. However, the remaining genotypes exhibited significant seasonal variation. While bringing out the differential performance of the genotypes across the seasons, the relatively better performance of the genotypes during the dry season was also observed. Even though, most of aus rices exhibit photoperiod insensitivity[155], this marginal variation may be attributed to more duration of sunshine in dry season compared to wet season[37,38]. BAAP was assembled with genotypes of a reduced flowering duration of 10-day window so that the genotypes undergo similar environmental conditions during the flower initiation to grain filling stage[47]. There was little to no significant seasonal variation observed in the measured soil properties. Out of five genetics groups reported in BAAP, group 3 performed better for the key traits such as SPGY, SPBY, SPSY and TN in the present study[156]. Interestingly dry season brought out the relative best performance also in the genetic groups analyses. The origin of BAAP genotypes was only shown as India and Bangladesh with no further locations within the countries, thus much difference was not observed for geographical adaptation of 201 genotypes.

NUE of rice was evaluated by many groups ranging from a single genotype to 180 genotypes (mostly released varieties) of rice under graded levels of N for understanding the association of agro -physiological traits with grain yield[17,34,70,118]. Evaluation of the vast and unutilized rice germplasm for their possible genetic potential could be a promising approach for identifying genotypes with NUE[39]. Especially landraces of rice could be a potential source for NUE as these genotypes are adapted to low input conditions[38]. However, efforts for screening of large-scale germplasm accessions in the field for NUE were scarce because there were no conscious breeding programs aimed at NUE in rice. With the advent of GWAS, hundreds of rice genotypes were evaluated for their NUE through either hydroponics or graded N levels at field conditions and promising donors are being identified[38,40,41]. The data recorded in the current study is being subjected to GWAS for identification of genomic regions associated with NUE in rice.

Conclusion

Field screening of a set of 201 BAAP rice genotypes under graded N application rate (0, 50, 100 kg N ha^{-1}) for two wet seasons (2021 and 2022) and one dry season (2022) revealed the existence of wide genotypic variability for grain yield and other traits. Occurrence of common tolerant genotypes between N0 and N50 revealed the genotype plasticity for both N0 and N50 conditions. Heat map clustering revealed SPGY, SPBY and SPSY as sensitive to low N application. Improved varieties viz., IR64-21, M202, BINA Dhan 5, BRRI Dhan 47 found to be tolerant to low N will aid in immediate NUE breeding programmes and promising landrace donors viz., Chhola Boro (2), Padma Sail, FR13A, Deshi Boro, Dula Aus and others have been identified which can be deployed as donors for NUE in rice.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request and will also be made available in the NERC EDS Environmental Information Data Centre from May 2026 (https://doi.org/10.5285/1e20a1c8-6aeb-4365-866d-71b24c497586).

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Author contributions

C.N. conceptualized and designed the study. B.S. and K.S. conducted the field experiments. B.S. and V.J. carried out the data analysis. B.S. prepared the manuscript. C.N., D.S.R., D.S. and A.P. edited the manuscript. All authors contributed to the article and approved the submitted version.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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