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#### Siddharth Panda

(1) Crop Improvement Division,  
Indian Council of Agricultural  
Research (ICAR)-National Rice  
Research Institute (NRRRI),  
Cuttack, Odisha, India  
(2) Department of Plant  
Breeding and Genetics, Odisha  
University of Agriculture and  
Technology, Bhubaneswar,  
Odisha, India

#### Debendranath Bastia

Department of Plant Breeding  
and Genetics, Odisha University  
of Agriculture and Technology,  
Bhubaneswar, Odisha, India

#### Annamalai Anandan

Crop Improvement Division,  
Indian Council of Agricultural  
Research (ICAR)-National Rice  
Research Institute (NRRRI),  
Cuttack, Odisha, India

#### Corresponding Author:

##### Siddharth Panda

(1) Crop Improvement Division,  
Indian Council of Agricultural  
Research (ICAR)-National Rice  
Research Institute (NRRRI),  
Cuttack, Odisha, India  
(2) Department of Plant  
Breeding and Genetics, Odisha  
University of Agriculture and  
Technology, Bhubaneswar,  
Odisha, India

## Assessing genetic variability of Bengal and Assam Aus panel rice lines under low nitrogen soil status

Siddharth Panda, Debendranath Bastia and Annamalai Anandan

#### Abstract

Nitrogen deficiency is more pronounced in rice especially in the upland aerobic soils. Compensating rice cultivars with Nitrogen Use Efficiency traits would require identifying the component traits involved and the level of dependence between these traits, in the present study 204 Bengal Assam Aus Panel rice lines were used for studying their response under low nitrogen soil status. It was found that the traits like chlorophyll index and days to 50% flowering were the most influenced trait under low nitrogen. There was high correlation of grain yield per plant with flag leaf width, panicle number and days to 50% flowering. The highest contribution was from the trait flag leaf width and panicle length.

**Keywords:** Assam Aus panel rice lines, low nitrogen soil, *Oryza sativa*

#### Introduction

Rice (*Oryza sativa* L.), the second most cultivated crop in the world, nurtures billions of lives including humans and animals, and is of prime importance in developing nations. Especially in Asia where it is the sole source of energy (carbohydrate) for a majority of the population. The nutritional content of rice grain is 80% carbohydrates, 7–8% protein, 3% fat, and 3% fibre (Chaudhari *et al.*, 2018) [4]. India's total rice production was 127.93 million metric tons in the year 2021-2022 (www.indiastat.com). As an energy-giving food, the consumption of rice is as high as 103.5 million metric tons in India and 509.87 million metric tons (estimated) worldwide in the crop year 2020-21 (www.fao.org/worldfoodsituation). notable increase in the grain yield of rice in the 1960s but eventually the growth rate declined and was almost stagnant by the mid-1980s (Dobermann *et al.*, 2004) [5]. This decline can be attributed to multiple reasons. The most significant being the incessant application of fertiliser and the reduced nutrient use efficiency of the newly developed varieties. This was the case despite the development of varieties with higher yield potential. This motivated farmers to resort to high blanket application of fertilisers (especially nitrogen) in order to suffice the shortcomings of yield leading to deterioration of soil (physically and chemically), declining water table and also damaging the soil fauna (Srivastava *et al.*, 2020) [12]. the total nitrogenous fertiliser applied, the nitrogen recovery efficiency remains less than 30% in the rainfed areas while it ranges between 20-30% under irrigated field conditions (Roberts, 2008) [10]. Rice is being grown in diverse ecological systems: this has led to diverse adaptive features, with higher degrees of variability between genotypes (Panda, Bhatt, *et al.* 2021) [7]. This paper studies the variability and correlation of Bengal and Assam Aus Panel under 50% nitrogen status of soil in order to understand the effect of low nitrogen on the panel and highlight the traits influenced by it. The peculiarity of the aus rice lines is that they harbour many beneficial novel genes absent in other indica or japonica cultivars, such as *Pup1*, *Sub-1*, *Xa5* etc. This would mainly cater to the needs of resource poor upland rice cultivation systems where nutrient use is an important factor deciding the farmer's income (A Anandan *et al.* 2021.) [2].

#### Materials and Methods

##### Experimental site

The nitrogen deficient trial was undertaken in field condition at ICAR-National Rice Research Institute, Cuttack during Rabi 2021 in transplanted system of rice cultivation. The soil was sandy clay loam with 0.53% organic carbon, 240 kg/ha of N, 26.3 kg/ha of P, 164.05 K kg/ha, 51.6% sand, 18% silt, 30.4% clay with bulk density of 1.41 g/cm<sup>3</sup>. In order to maintain a nitrogen deficient condition only 50% of the total recommended dose of N was applied.

The recommended dose was 80:40:40:N:P2O:K2O, while the applied fertilizer in the trial was 40:40:40::N:P2O:K2O. A total of 203 BAAP lines were used for sowing. Transplanting was done at 28 days after germination in a single row of each genotype in four replications. The planting followed randomized block design in four replications. The spacing was maintained at 20 cm between rows and 15 cm between plants. After each row of entry one row of Naveen variety was grown to ensure uniform competition in the field. Standard agronomic practices were followed with respect to irrigation schedule and controlling weed in the field. Six plants were taken from an entry in each replication to record the observations and the mean was calculated using the values recorded from the six plants in each replication for further statistics. The traits studied were chlorophyll index of the flag leaf, days to 50% flowering, number of tillers, number of productive tillers, flag leaf length, flag leaf width, panicle length and grain yield per plant.

## Results

All the traits studied displayed significant differences among the genotypes and there was no significant difference among the replications for these genotypes. The ANOVA for the above-mentioned traits in the nitrogen deficient trial is presented in table 1 and the data distribution is depicted in Fig.1.

The chlorophyll Index was measured using SPAD. The SPAD values ranged from 20.33 in the accession Kele Bari to 42.01 in AUS453. The mean value for chlorophyll index in the panel was 30.95 with a standard deviation of 4.66. The PCV (14.7) was higher than GCV (10.46) with moderate heritability (0.51). The data here shows a skewness of 0.17 and kurtosis of 0.34. The days to 50% flowering was in the range from 80 (Assam4) to 96.33 (Raj mundo) days. The mean was 87days, the CV (4.97%) was low compared to other traits. The PCV (4.05) was higher than the GCV (3.32) and the heritability was 0.67. The data distribution here shows a skewness of 0.36 and kurtosis of 0.4. A considerable amount of variation was seen, as plant height ranged from 81.29cm (Zhenshan 97 B) to 165.02cm (AUS321). The mean plant height was 118.95cm with a standard deviation of 12.23. The PCV (14.14) was higher than the GCV (11.48) and the heritability was 0.67 with a genetic advance value of 19.23. The data distribution here shows almost symmetrical skewness of -0.4. Flag leaf length as a trait displayed variation among the genotypes with the lowest at 22.97cm (Zhenshan 97B) and the highest was 43.87cm (AUS362) with a mean of 31.16cm. The heritability (0.37) was low with a higher PCV value of 17.47 and lower GCV value of 10.56. The data distribution here shows a positive skewness of 0.51. There was a wide variation among the genotypes for flag leaf width ranging from 0.80cm in (AUS paddy white) to 2.98cm (Chhola boro 2). The mean flag leaf width was 1.43cm with a standard deviation of 0.25. There was moderate heritability with 0.47 and genetic advance of 15.58. The data distribution here shows a positive skewness of 0.57. Panicle length ranged from 17.58cm (AUS paddy white) to 31.10cm (AUS99). The mean was 24.08cm with a cv of 11.13%. The heritability (0.49) was moderate. The distribution of data for this trait in the panel is positively skewed (0.72). Number of tillers for the

panel ranged from a minimum of 7.06 (AUS440) to a maximum of 21.20 (Shada boro). The mean number of tillers per plant was 13.05 with a CV of 19.28%. The heritability was 0.44 and the genetic advance was 25.81. The data distribution here shows symmetrical distribution with a skewness of 0.39. Number of productive tillers ranged from 3.61 in LI-JIANG-XIN-TUAN-HEI-GU to 15 in KALASU. The mean number of productive tillers per plant was 8.59 and the CV was 18.28%. The heritability was moderate with 0.41. The data distribution here shows an almost symmetrical skewness of 0.28. Grain yield per plant showed a wide range of values with the lowest being 2gms (AUS paddy white) to as high as 20.36gms (ARD11600). The mean grain yield per plant in the panel was 11.45gms with a CV of 17.98%. The heritability (0.35) was low and genetic advance of 24.90. The data distribution here shows an almost symmetrical skewness of 0.39.

## Correlation analysis and Principal component analysis

A considerable positive link between grain production per plant and flag leaf width (0.43), panicle length (0.30), and the number of productive tillers was underlined by the Pearson's correlation of the aforementioned parameters under the nitrogen deficiency condition (0.37). Days to 50% flowering significantly positively correlated with both the number of tillers (0.22) and the average grain yield per plant (0.15). Number of tillers and flag leaf width revealed a significant negative correlation (-0.21). The results of the correlation analysis for the BAAP lines under nitrogen deficient trial are presented in Table 2 and Figure 2. The PCA results further highlighted the amount variation contributed by each trait and the nature of relationship the traits studied (Table 4.5). Only three PCs had eigenvalues greater than one, leading to the consideration of three primary axes for additional deductions. Between the genotypes in the panel and specific phenotypes, PC1 accounted 28.5% of the variation while PC2 explained 20.3%. The most variable factor in PC1 was panicle length (25.62), followed by flag leaf width (22.03). The most significant variations in PC2 and PC3 were caused by the number of productive tillers (31.93) and chlorophyll index (38.46), respectively. The PCA biplot for yield and yield attributing traits under nitrogen deficient trial is presented in Fig 3.

**Table 1:** ANOVA of BAAP lines for yield and yield attributing traits under nitrogen deficient trial

Traits	Mean Sum of Squares		
	Genotype	Replication	Error
Chlorophyll Index (SPAD)	51.14**	30.08	10.2
Days to 50% flowering	37.41**	14.23	4.06
Plant height (cm)	843.41**	432.28	96.28
Flag Leaf length (cm)	62.09**	10.46	8.81
Flag Leaf width (cm)	0.64**	0.19	0.1
Panicle length (cm)	17.03**	9.56	3.46
Number of tillers	32.38**	22.9	7.9
Number of productive tillers	15.96**	7.63	4.28
Grain yield per plant (g)	32.33**	20.4	10.31

Treatment degrees of freedom = 202, Replication degrees of freedom = 3

\*, \*\* Significant at 0.05 and 0.01 levels, respectively

**Table 2:** Estimates of variability of BAAP lines for yield and yield attributing traits under nitrogen deficient trial

Traits	RANGE	MEAN	SD	CD (5%)	CV (%)	GCV	PCV	h <sup>2</sup> <sub>bs</sub>	GA	SKEWNESS	KURTOSIS
Chlorophyll Index	20.33 - 42.01	30.95	4.6	4.44	14.97	10.46	14.7	0.51	15.34	0.17	0.34
Days to 50% flowering	80.00 – 96.33	87.00	3.53	2.79	4.05	3.32	4.05	0.67	5.61	0.36	0.4
Plant height (cm)	81.29 – 165.02	118.95	12.23	13.62	10.31	11.48	14.14	0.66	19.23	-0.04	0.71
Flag Leaf length (cm)	22.97 - 43.87	31.16	5.55	6.05	17.85	10.56	17.47	0.37	13.14	0.51	0.42
Flag Leaf width (cm)	0.80 - 2.98	1.43	0.25	18	17.46	11.07	16.19	0.47	15.58	0.57	1.94
Panicle length (cm)	17.58 - 31.10	24.08	2.68	2.58	11.13	7.65	10.88	0.49	11.08	0.72	1.05
Number of tillers	7.06 - 21.20	13.05	2.89	3.9	19.28	18.96	20.7	0.44	25.81	0.39	0.2
Number of Productive tillers	3.61-15.00	8.59	1.82	2.87	18.28	19.88	21.23	0.41	26.05	0.28	-0.33
Grain yield per plant (g)	2.00 - 20.36	11.45	2.67	4.46	17.98	20.49	34.78	0.35	24.9	0.39	0.03

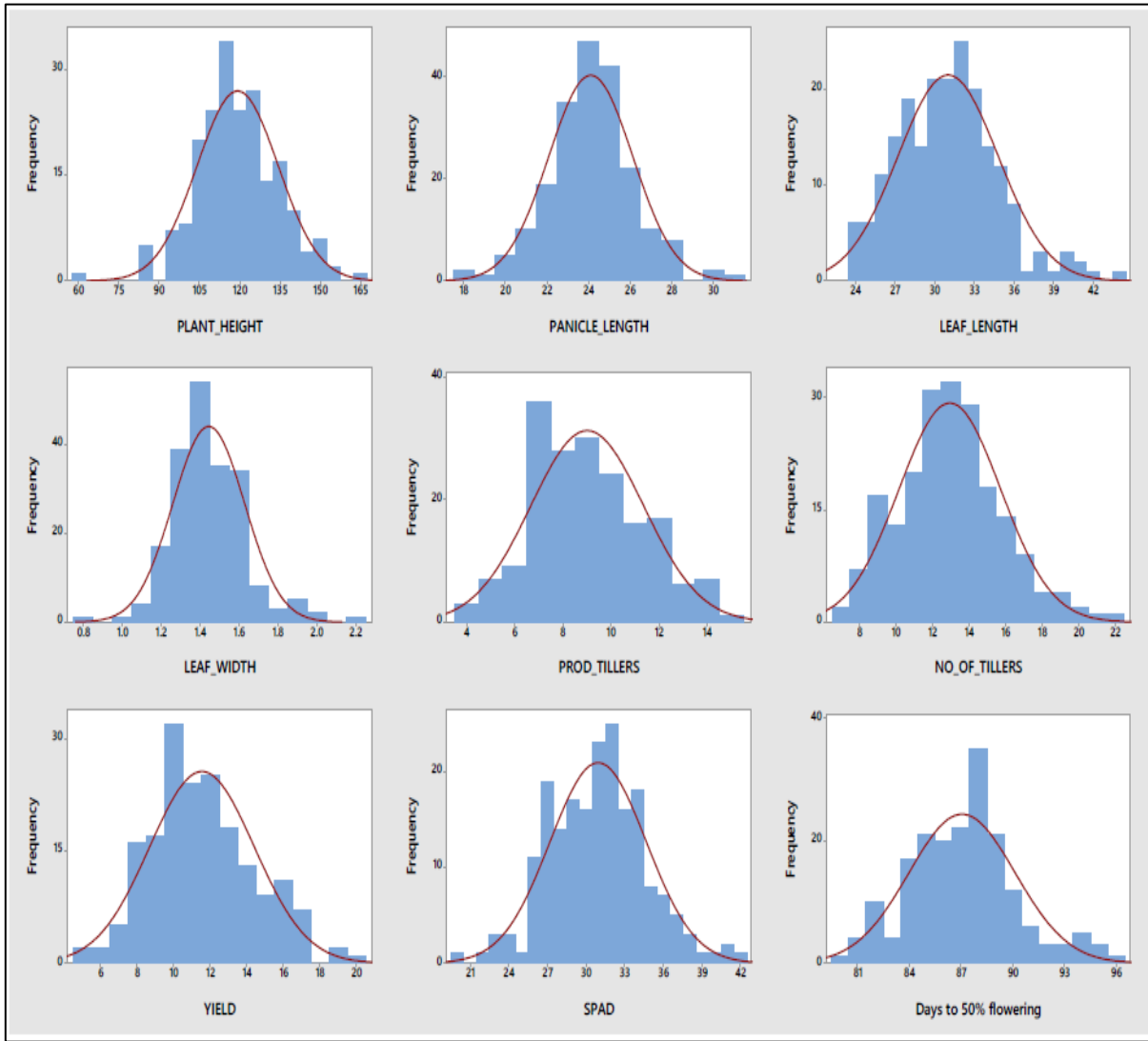
**Table 3:** Estimates of correlation among yield and yield attributing traits under nitrogen deficient trial

	Chlorophyll Index	Days to 50% flowering	Plant height	Flag Leaf length	Flag Leaf width	Panicle length	Number of tillers	Number of productive tillers	Grain yield per plant
Chlorophyll Index	1								
Days to 50% flowering	0.125	1							
Plant height	0.012	-0.042	1						
Flag Leaf length	0.029	-0.044	0.558**	1					
Flag Leaf width	0.071	-0.013	0.053	0.119	1				
Panicle length	0.016	-0.073	0.149*	0.161*	0.585**	1			
Number of tillers	0.020	0.074	0.116	-0.077	-0.211**	-0.039	1		
Number of productive tillers	0.087	0.222**	-0.007	-0.043	-0.136	0.113	0.247**	1	
Grain yield per plant	0.030	0.153*	0.057	0.082	0.436**	0.307**	0.079	0.377**	1

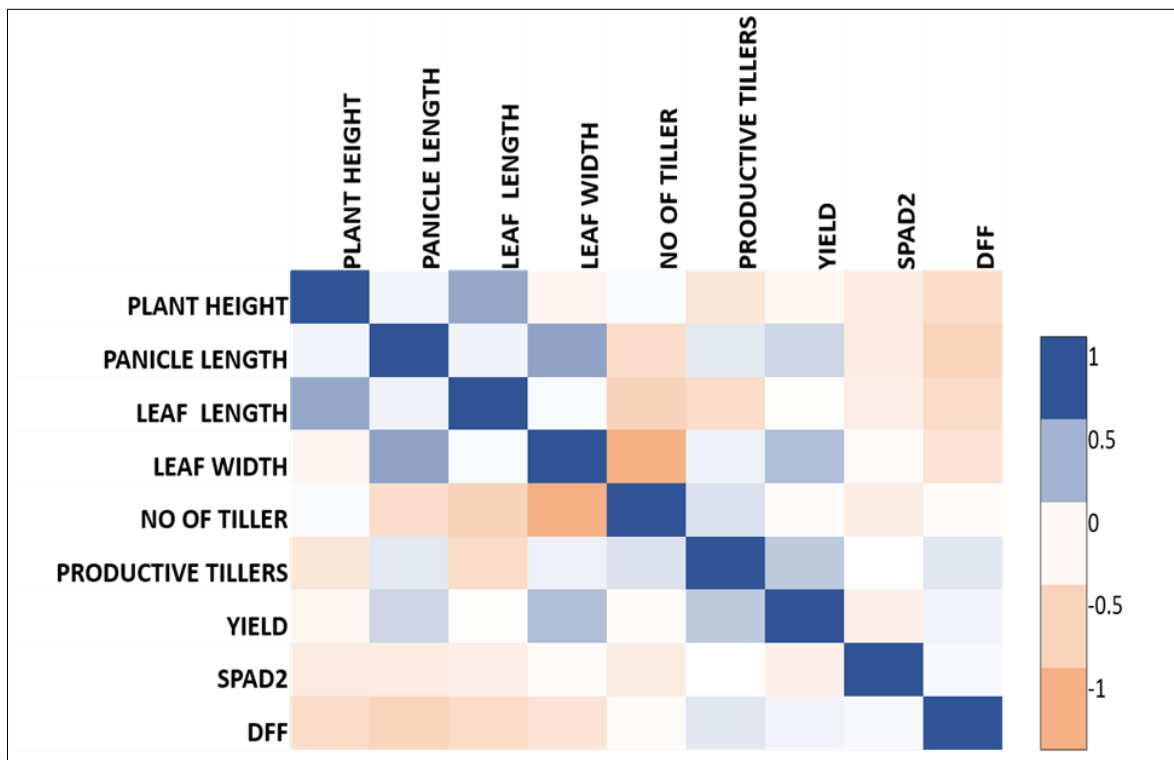
\*, \*\* Significant at 0.05 and 0.01 levels, respectively

**Table 4:** Estimates of eigen values and contribution of yield and yield attributing traits towards the major principal components under nitrogen deficient trial

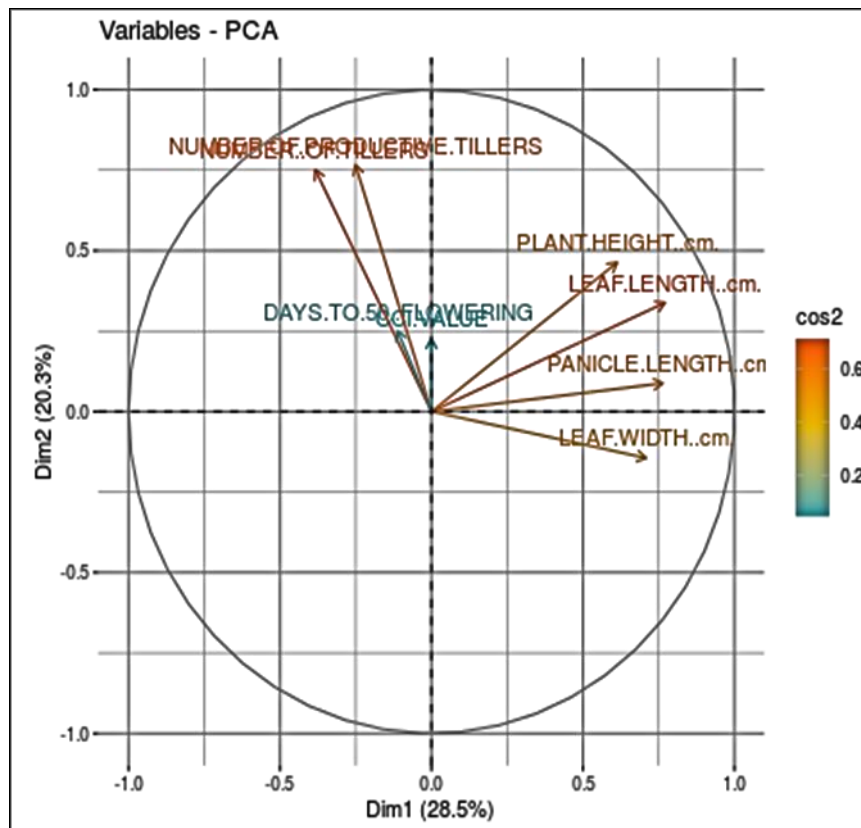
Traits	PC1	PC2	PC3
Eigen values	2.27	1.77	1.14
Chlorophyll Index	0.47	4.51	38.46
Days to 50% flowering	0.56	2.26	31.24
Plant height (cm)	16.85	8.51	8.72
Flag Leaf length (cm)	16.56	5.36	1.16
Flag Leaf width (cm)	22.03	0.61	10.75
Panicle length (cm)	25.62	0.14	0.003
Number of tillers	5.91	30.31	7.57
Number of Productive tillers	2.4	31.93	0.07
Grain yield per plant (g)	0.12	16.33	1.99



**Fig. 1:** Morphological data distribution of the BAAP lines for yield and yield attributing traits under nitrogen deficient trial



**Fig 2:** Correlation heat map for yield and yield attributing traits under nitrogen deficient trial



**Fig 3:** PCA biplot for yield and yield attributing traits under nitrogen deficient trial

## Discussion

The accessions employed in this study showed considerable mean sum of squares, according to the analysis of variance, indicating that the features under examination had a lot of variability. One of the accessions on the panel was the popular indica rice variety; Swarna. Swarna's reaction to N deficit can be utilised as a benchmark for other accessions in the panel when comparing it as an enhanced variety. The low levels (i.e., below 30 for 76 accessions) of nitrogen content maintained in the field were the cause of the low chlorophyll index. Similar results were also reported by Swain and Sandip (2010)<sup>[13]</sup>, Yang *et al.* (2014)<sup>[15]</sup>. This is due to the fact that nitrogen is a necessary component of chloroplasts, with reports stating that 80% of leaf nitrogen is diverted there, with 50% of that nitrogen going toward the production of photosynthetic proteins (Xiong *et al.*, 2015)<sup>[14]</sup>. The trait can be improved by selection, and the heritability was 0.51. More than half of all accessions were flowering sooner than the average, with the mean days to 50% flowering being 87 days. Another important feature that is affected by nitrogen status in the soil is flowering. Early flowering is accelerated by low nitrogen status, and normally higher nitrogen doses cause delayed flowering and ripening. Similar results were reported by Sanagi *et al.* (2021)<sup>[11]</sup> and Zhang *et al.* (2021)<sup>[16]</sup>. Leaf width is a crucial characteristic that influences leaf area and, in turn, the effectiveness of a plant's photosynthetic process. (Rahman *et al.*, 2013)<sup>[9]</sup>. The mean flag leaf width in this study was 1.43 cm, with a moderate heritability that might be enhanced through selection. With the exception of plant height, all of the panel's features had mean values that were lower than Swarna's performance, which had higher leaf width (1.82 cm) and more prolific tillers (12.66), while still maintaining a SPAD score of 31.99. Except for flag leaf width, flag leaf length, and panicle length, which were positively skewed, all the examined attributes in the nitrogen-

deficient trial showed symmetric distribution in the population. A greater chlorophyll index would result from a larger dose of N, which would also improve other yield-attributing traits like productive tillers, leaf width, etc. However, identifying plants that have superior N usage efficiency even under deficient soil conditions would be helpful in further deciphering the genetic regulations governing them. These results are in support of previous reports by Agahi *et al.* (2007)<sup>[1]</sup>, Khaliq *et al.* (2008)<sup>[6]</sup> and Rahman *et al.* (2013)<sup>[9]</sup>. Flag leaf width and panicle length in the first component were identified by the principal component analysis as the main sources of variance. The distributional data for these traits were positively skewed, which explains the trait's variability. Grain yield per plant was discovered to be strongly linked with flag leaf width and panicle length based on the correlation and PCA analyses. This work can further be studied for genome wide association studies in the BAAP panel (Annamalai Anandan *et al.* 2022)<sup>[3]</sup>. Apart from the above mentioned traits a focused study on root morphological traits is also needed for a future model nutrient use efficient crop (Panda, Majhi, *et al.* 2021)<sup>[8]</sup>.

## Conclusion

The present study has identified BAAP lines that can be used in identification of QTLs responsible for low N tolerance and also serve as a source of breeding material for developing low N tolerant rice cultivars through classical breeding approach. It is also suggested that the advances made in molecular and genomic tools combining traditional science of plant breeding may facilitate to study the genetic differences in NUE, bringing N-efficient crops.

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