



Evaluation of Assam rice (*Oryza sativa*) genotypes for nitrogen use efficiency

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ABSTRACT

We assessed genetic diversity in 26 rice genotypes including 21 landraces and five improved varieties grown in three different nitrogen doses, viz. 0, 40, and 60 kg/ha during *kharif* 2014–15, and identified superior nitrogen use efficient genotypes for lowland rice ecosystem. The presence of significant genotypic differences was registered for 12 quantitative traits of nitrogen use efficiency, grain yield, and its related traits. Mahalanobis distance-based clustering grouped the genotypes into seven clusters. The maximum contribution towards divergence was due to flag leaf area followed by physiological nitrogen use efficiency and grain yield/plant. Principal component analysis performed on the 12 quantitative traits revealed that the first four principal components accounted for 83.57% variance among the genotypes. Grain yield/plant (0.43), nitrogen utilization efficiency (0.39), and nitrogen harvest index (0.37) accounted most for principal component 1. The genotypes namely Lothabor, Betguti Sali, and Luhasali were found to be suitable for the lowland rice ecosystem.

Keywords: Genetic divergence, Lowland rice ecosystem, Principal component analysis, Rice landraces, Varying nitrogen regimes

Rice (*Oryza sativa* L.) is a principal crop of the North-Eastern (NE) region of India. Majority of rice area in the region is under upland condition and exposed to biotic and abiotic stresses resulted in poor yield. Nitrogen deficiency is the one of the major cause of low productivity of rice in the NE. More than 50% of applied nitrogen is not assimilated by the rice plant, particularly when nitrogen fertilizer is applied using conventional broadcast methods (Islam *et al.* 2016). The remaining nitrogen is lost through different mechanisms including ammonia (NH₃) volatilization, nitrification-denitrification, leaching, and surface runoff (Rochette *et al.* 2013). Low recovery of nitrogen by the plant results in poor yield per unit area. To tackle such adverse environmental conditions and to maximize crop yield, farmers often use more nitrogen fertilizers than the optimum requirement (Yadav *et al.* 2013). As a result, the application of nitrogenous fertilizers is one of the major investments for rice farmers in developing countries.

Excessive nitrogenous fertilizer application may not result in yield improvement but will lead to serious environmental problems (Yadav *et al.* 2013). Hence, to ensure food security and environmental sustainability, agricultural production must be improved by improving land productivity and resource use efficiency. Therefore, breeding for NUE is crucial not only to increase crop yield but also to reduce production costs and environmental pollution.

The NE region is endowed with a rich source of genetic diversity in rice. The traditional varieties continue to occupy a significant role in the rice cultivation of NE India because of the region's predominantly rainfed agriculture and consequent climatic and edaphic variations. There is an enormous opportunity to isolate the outstanding NU efficient cultivars for further breeding high-yielding NU efficient varieties suitable for the rainfed lowland rice ecosystems. Therefore, this study was undertaken to understand the nature and the magnitude of quantitative variation for NUE and related traits and identify high-yielding nitrogen use efficient rice genotypes for low input lowland rice ecosystem to help in designing effective breeding strategies for the region.

MATERIALS AND METHODS

A set of 26 rice genotypes consist of 21 landraces (Lothabor, Myochang, Luhasali, Kolabor, Mohmda Nneng, Khauji, Ronga Bora, Betguti Sali, Abhimanyu, Jahinga, Jengoni Bora, Betguti, Moina Bora, Aki Bora, Doombor, Bhog Prasad, Rongdoi, Mal Bhog, Mou Bora, Kabori and Kon Joha) and five improved varieties (Aghoni Bora, Gitesh,

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Manohar Sali, Mashuri, and Ranjit) were tested under three different nitrogen environments (E_1 : 0 kg N, E_2 : 40 kg N, and E_3 : 60 kg N/ha) with fixed P_2O_5 and K_2O @ 20 kg/ha during *kharif* 2014 and 2015. The genotypes were evaluated in randomized block design with three replications at Assam Agricultural University (AAU), Jorhat. The nitrogen, phosphorus, and potassium were applied in the form of urea, single super phosphate (SSP), and muriate of potash (MOP). One-half of the total requirement of urea was applied at the time of final land preparation as a basal dose. The remaining urea was applied in two equal splits as a top dressing at the tillering and panicle initiation stage. Full doses of SSP and MOP along with vermicompost @ 10 t/ha were applied at the time of final land preparation. Thirty-five days old seedlings were transplanted in the main field on 17th July 2014 and 2015 with a spacing of 20 cm × 20 cm. The field soil characteristics were recorded from three randomly selected sites of each experimental plot before sowing and planting. The unpredictable environmental conditions during the crop period of 2014 and 2015 were mainly due to differences in rainfall intensity and light intensity. The yearly variations in maximum/minimum temperatures and relative humidity were negligible. The total rainfall during the vegetative and reproductive period was 797.3 mm and 240.8 mm, respectively in 2014 while the same was 955.9 mm and 152.3 mm in 2015. The crop of 2014 received bright sunshine hours of 4.4 and 6.0 during the vegetative and the reproductive period, respectively as against 6.0 and 5.5 during the same period of 2015.

Twelve quantitative traits including NUE, yield and its related traits were recorded at the appropriate stages of the rice plants. For estimation of NUE-related traits, the samples were oven-dried at 70°C up to constant weight. Grain nitrogen content was determined by Micro-Kjeldahl's method, while physiological nitrogen use efficiency (PNUE) (Matsunami *et al.* 2013), nitrogen utilization efficiency (NUtE) (Moll *et al.* 1982), and nitrogen harvest index (NHI) (Singh *et al.* 1998) was estimated following standard procedures described earlier as per the following formulae:

$$\text{PNUE (kg per kg)} = \frac{\text{Biomass production}}{\text{Amount of nitrogen in the plant at maturity}}$$

$$\text{NUtE (kg per kg)} = \frac{\text{Gain yield}}{\text{Amount of nitrogen in the plant at maturity}}$$

$$\text{NHI (\%)} = \frac{\text{Grain nitrogen}}{\text{Amount of nitrogen in the plant at maturity}} \times 100$$

The replication-wise data of both the years were subjected to a pooled analysis of variance (ANOVA) in Windostat v. 9.2. For diversity analysis, the variance due to genotype × environment interaction and pooled error variance were taken together as error variance. Genotypic variances were tested against error variances by applying F-test for significance. The genetic divergence was assessed through D^2 statistics of Mahalanobis (1936) and grouping of the genotypes into clusters was done by Tocher's method (Rao 1952). The principal component analysis (PCA) was performed by using statistical software R.

RESULTS AND DISCUSSION

Genetic divergence analysis: Pooled ANOVA (data not shown) revealed significant genotypic differences for all the traits. Further, Mahalanobis D^2 statistics were performed to find out the nature and magnitude of genetic diversity so that diverse cross combinations could be selected for further successful hybridization programs. All the 26 rice genotypes were grouped into seven clusters based on the D^2 analysis (Supplementary Table 1). The maximum number of genotypes were retained in cluster VI (9) followed by cluster III (5) and cluster IV (4). Cluster VII consists of only one genotype. The highest inter-cluster distance was observed between cluster V and cluster VII followed by cluster II and cluster VII, whereas the highest intra-cluster distance was observed in cluster VI. The maximum contribution in the manifestation of genetic divergence (Supplementary Table 2) was exhibited by flag leaf area (27.38%) followed by PNUE (19.38%) and grain yield/plant (14.15%). Cluster III had the highest mean value for total chlorophyll, biological yield, harvest index, PNUE, NUtE, and grain yield/plant (Supplementary Table 2). Similarly, cluster VII exhibited maximum mean values for NR activity, grain nitrogen, and NHI; and minimum mean values for root volume and flag leaf area. Thus, the genotypes from these clusters seem to be promising for most of the traits. The result of the D^2 analysis might be useful to exploit heterosis and creating substantial variability in advanced generations concerning the traits under study. The genotypes from diverse groups might be used as potential donors in hybridization programs to obtain desirable recombinants (Padmaja *et al.*

Table 1 The four most informative principal components and the top three traits contributing towards them

Principal component	Eigen value	Variability (%)	Cumulative variability (%)	Traits		
				Rank 1	Rank 2	Rank 3
Comp. 1	2.19	39.91	39.91	Grain yield/plant (0.43)	Nitrogen utilization efficiency (0.39)	Nitrogen harvest index (0.37)
Comp. 2	1.57	20.45	60.36	Root length (0.46)	Physiological nitrogen use efficiency (0.42)	Root volume (0.33)
Comp. 3	1.22	12.42	72.78	Harvest index (0.59)	Nitrogen utilization efficiency (0.21)	Flag leaf area (0.21)
Comp. 4	1.14	10.79	83.57	Root volume (0.54)	Harvest index (0.33)	Root length (0.32)

Table 2 Identification of genotypes based on *at par* performance with respect to important NUE related traits

Trait	Highest	Lowest
Grain yield/plant (g)	Lothabor	Mou Bora
	Betguti Sali	Kon Joha
	Luhasali	Mashuri
Nitrogen harvest index (%)	Lothabor	Mou Bora
	Aki Bora	Kon Joha
	Ranjit	Betguti
Nitrogen utilization efficiency (kg/kg)	Lothabor	Mashuri
	Luhasali	Ranjit
	Betguti Sali	Mou Bora
Physiological nitrogen use efficiency (kg/kg)	Aki Bora	Aghoni Bora
	Lothabor	Mashuri
	Luhasali	Ronga Bora
Grain nitrogen (%)	Lothabor	Betguti
	Ranjit	Kon Joha
	Betguti Sali	Mou Bora
Nitrate reductase activity ((μ mol NO ₂ ⁻ g ⁻¹ fr. L wt. per h)	Kabori	Khauji
	Lothabor	Mohmda Nneng
	Ranjit	Luhasali
Root length (cm)	Betguti Sali	Ranjit
	Aki Bora	Luhasali
	Gitesh	Ronga Bora

contributed 20.45%, 12.42%, and 10.79%, respectively of the total variation. Grain yield/plant (0.43), NUtE (0.39) and NHI (0.37) were the top contributors to the PC1 while root length (0.46), PNUE (0.42) and root volume (0.33) contributed to the PC2. Harvest index (0.59), NUtE (0.21) and flag leaf area (0.21) contributed to the PC3. Also, root volume (0.54), harvest index (0.33) and root length (0.32) contributed to PC4. Thus, this information might be kept into consideration during the utilization of these traits in the breeding program. Better performing outliers concerning the first two PCs were identified (Fig 1). The PCA helped in identifying the genotypes namely Lothabor, Kolabor, Kabori, and Luhasali that performed well concerning PC1 and PC2.

A better understanding of the genetic basis of NUE-related traits and grain yield in rice could be very important for food security and environmental benefits (Zhou *et al.* 2017). Therefore, we identified contrasting parents for the traits of interest (Table 2) for recombination breeding. For instance, mapping population for detection of quantitative trait loci (QTLs) related to NUtE could be developed from the crosses between nitrogen use efficient genotypes Lothabor, Luhasali, and Betguti Sali with inefficient genotypes Mashuri, Ranjit, and Mou Bora. QTLs for grain yield could be mapped from the cross combination of high-yielding genotypes Lothabor, Betguti Sali, and Luhasali with low performing genotypes Mou Bora, Kon Joha, and Mashuri. The mapping population derived from the crosses of Aki Bora, Lothabor, and Luhasali with Aghoni Bora, Mashuri, and Ronga Bora could be used for mapping QTLs for PNUE.

The present study could be considered as a preliminary

2010). A wide spectrum of variability could be expected in segregating generations from crosses involving genotypes belonging to clusters III (Lothabor, Luhasali, Kolabor, Jahinga, Gitesh), cluster V (Betguti, Mou Bora), and cluster VII (Ranjit) to isolate high-yielding nitrogen use efficient genotypes.

Principal component analysis and identification of desirable genotypes: Principal component analysis has been a powerful multivariate analysis for data reduction which removes interrelationships among the components and has proved its utility in the conservation and utilization of germplasm (Smith *et al.* 1995). In the present study, PCA revealed that the first four principal components (PCs) having greater than one Eigen value contributed 83.57% of the total variation among the 26 rice genotypes (Table 1). The PC1 contributed 39.91%, whereas PC2, PC3, and PC4

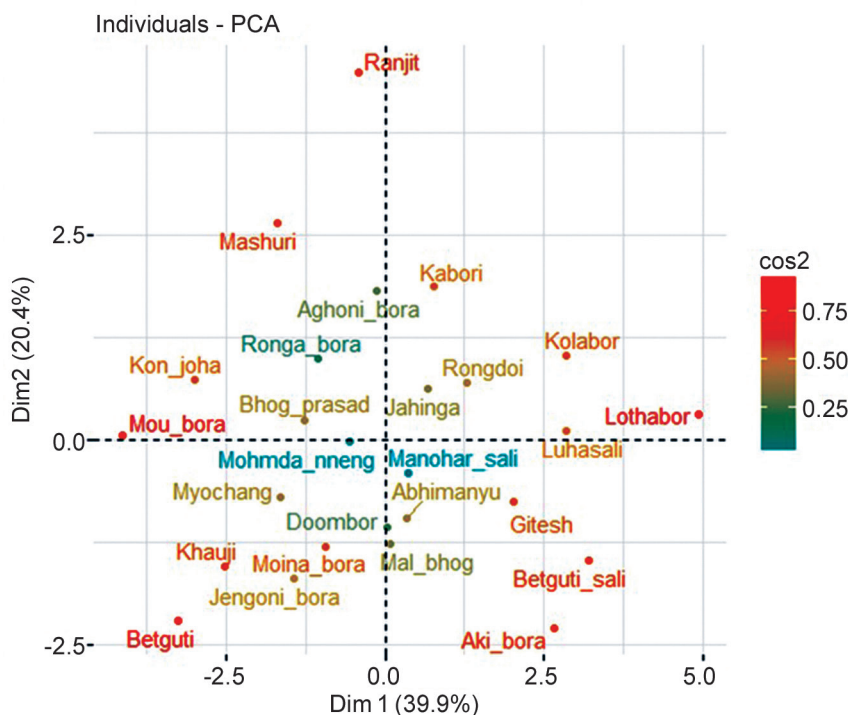


Fig 1 Scatter diagram showing the distribution of rice genotypes with respect to the first two principal components.

study toward the development of nitrogen efficient high-yielding varieties. The experiment identified potential genotypes like Lothabor, Betguti Sali, and Luhasali having high grain yield/plant along with high NUE which could be directly used in the lowland rice ecosystem or crossed with diverse parents for breeding high-yielding nitrogen use efficient genotypes for nitrogen limiting soils. The contrasting genotypes Lothabor vs Mou Bora for grain yield and NUE-related traits could be used for developing mapping populations. Recent molecular studies have focused on QTL mapping for grain yield and NUE related traits (Nguyen *et al.* 2016, Zhou *et al.* 2017) and with the availability of different molecular marker-based platforms in rice, mapping population for the traits of interest could be a useful resource for genetic analysis and precision breeding.

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