



Research Article

Phylogenetic studies on assam rice (*Oryza sativa* L.) accessions for nitrogen use efficiency

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Abstract

In the present investigation, phylogenetic analysis was carried out on 26 rice genotypes for nitrogen use efficiency (NUE) related traits and grain yield in order to identify similar performing genotypes over varying nitrogen (N) level. The phylogenetic trees at both N_0 and N_{60} clustered all the genotypes into four main groups. To identify genotypes performing uniformly over the different N levels, a maximal length MAST (maximum agreement sub-tree) on rooted trees was constructed for comparison between the clustering at N_0 and N_{60} , which identified seven genotypes grouped into four clusters in the MAST. Phylogenetic analysis revealed that for NUE related traits and grain yield, the clustering pattern for these seven genotypes was independent of the N levels. The study further suggests stability analysis to get more realistic picture.

Key words

Cluster, nitrogen use efficiency, N level, phylogenetic tree, rice

Introduction

Rice (*Oryza sativa* L.) is the world's most important staple food crop of more than 60% of the world's population (Das and Sarma, 2015). Future increases in rice production required to feed the growing population will rely primarily on the genetic improvement of rice cultivars. Low NUE comprises a major problem for rice production among the various factors that significantly reduce rice productivity. Efficient use of N in agricultural practice can increase yield, decrease production costs and reduce the risk of environmental pollution (Zhang *et al.*, 2009). By developing resource-efficient varieties, plant breeders can contribute to improve the sustainability of agricultural ecosystems.

Northeast (NE) India is considered to be an important hotspot of rice cultivars since long. Therefore, indigenous rice germplasm of NE India, especially Assam rice collection is enriched with wide genetic diversity and valuable gene system for yield and adaptability. The genetic diversity present in the collections provides an opportunity to identify the outstanding N use efficient lines/varieties for use in low, high or for all environments or for further breeding of high yielding N use efficient varieties suitable for the rainfed lowland rice ecosystem. In the present investigation, phylogenetic studies were carried out on 26 rice genotypes in order to identify similar performing genotypes over varying N levels.

Materials and Methods

A set of 26 rice genotypes consisting of 21 landraces and 5 high yielding varieties of Assam

(Table 1) were tested under two different environments- E_1 : 0 kg N ha⁻¹ (N_0) and E_2 : 60 kg N ha⁻¹ (N_{60}) with fixed P and K @ 20 kg ha⁻¹ during two successive Kharif seasons (2014 and 2015). The genotypes were evaluated in randomized block design with three replications at the Instructional-cum-Research Farm of Assam Agricultural University, Jorhat. One half of the total requirement of urea and full doses of SSP and MOP along with vermicompost @ 10 t ha⁻¹ were applied at the time of final land preparation as a basal dose. The remaining urea was applied in two equal splits as top dressing at tillering and panicle initiation stage.

Thirty-five days old seedlings were transplanted in the main field on 17th July 2014 and 2015. A single seedling was planted per hill with a spacing of 20 × 20 cm. Standard agronomic practices and plant protection measures were adopted to raise a healthy crop.

A random sample of 10 hills/plot was observed for 9 different traits, directly or indirectly related to N assimilation namely, chlorophyll *a* (CHL *a*), chlorophyll *b* (CHL *b*), total chlorophyll (TCHL), nitrate reductase activity (NRA), grain N (Ng), physiological N use efficiency (PNUE), N utilization efficiency (NUE), N harvest index (NHI) and grain yield (GY). For estimation of NUE related traits, the samples were taken at booting stage and oven dried at 70°C up to constant weight.

The N-content was determined by Micro-Kjeldahl's method (Jackson, 1974), while PNUE (Singh *et al.*, 1998), NUtE (Ayneband *et al.*, 2014) and NHI (Dawson *et al.*, 2008) was estimated following standard procedures described earlier and as per the following formulae:

$$\text{PNUE (kg kg}^{-1}\text{)} = \frac{\text{Biomass production}}{\text{Amount of N in the plant at maturity}} \times \text{Grain yield}$$

$$\text{NUtE (kg kg}^{-1}\text{)} = \frac{\text{Grain yield}}{\text{Amount of N in the plant at maturity}}$$

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

To study the genetic diversity among genotypes at N_0 and N_{60} level, phylogenetic analysis was done based on the performance of 26 genotypes. Dissimilarity matrix of usual Euclidean distances among the genotypes was worked out and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) hierarchical clustering method (Sokal and Michener, 1958) was used to construct the phylogenetic tree. The analysis was done using the software DARwin v. 6. Pearson correlation coefficients were worked out in Excel 2007.

Results and Discussion

Phylogenetic analysis constructed phylogenetic trees at N_0 and N_{60} for comparing the genotypic performance are presented in Fig. 1 and 2, respectively. The phylogenetic tree at N_0 clustered the genotypes studied into four main groups (Fig. 1). Cluster I consisted of 15 genotypes indicating the closest relationships between these genotypes. The cluster II exhibited six genotypes which were highly dissimilar from the genotypes of other clusters. The genotypes Betguti Sali, Myochang and Ronga Bora were clustered together under group III; whereas Khauji and Mohmda Nneng belonged to cluster IV.

Similarly, genotypes under N_{60} were grouped into four clusters, cluster I and II comprised of fifteen and six genotypes, respectively (Fig. 2). In cluster III, four genotypes showed the closest relationships with each other and this cluster could be further subdivided into two sub-clusters; sub-cluster III-A had Mashuri and Bhog Prasad, and III-B included Kolabor and Lothabor. The cluster IV with only Manohar Sali was distant from the remaining genotypes.

To identify genotypes performing uniformly over the different N levels, a maximal length MAST on the rooted tree was constructed for comparison between the clustering at N_0 and N_{60} , which identified seven genotypes into four -maximum agreement sub-trees (Fig. 3). The sub-tree I with

Mohmda Nneng and Khauji, II with Betguti Sali and Myochang, III with Lothabor and Bhog Prasad and IV with Manohar Sali were in agreement in both the N levels. Phylogenetic analysis revealed that for NUE related traits and grain yield, the clustering pattern for these seven genotypes was independent of the N levels suggesting that these genotypes were stable in performance for the traits. This was corroborated by the presence of significant correlation coefficients of grain yield with PNUE, NUtE and NHI at both N_0 and N_{60} level (Table 2). Grain yield was also correlated with CHL *b* at N_0 and Ng at N_{60} . These yield components were mostly correlated *inter se*. Significant association of grain yield with NUtE (Fageria *et al.*, 2010), NUE (Wei *et al.*, 2011; Zhu *et al.*, 2016; Naveen and Uma, 2016), NHI (Lakew, 2015) and Ng (Lakew, 2015) was also reported in earlier studies. The cluster means of the MAST revealed that the genotypes of cluster II and III viz., Myochang/Betguti Sali and Lothabor/Bhog Prasad had high mean grain yield along with concomitantly high performance for the NUE related traits (Table 3).

It can be concluded that genotypes Manohar Sali, Mohmda Nneng, Khauji, Myochang, Betguti Sali, Lothabor and Bhog Prasad could be considered as potential genotypes for general adaptability for both N limiting and non-limiting conditions. The latter four genotypes, being high yielders, could be directly used for low input farming systems. Stability of these genotypes over varying levels of N however needs to be carried out for more realistic information.

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Table 1. Details of genotypes used in the present investigation

Sl. No.	Genotype	Pedigree	Origin	Sl. No.	Genotype	Pedigree	Origin
1	Lothabor	Landrace	Assam, India	14	Aki Bora	Landrace	Assam, India
2	Myochang	Land race	Assam, India	15	Doombor	Land race	Assam, India
3	Luhasali	Land race	Assam, India	16	Bhog Prasad	Land race	Assam, India
4	Kolabor	Land race	Assam, India	17	Rongdoi	Landrace	Assam, India
5	Mohmda Nneng	Land race	Assam, India	18	Mal Bhog	Land race	Assam, India
6	Khauji	Land race	Assam, India	19	Mou Bora	Land race	Assam, India
7	Ronga Bora	Land race	Assam, India	20	Kabori	Land race	Assam, India
8	Betguti Sali	Land race	Assam, India	21	Kon Joha	Land race	Assam, India
9	Abhimanyu	Land race	Assam, India	22	Aghoni Bora	Gandhi Bora/Kmj 1-52-2	Assam, India
10	Jahinga	Land race	Assam, India	23	Gitesh	Akisali/Kushal	Assam, India
11	Jengoni Bora	Land race	Assam, India	24	Manohar Sali	Lati Sail/Guachari	Assam, India
12	Betguti	Land race	Assam, India	25	Mashuri	Taichung 65/Mayang Ebos 6080/2	Malaysia
13	Moina Bora	Land race	Assam, India	26	Ranjit	Pankaj/Mashuri	Assam, India



Table 2. Correlation matrix (Pearson) at N₀ (below diagonal) and N₆₀ (above diagonal)

Trait	CHL <i>a</i>	CHL <i>b</i>	TCHL	NRA	Ng	PNUE	NUtE	NHI	GY
CHL <i>a</i>		0.961	0.998	-0.070	0.237	0.116	0.262	0.237	0.263
CHL <i>b</i>	0.871		0.977	-0.043	0.268	0.117	0.243	0.318	0.240
TCHL	0.961	0.940		-0.064	0.246	0.117	0.259	0.257	0.259
NRA	-0.109	-0.199	-0.142		0.231	0.069	-0.116	0.123	-0.014
Ng	0.283	0.036	0.219	0.454		0.339	0.635	0.785	0.803
PNUE	0.109	0.271	0.117	-0.191	-0.237		0.733	0.514	0.640
NUtE	0.204	0.351	0.223	-0.225	-0.183	0.834		0.683	0.952
NHI	0.381	0.221	0.340	0.143	0.698	0.372	0.464		0.675
GY	0.322	0.390	0.318	-0.019	0.202	0.708	0.906	0.669	

Values in bold are different from 0 with a significance level alpha=0.05

Table 3. Cluster means for the N-use related traits and grain yield of the MAST

Cluster	CHL <i>a</i>	CHL <i>b</i>	TCHL	NRA	Ng	PNUE	NUtE	NHI	GY
I	3.01 (2)	0.78 (2)	3.79 (2)	29.19 (4)	1.52 (3)	19.63 (4)	7.53 (3)	70.67 (4)	15.92 (4)
II	3.32 (1)	0.90 (1)	4.21 (1)	42.18 (3)	1.59 (2)	23.27 (1)	8.52 (2)	71.85 (3)	18.47 (2)
III	2.89 (3)	0.75 (3)	3.57 (3)	67.62 (2)	1.59 (2)	21.54 (2)	8.76 (1)	72.60 (2)	18.61 (1)
IV	2.63 (4)	0.64 (4)	3.30 (4)	78.88 (1)	1.67 (1)	21.07 (3)	6.93 (4)	72.93 (1)	15.96 (3)

Figures in parentheses are the ranks for the cluster means

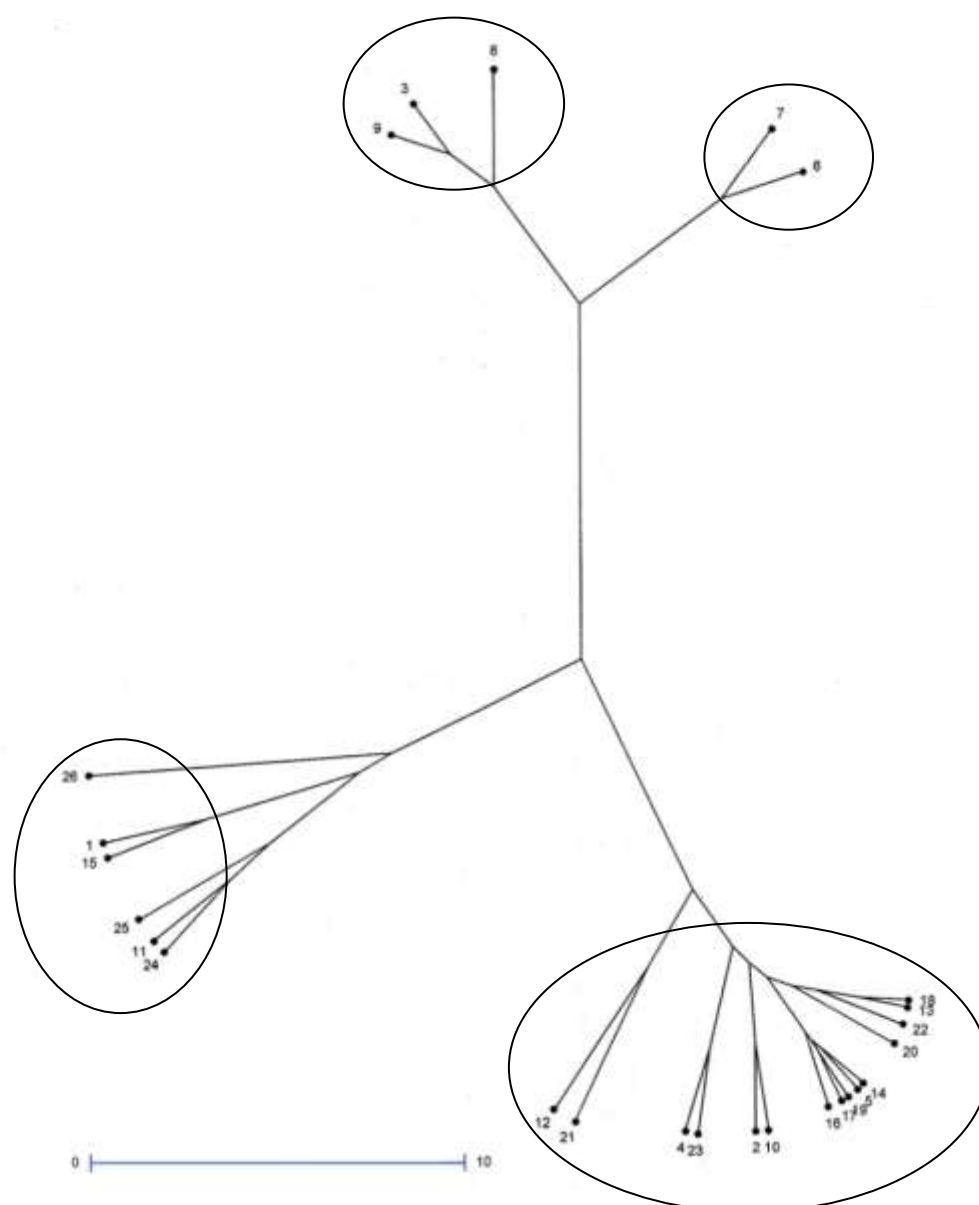


Fig. 1. UPGMA based clustering of the 26 genotypes for NUE related traits and grain yield at N₀

- | | | |
|----------------|-----------------|-----------------|
| 1 Kabori | 10 Abhimanyu | 18 Rongdoi |
| 2 Lothabor | 11 Jahinga | 19 Mal Bhog |
| 3 Myochang | 12 Jengoni Bora | 20 Mou Bora |
| 4 Luhasali | 13 Betguti | 21 Kon Joha |
| 5 Kolabor | 14 Moina Bora | 22 Aghoni Bora |
| 6 Mohmda Nneng | 15 Aki Bora | 23 Gitesh |
| 7 Khauji | 16 Doombor | 24 Manohar Sali |
| 8 Ronga Bora | 17 Bhog Prasad | 25 Mashuri |
| 9 Betguti Sali | | 26 Ranjit |

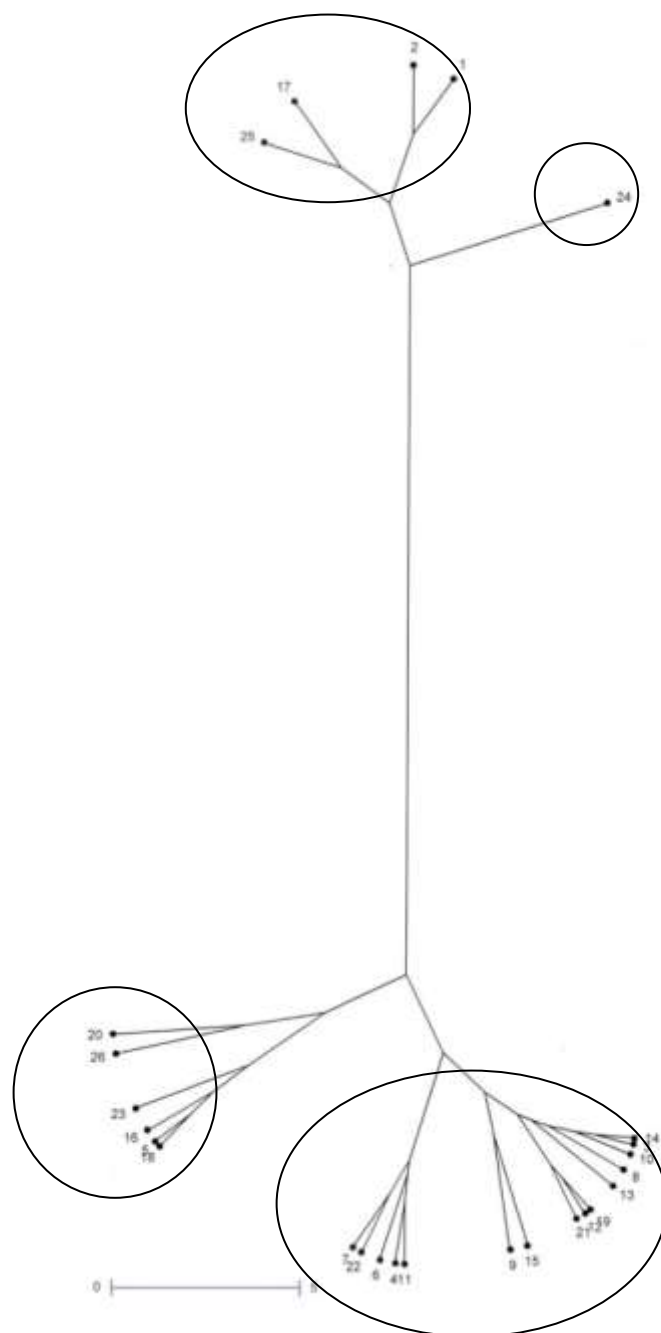


Fig. 2. UPGMA based clustering of the 26 genotypes for NUE related traits and grain yield at N₆₀

- | | | |
|----------------|-----------------|-----------------|
| 1 Kabori | 10 Abhimanyu | 18 Rongdoi |
| 2 Lothabor | 11 Jahinga | 19 Mal Bhog |
| 3 Myochang | 12 Jengoni Bora | 20 Mou Bora |
| 4 Luhasali | 13 Betguti | 21 Kon Joha |
| 5 Kolabor | 14 Moina Bora | 22 Aghoni Bora |
| 6 Mohmda Nneng | 15 Aki Bora | 23 Gitesh |
| 7 Khauji | 16 Doombor | 24 Manohar Sali |
| 8 Ronga Bora | 17 Bhog Prasad | 25 Mashuri |
| 9 Betguti Sali | | 26 Ranjit |

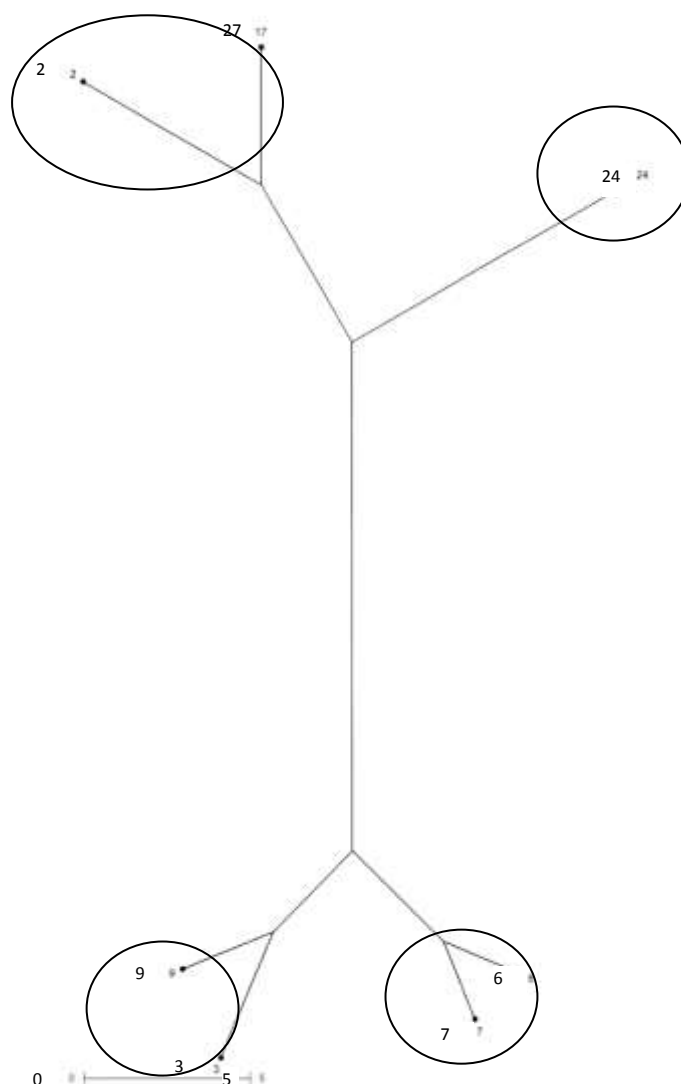


Fig. 3. Maximum agreement sub-tree for the UPGMA based clustering of the genotypes at N_0 and N_{60}

2 Lothabor
3 Myochang
6 Mohmda Nneng

7 Khauji
9 Betguti Sali

17 Bhog Prasad
24 Manohar Sali