Original Research Article

Phenotyping Rice (*Oryza sativa*. L) Genotypes for Nitrogen Use Efficiency

ABSTRACT:

Aim: The present study was aimed to identification of rice genotypes from the existing germplasm or development of rice cultivars that can make the best use of N in low nitrogen soils is essential for the sustainability of agriculture

Study design: The experiment was laid out in split plot design with three nitrogen levels as main plots and twenty five rice genotypes as subplots.

Place of study: Screening experiment was conducted during December 2021-April 2022 at wetland, Tamil Nadu Agricultural University, Coimbatore.

Methodology: Twenty five rice genotypes were cultivated with three nitrogen levels 0% Recommended Dose of Nitrogen (RDN) (T0), 50% RDN (T1), 100% RDN(T2control) under field condition, morphological traits such as plant height, leaf area; number of tillers, total dry weight and leaf nitrogen were recorded along with leaf nitrogen and photosynthetic nitrogen use efficiency (PNUE). Two way ANOVA and Cluster analysis were used to analyze the data.

Result: The results revealed that among twenty five rice genotypes, G3 recorded the highest photosynthetic nitrogen use efficiency (25.10) followed by G7 (22.62) under 0% RDN (T0). The lowest PNUE was recorded in G11 (5.99) 100% RDN (T2). Across the genotypes G3, G7 and G11 genotypes exhibited highest NUE.

Conclusion: Observations relies that the rice genotype G3, G7 and G1 exhibit high nitrogen use efficiency.

Key words: *Rice genotypes, nitrogen treatments, Photosynthetic nitrogen use efficiency.*

1. INTRODUCTION:

Rice *(Oryza sativa L.)* is a cornerstone of the population's diet of East and South Asia, the Middle East, the West Indies and the Latin America. It is an excellent source of niacin, thiamine, riboflavin, and dietary fiber, and it has about 13% and 20% protein and dietary energy, respectively [1]. The majority of the world's population relies on rice for 50-80% of their daily dietary requirements [2]. Rice is grown in over 100 countries, accounting for 90 percent of the earth's population. As per United State Department of Agriculture (USDA, 2019) China ranks first in rice production (211.4 million metric tons) followed by India (117 million metric tons).

Rice production is influenced by a number of factors including climate, soil physical condition, soil fertility and water management, as well as sowing date, cultivar, seed rate, weed control and nutrient management. Among the fertilizers, proper nitrogen (N) management is required to accelerate plant growth and development. N is required for the formation of cell components and plant tissue in rice. Although nitrogen is abundant in the atmosphere, only legumes can convert atmospheric N_2 to plant-available forms through a symbiotic biological process involving rhizobium bacteria and the plant roots. Nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) are plant-available inorganic forms of nitrogen. However, urea $(NH_2)_2CO$ is probably the most often used nitrogen fertilizer in rice. Globally, India stands third in N fertilizer consumption and second in fertilizer production [3]. In recent years, the expense of fertilizer concerns about long-term soil productivity and ecological stability in relationship to nitrogen fertilizers has emerged as challenging issues [4]. The interaction between rice plant development, yield and N consumption is becoming more understood at the physiological and molecular levels.

Nitrogen use efficiency of rice is low compared to other cereals, a major part of N applied to rice released as gaseous N, affecting environment and reducing economic efficiency. It is necessary at this juncture to recognize the importance of identifying or developing genotypes with high NUE in rice to reduce the cost of cultivation and also to protect the environment [5]. Attempts have been made using cluster analysis to disperse rice genotypes into qualitative groups for better NUE based on similar response [6]. With this background, present study was aimed to identification of rice genotypes from the existing germplasm or development of rice cultivars that can make the best use of N in low nitrogen soils is essential for the sustainability of agriculture.

2. MATERIALS AND METHODS:

2.1 LOCATION DESCRIPTION

The field trail was conducted in the field no.A7 during December 2021- April 2022, Tamil Nadu Agricultural University, Coimbatore which is geographically placed under western agro climatic zone of Tamil Nadu at 11° N latitude and 77° E longitude with an altitude of 426.72 m above mean sea level. The seeds of 25 genotypes collected from the Department of Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agriculture University, Coimbatore. The list of rice genotypes used in the study was given in table 1.

2.2 Weather and climate

During cropping period December 2021- April 2022, the average maximum and minimum temperature were 32.06°C and 21.83°C, respectively. The total rainfall received during the cropping period was 121.5 mm. The maximum and minimum relative humidity was 83.65 percent and 46.46 percent respectively. The average bright sunshine hour was 7.06 and the mean wind velocity recorded was 4.96 km hr^{-1}.

2.3 Experimental Details

2.3.1 Field experiment

The field experiment designed with split plot with three nitrogen levels as main plots and 25 rice genotypes as sub plots with three replications. The crop at each plot was treated with three levels of nitrogen fertilizer *viz*. 0% Recommended Dose of Nitrogen (RDN), 50% RDN and 100% RDN (as control). Nitrogen was applied as per treatment in each plot in the form of Urea (46 % N) in four equal splits as basal and top dressing at active tillering, panicle initiation and 50 % flowering stages. In the main field 25 days old seedlings were transplanted manually with the spacing of 25×25. All other management practices were followed uniformly for raising crops as per the Crop production technique of Agricultural Crops (2020). Morphological traits *viz.* plant height (cm), number of tillers; leaf area (cm^2), total dry weight (g), and leaf nitrogen were recorded. The plant height was measured from the base of the shoot to the longest leaf at active tillering stage. After the panicle initiation, the plant height was measured from the base of the shoot to the panicle tip and expressed in centimeter (cm). The number of tillers per plant was counted manually. Leaf area was measured by the maximum length and breadth of the leaves in the tagged plants of each genotype; leaf area was calculated by (L×B×K). It is expressed in cm^2 plant^{-1}. For total dry weight the harvested plants were shade dried for 2 days and then the plant samples were kept in hot air oven at 80^{0}C for 48hours and the samples were weighed and expressed as gram plant^{-1}.

Leaf nitrogen was estimated by micro-kjeldhal method [7]. About, 1g of powdered leaf sample was transferred into the conical flask and diacid mixture (Sulphuric acid & Perchloric acid (5:2)) was added and then the mouth is closed with funnel and left for overnight digestion. Further digestion was carried by placing it in sand bath until clear solution was obtained. The contents were filtered and transferred to 100 ml volumetric flask using minimum quantity of water and volume made upto 100 ml. About, 10 ml of sample mixed with 10 ml of 40% sodium hydroxide was taken at collection chamber in *Kel plus* nitrogen analyzer with 2% boric acid in tall form beaker and add double indicator (Bromocresol Green And Methyl Red) at the delivery end. The distillation was carried out, the colour of boric acid changes to blue indicating the end of distillation. Then the boric acid was titrated against 0.1N Sulphuric acid. The colour changes from blue to wine red, the end point and titer value was noted and further calculation was carried out. The nitrogen calculated was expressed in mg g^{-1} of sample.

Photosynthetic nitrogen use efficiency is defined as the rate of CO_2 assimilation per unit of leaf Nitrogen [8,9]

Photosynthetic N use efficiency =Net photosynthesis rate / Leaf N content

3. Statistical analysis

The data was arranged in split plot design with three replication used to statistically analyze each parameter. Specific pair wise difference between means was evaluated at 0.05 significance level using Fisher's least significance difference (LSD) test. The interrelationship between the recorded Photosynthetic NUE parameter was evaluated using cluster analysis. Statistical tool R-Studio was used to conduct all test analysis.

4. Results and Discussion:

4.1 Effect of nitrogen on rice genotypes:

The main effects of nitrogen levels and genotypes as well as the interaction effects between them were significant in parameters such as plant height (PH), number of tillers (NT), leaf area (LA), total dry weight (TDW) and leaf nitrogen (LN) (Table 2).

4.2 Assessment of rice genotypes under different nitrogen levels

Rice genotypes differed significantly in traits such as, PH, NT, LA, TDW and LN at 0% RDN, 50% RDN and 100% RDN.

4.2.1 Plant height

Plant height is a critical trait represents of plant the growth and development. The plant height was significantly influenced by three nitrogen levels of twenty five rice genotypes are presented in table 3. The result showed that, the plant height reduced significantly in treatments 0% RDN (T0) (15.29%) & 50% RDN (T1) (6.92 %) compared with 100% RDN (T2). The rice genotypes G5, G14, G21 showed the least percent change where the plant height decreased 4.22%, 6.48% and 6.8% respectively at 0% RDN (T0) and 0.82%, 1.94%, 0.28% respectively at 50% RDN (T1) compared with 100% RDN (T2). In genotypes like G12 (23.36%, 13.29%), G16 (25.12%, 13.32%) and G19 (23.19%, 14.21%) plant height was decreased highly at 0% RDN and 50% RDN in compared with 100% RDN. The above result was accordance with Manzoor [10] that the increase in plant height was noted with increased nitrogen application which enhances vegetative growth. Increasing the amount of nitrogen fertilizer engendered the plant to grow tall as this increased the pace at which nitrogen translocated from the culm to the leaves and produced photosynthates, which improved the mobilization of nutrients to the growing panicle. The plant height increases in response to supply of nitrogen fertilizers due to nitrogen availability which increases the leaf area resulting in enhancement of photo assimilation and dry matter accumulation.

4.2.2 Number of tillers

The growth and productivity of cereal crops is correlated with the number and tiller development [11]. The number of productive tillers per plant directly influences the total number of tillers, which in turn impacts grain yield. The data on number of tillers influenced by three nitrogen levels of twenty five rice genotypes are presented in table 3. The result showed that, the number of tillers reduced significantly among the treatments in compared with 100% RDN (T2). The reduction was about 36.76 % in 0% RDN (T0) and 20.09 % in 50% RDN (T1). Among 25 rice genotypes, G4, G5, G14 showed the least percent change where the number of tillers decreased to 17.61%, 19.99% and 23.23% respectively at 0% RDN (T0) 4.60%, 4.65% and 2.30% respectively at 50% RDN (T1) compared with 100% RDN (T2). In genotypes like G9 (52.06%, 40.60%), G13 (55.70%, 42.34%) and G19 (47.40%, 42.16%) number of tillers decreased more at 0% RDN and 50% RDN in compared with 100% RDN. In accordance with the above result, [12] reported that increase in nitrogen supply increases the number of tillers at various stages in rice genotypes. Nitrogen supply involves directly or indirectly in cell enlargement, cell formation and production of new tissues. [13] also reported that the reduction in nitrogen supply caused decrease in number of tillers.

4.2.3 Leaf area (LA)

Leaf area is an important parameter closely related to physiological process which controls dry matter production and yield. The values on leaf area influenced by three nitrogen levels of twenty five rice genotypes are presented in table 3. The result showed that, leaf area significantly decreased among treatments when compared to 100% RDN (T2), leaf area decreased by around 25.45% in 0% RDN (T0) and 17.97 % in 50% RDN (T1). Among 25 rice genotypes, G11, G23, G25 showed the least percent change where the leaf area decreased to 10.48%, 11.91% and 6.65% respectively at 0% RDN (T0) and 9.55%, 9.70% and 4.48% respectively at 50% RDN compared with 100% RDN (T2). In genotypes like G3 (41.19%, 28.94%), G16 (40.82%, 29.24%) and G21 (43.39%, 36.97%) recorded lesser leaf area highly at 0% RDN (T0) and 50% RDN (T1) compared with 100% RDN (T2). Since the green leaves surface area determines the production of photosynthate, leaf area is a critical component in the generation of dry matter [14]. It was observed that influence of nitrogen enhances the rate of leaf expansion leading to interception of solar radiation enhancement.

4.2.4 Total dry weight

Total dry weight is a significant factor to measure the total photosynthates produced over time which is influenced by the nitrogen application. The result on total dry weight influenced by three nitrogen levels of twenty five rice genotypes are presented in table 3. The result showed that, total dry weight reduced significantly among the treatments compared with 100% RDN, the reduction was about 49.30% in 0% RDN and 21.67 % in 50% RDN. Among 25 rice genotypes, the rice genotypes G4, G7, G22 shows the least percent reduction where the total dry weight decreased 23.75%, 28.94% and 16.62% respectively at 0% RDN (T0) and 5.99%, 6.84% and 2.66% respectively at 50% RDN (T1) compared with 100% RDN (T2). In genotypes like G5 (73.66%, 52.52 %), G9 (72.87%, 42.28%) and G14 (73.83%, 39.33%) were recorded lesser total dry weight highly at 0% RDN and 50% RDN in compared with 100% RDN (T2). The above result was accordance with [15], that maximum accumulation of dry matter was noticed during grain filling stage with higher nitrogen level compared with lower nitrogen level pants.

4.2.5 Leaf Nitrogen

The variation in leaf nitrogen is responsible for leaf photosynthetic rate, increase in CO_2 concentration, carboxylation capacity and *Rubisco* activity (Li *et al*., 2013).The leaf nitrogen influenced by three nitrogen levels of twenty five rice genotypes is presented in table 3. According to the results, leaf nitrogen decreased significant when compared to 100% RDN, it decreased by about 58.71 % in 0% RDN (T0). Among 25 rice genotypes, G19, G21, G24 showed the least percent change where the leaf nitrogen decreased 49.24%, 46.59% and 43.90% respectively at 0% RDN (T0) and 38.43%, 37.60% and 34.49% respectively at 50% RDN (T1) compared with 100% RDN (T2). In genotypes like G2 (68.91%, 61.72%), G12 (65.34%, 60.18%) and G14 (66.40%, 62.41%) leaf nitrogen decreased more at 0% RDN and 50% RDN in compared with 100% RDN (T2). The above investigation was in line with [16] who stated that plant N content delays leaf senescence and duration of photosynthesis, which lead to greater N allocation and higher mesophyll conductance [17]. Increasing the nitrogen content in leaf and delaying senescence especially flag leaf could enhance the photosynthetic efficiency biomass.

4.2.6 Photosynthetic nitrogen use efficiency (PNUE)

A significant fraction of the total leaf nitrogen is made up of photosynthetic proteins, such as vast amounts of *Rubisco* and to chlorophyll-protein complexes. High N content results in reduction of PNUE that indicates a less accumulation of *Rubisco* [18]. Photosynthetic nitrogen use efficiency (PNUE) differed with genotype and also with nitrogen which reduced with increased nitrogen application and leaf nitrogen content. Among the genotypes, the highest value of PNUE index (Fig. 1) was observed in genotype G3 followed by G7, G22 G14 in the entire treatments 0% RDN (T0), 50% RDN (T1) and 100% RDN (T2). In genotypes like G12, G16 & G24 higher PNUE index was observed in 50% RDN (T1).Photosynthetic nitrogen use efficiency is defined as the rate of CO_2 assimilation per unit leaf N [19]. PNUE is essential criteria for evaluating the efficiency of plants use N to sustain growth and photosynthesis. It is frequently identified that PNUE is favorably correlated with leaf N content per area [20, 9].

4.3 Cluster analysis

Based on individual parameters, it is difficult to discuss nitrogen use efficiency of all twenty five investigated genotypes. Therefore, we applied cluster analysis to compare photosynthetic nitrogen use efficiency in all treatments and in three stages (active tillering, 50% flowering and physiological maturity) simultaneously. The classification tree (Fig.2), which takes account of all treatments, demonstrated that the high nitrogen use efficient genotypes were G3 & G7 at all three stages. The high nitrogen use efficient rice genotypes were grouped at various clusters, at physiological maturity stage rice genotypes such as G3 & G7 present in group I, at 50% flowering stage G3 & G7 were grouped in group III along with G1 are also nitrogen use efficient, at active tillering the genotype G3 & G7 were grouped under group V. It was also found that lowest nitrogen use efficient genotype was G11 which was grouped under group VII at active tillering & 50% flowering, in group VI at physiological maturity where G23 also performed low nitrogen use efficiency. Based on these results, it is concluded that G3, G7, G1genotypes were found to higher nitrogen use efficient and G11& G23 were lower nitrogen use efficient genotype.

5. Conclusion:

In the present investigation, we demonstrated the presence of substantial difference among the rice genotypes against nitrogen use efficiency. The rice genotypes G3 (AMPL 485), G7 (AMPL 451), G1 (AMPL 446) has recorded higher nitrogen use efficiency where as G11 (AMPL 254) & G23 (AMPL 259) has recorded lower nitrogen use efficiency. The genetic variations constitute a stable platform for describing the rice genotypes that may be cultivated and helpful for improving crop output and assessing the degree of efficiency in various genotypes for use in breeding programmes. Identifying and selecting the most nitrogen use efficient genotypes is very important for agriculture to improve quality and yield in rice.

Table.1. List of rice genotypes used in the study

Genotype Number	Genotype Name
G1	AMPL 446
G2	AMPL 494
G3	AMPL 485
G4	AMPL 330
G5	AMPL249
G6	AMPL478
G7	AMPL451
G8	AMPL 469
G9	AMPL 430
G10	AMPL501
G11	AMPL254

G12	AMPL287
G13	AMPL 475
G14	AMPL 258
G15	AMPL 452
G16	AMPL 295
G17	AMPL 244
G18	CO 43
G19	CO(R) 50
G20	DRR DHAN 40
G21	CO51
G22	AMPL 470
G23	AMPL 259
G24	AMPL 306
G25	AMPL 269

AMPL-Associated Mapping Line

Table .2. Analysis of variance (mean squares) for morphological traits of twenty five rice genotypes in three different nitrogen levels

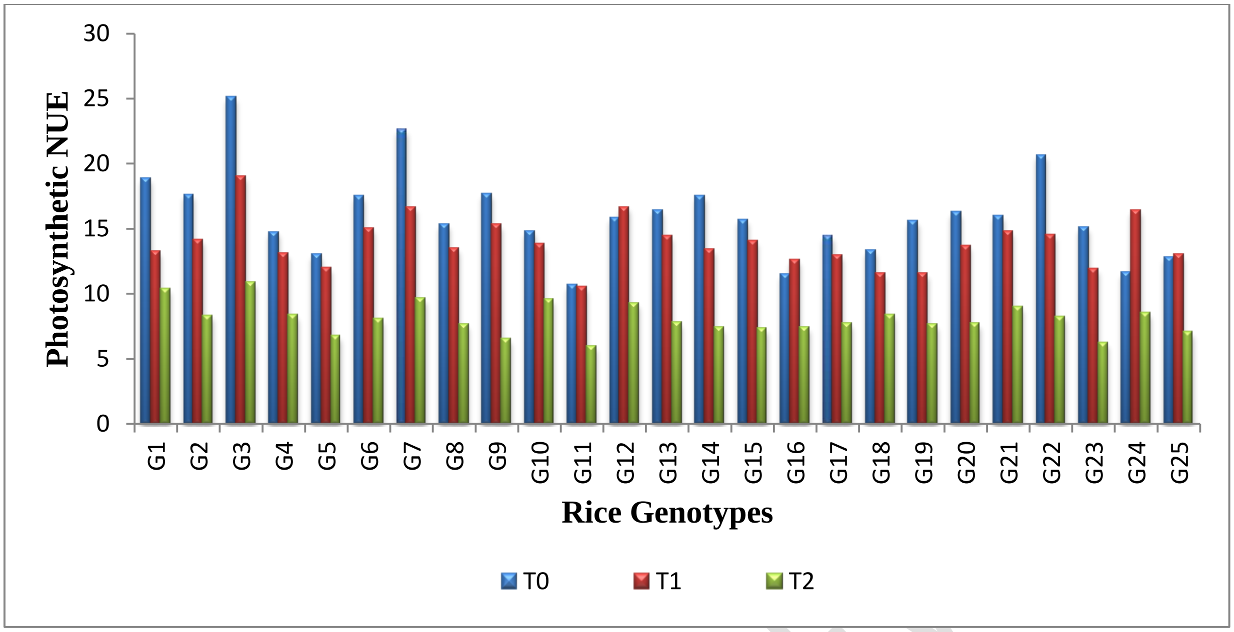
TRAIT	Source of Variation			
	PHYSIOLOGICAL MATURITY			
	T	**G**	**T*G**	**Error**
df	2	24	48	144
Plant height	4955.4**	2660.2**	54.9**	26.57
Number of tillers	1799.5**	107.21**	14.79**	1.54
Leaf area	6033161**	767351**	58294**	9820
Total dry weight	73155**	8316**	1030**	28.12
Leaf nitrogen	88.31**	0.288**	0.87**	0.015

T-Nitrogen treatments; G-Rice genotypes; df- degrees of freedom; Error = within group variance; * $p \leq 0.001$; **$p \leq 0.01$

Table.3. Effect of different nitrogen levels on morphological traits of rice genotypes

Genotype	PH		NT		LA		TDW		LN	
	% change over 100% RDN		% change over 100% RDN		% change over 100% RDN		% change over 100% RDN		% change over 100% RDN	
	0% RDN	50% RDN	0% RDN	50% RDN	0% RDN	50% RDN	0% RDN	50% RDN	0% RDN	50% RDN
G1	-7.85	-2.32	-36.03	-24.26	-36.76	-24.27	-45.12	-15.56	-57.50	-48.83
G2	-20.18	-7.44	-23.75	-7.86	-18.96	-25.45	-46.80	-16.97	-68.91	-61.72
G3	-17.97	-10.47	-26.12	-21.38	-35.33	-26.87	-56.80	-37.42	-56.53	-53.29
G4	-13.11	-2.15	-17.61	-4.60	-38.80	-22.83	-23.75	-5.99	-49.57	-43.21
G5	-4.22	-0.82	-19.99	-4.65	-36.95	-20.93	-73.66	-52.52	-59.29	-52.07
G6	-19.09	-10.50	-32.05	-11.53	-10.48	-9.55	-48.87	-25.37	-58.77	-49.83
G7	-8.64	-3.22	-38.77	-14.08	-24.01	-13.44	-28.94	-6.84	-64.52	-50.17
G8	-11.23	-5.63	-35.23	-23.92	-17.94	-13.61	-53.68	-17.22	-61.67	-46.55
G9	-19.80	-3.92	-52.06	-40.60	-14.44	-11.49	-72.87	-42.28	-63.84	-45.33
G10	-13.73	-9.61	-45.21	-5.99	-19.54	-14.41	-33.57	-14.62	-58.33	-54.16
G11	-15.65	-3.72	-31.13	-4.95	-40.82	-29.24	-51.50	-12.90	-62.82	-59.88
G12	-23.36	-13.29	-44.84	-38.16	-17.14	-10.61	-67.65	-14.53	-65.34	-60.18
G13	-20.52	-7.86	-55.70	-42.34	-24.76	-28.39	-64.64	-38.96	-60.81	-54.83
G14	-6.48	-1.94	-23.23	-2.30	-43.39	-36.97	-73.83	-39.33	-66.40	-62.41
G15	-12.15	-9.99	-40.37	-39.71	-25.62	-13.37	-31.57	-11.64	-62.38	-51.84
G16	-21.20	-8.53	-43.96	-19.67	-11.91	-9.70	-46.61	-20.10	-55.28	-42.72
G17	-25.12	-13.32	-44.19	-13.97	-16.87	-13.55	-65.98	-38.92	-57.52	-54.92
G18	-13.88	-3.68	-33.33	-18.55	-14.70	-10.31	-56.55	-28.97	-49.24	-38.43
G19	-23.19	-14.21	-47.40	-42.16	-19.97	-14.91	-29.70	-10.00	-59.23	-42.91
G20	-17.07	-10.95	-40.74	-15.02	-32.50	-23.05	-55.17	-9.36	-57.85	-48.11
G21	-6.80	-0.28	-38.12	-32.85	-6.65	-4.48	-32.80	-13.96	-46.59	-37.60
G22	-7.92	-8.21	-34.88	-33.72	-41.19	-28.94	-16.62	-2.66	-64.81	-52.14
G23	-12.20	-5.20	-31.23	-12.76	-19.72	-15.69	-40.44	-12.90	-62.48	-48.70
G24	-18.03	-7.21	-42.47	-14.43	-38.02	-12.65	-54.41	-7.34	-43.90	-34.49
G25	-11.65	-5.14	-37.04	-9.32	-30.35	-19.69	-29.59	-7.62	-52.71	-49.45
MEAN	-15.29	-6.92	-36.76	-20.09	-25.45	-17.97	-49.30	-21.67	-58.71	-49.79

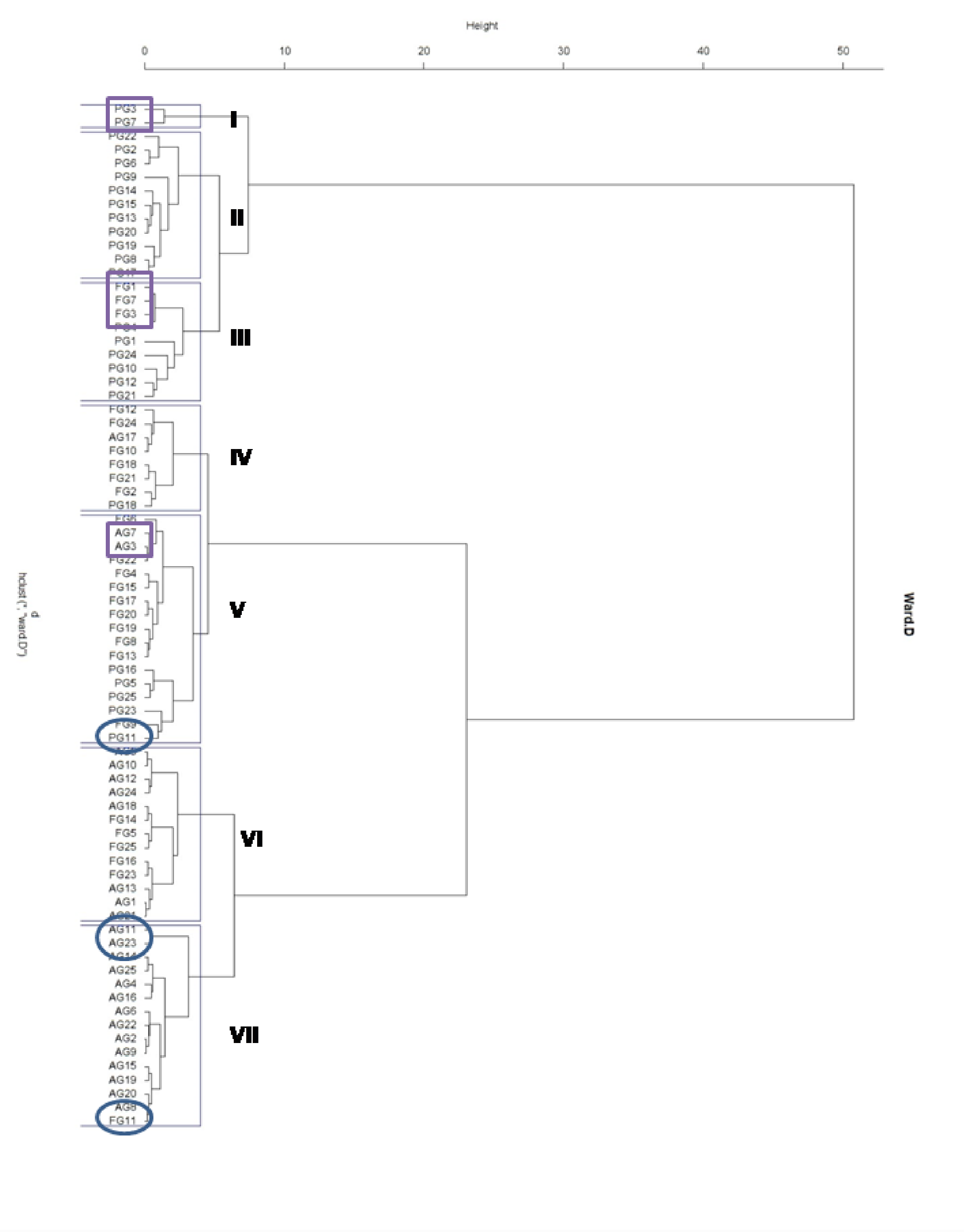
PH- Plant Height; NT-Number of tillers; LA-Leaf Area; TDW-Total Dry Weight; LN-Leaf Nitrogen



0% RDN-T0; 50% RDN-T1; 100% RDN-100%

Fig.1. PNUE of rice genotypes influenced by Nitrogen levels

Fig.2. Results of hierarchical agglomerative cluster analysis based on photosynthetic nitrogen use efficiency in three treatments. The high PNUE and low PNUE genotypes in cluster are in frame.



Reference:

1. FAO W. Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria. 2009; 240.
2. Khush GS. What it will take to feed 5.0 billion rice consumers in 2030. Plant molecular biology. 2005 Sep;59(1):1-6.
3. Gowariker V, Krishnamurthy VN, Gowariker S, Dhanorkar M, Paranjape K. The fertilizer encyclopedia. John Wiley & Sons; 2009 Apr 8.
4. Aulakh MS, Khera TS, Doran JW. Mineralization and denitrification in upland, nearly saturated and flooded subtropical soil II. Effect of organic manures varying in N content and C: N ratio. Biology and Fertility of Soils. 2000 May;31 (2):168-74.
5. Hirel B, Le Gouis J, Ney B, Gallais A. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. Journal of Experimental Botany. 2007 Jul 1; 58 (9):2369-87.
6. Vijayalakshmi P, Vishnukiran T, Kumari BR, Srikanth B, Rao IS, Swamy KN, Surekha K, Sailaja N, Subbarao LV, Rao PR, Subrahmanyam D. Biochemical and physiological characterization for nitrogen use efficiency in aromatic rice genotypes. Field Crops Research. 2015 Aug 1;179:132-43.
7. Yoshida, S. (1976). Physiological aspects of grain yield. Annual Review of Plant Physiology, 23(1), 437-464.
8. Good AG, Shrawat AK, Muench DG. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production?. Trends in plant science. 2004 Dec 1;9 (12):597-605.
9. Makino A, Mae T, Ohira K. Relation between nitrogen and ribulose-1, 5-bisphosphate carboxylase in rice leaves from emergence through senescence. Plant and Cell Physiology. 1984 Apr 1;25(3):429-37.
10. Manzoor Z, Awan TH, Zahid MA, Faiz FA. Response of rice crop (super basmati) to different nitrogen levels. J. Anim. Pl. Sci. 2006;16(1-2):52-5.
11. Li Y, Yang X, Ren B, Shen Q, Guo S. Why nitrogen use efficiency decreased under high nitrogen supply in rice (Oryza sativa L.) seedlings. Journal of Plant Growth Regulation. 2012 Mar;31(1):47-52.
12. Laroo, N., & Shivay, Y. S. (2011). Effect of nitrogen and sulphur levels on growth and productivity of scented rice (Oryza sativa). Current Advances in Agricultural Sciences (An International Journal), 3(1), 45-48.
13. Tanaka A, Garcia CV, Diem NT. Studies of the relationship between tillering and nitrogen uptake of the rice plant: 1. Observations on the leafing and tillering patterns of some rice varieties grown under different nitrogen levels. Soil Science and Plant Nutrition. 1965 Jan 1;11(1):9-13.

14. Cook MG, Evans LT. Nutrient responses of seedlings of wild and cultivated Oryza species. Field Crops Research. 1983 Jan 1;6:205-18.
15. Fageria NK, Baligar VC. Improving nutrient use efficiency of annual crops in Brazilian acid soils for sustainable crop production. Communications in Soil Science and Plant Analysis. 2001 Apr 30;32(7-8):1303-19.
16. Pannangpetch K. Relation between leaf senescence, leaf nitrogen content, and leaf photosynthesis. Simulation and Systems Analysis of Rice Production (SARP). (FWT Penning de Vries, HH van Laar & MJ Kropff, eds.). Pudoc, Wageningen. 1991:239-49.
17. von Caemmerer S, Evans JR. Temperature responses of mesophyll conductance differ greatly between species. Plant, Cell & Environment. 2015 Apr; 38(4):629-37.
18. Li D, Tian M, Cai J, Jiang D, Cao W, Dai T. Effects of low nitrogen supply on relationships between photosynthesis and nitrogen status at different leaf position in wheat seedlings. Plant Growth Regulation. 2013 Jul;70(3):257-63.
19. Makino A, Osmond B. Effects of nitrogen nutrition on nitrogen partitioning between chloroplasts and mitochondria in pea and wheat. Plant Physiology. 1991 Jun;96(2):355-62.
20. Gu J, Zhou Z, Li Z, Chen Y, Wang Z, Zhang H. Rice (Oryza sativa L.) with reduced chlorophyll content exhibit higher photosynthetic rate and efficiency, improved canopy light distribution, and greater yields than normally pigmented plants. Field Crops Research. 2017 Jan 1; 200: 58-70.