

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(4): 1145-1155
www.biochemjournal.com
Received: 13-02-2026
Accepted: 15-03-2026

P Priyavarshini
Division of Genetics and Plant Breeding, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

N Devasena
Division of Genetics and Plant Breeding, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

SM Gowshik Bala
Division of Genetics and Plant Breeding, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

P Swetha
Division of Genetics and Plant Breeding, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

J Lydia Pramitha
Division of Genetics and Plant Breeding, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

B Balaganesh
Division of Soil Science and Agricultural Chemistry, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

Divya Balakrishnan
Senior Scientist, Indian Institute of Rice Research (IIRR), Hyderabad, Telangana, India

Corresponding Author:
N Devasena
Division of Genetics and Plant Breeding, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

Phenotypic diversity and correlation analysis of rice genotypes across contrasting nitrogen regimes

P Priyavarshini, N Devasena, SM Gowshik Bala, P Swetha, J Lydia Pramitha, B Balaganesh and Divya Balakrishnan

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i4Sn.8271>

Abstract

Rice growth, productivity, and Nitrogen Use Efficiency (NUE) are all influenced by nitrogen. This study used principal component analysis (PCA), hierarchical clustering, and correlation to evaluate the phenotypic variability and trait connections of rice genotypes cultivated hydroponically under high nitrogen (HN) and low nitrogen (LN) circumstances. Under HN conditions, correlation analysis showed weak and independent trait correlations; in contrast, LN conditions showed greater and more coordinated relationships between growth traits, indicating interactions brought on by stress. Genotypes clustered more closely under HN, according to PCA data, although LN had a higher dispersion and increased variability, especially along PC1 (25.2%) and PC2 (17.4%). According to hierarchical clustering, LN had higher inter-cluster distances (8-11) and greater genetic divergence than HN (6-9). While HN conditions resulted in more consistent trait expression, LN conditions generally enhanced phenotypic variability and successfully distinguished genotypes. These results highlight the necessity of examining rice genotypes in low-nitrogen environments to uncover cultivars that are stress-tolerant and nitrogen-efficient, which will support the creation of sustainable agricultural modification methods.

Keywords: Rice, nitrogen, PCA, correlation, cluster, hydroponics

Introduction

Over half of the world's population is fed by rice, a vital cereal crop that is a member of the Poaceae family of grasses [1]. Overall rice production in 2021-2022 was predicted to be 130.29 million tons [2]. Nitrogen fertilizers are therefore required to produce enough food to support the world's expanding population, and N is an essential nutrient for agriculture and the security of the global food supply [3]. One of the most important macronutrients needed for crop growth and development is nitrogen (N), which is also an essential component of ATP, amino acids, chlorophyll, and nucleic acids [4]. In plants, nitrogen is vital for influencing root morphology, growth, and managing a number of developmental processes [5].

Clear indicators of nitrogen deficit in rice plants include chlorosis, slowed development, fewer tillers, and an overall decrease in grain output [6]. Plant root system development is severely influenced by nitrogen shortage. Plants tend to devote more resources to root growth, which increases the root to shoot ratio, even though overall biomass is decreased. However, the initiation of lateral roots is inhibited and the specific root length decreases [7]. The root system in rice is especially important because it directly uses vital soil nutrients and makes it easier for nitrogen to be efficiently absorbed and transported. Thus, the shape and function of roots have a major role in determining crop productivity and yield outcomes [8]. The synthesis of amino acids, carbohydrates, photosynthesis, and other physiological processes are all hampered by insufficient nitrogen [9]. The principal photosynthetic pigment, chlorophyll A, is mostly produced utilizing nitrogen, and the chloroplast is the necessary organelle for photosynthesis. Thus, a lack of nitrogen can seriously hinder plants' ability to photosynthesize [10]. It also has an impact on leaf function, root architecture, and seed development [11]. Excessive use of nitrogen fertilizers combined with improper application procedures frequently leads to low nitrogen use efficiency, with 50-70% of applied nitrogen being lost to the environment [12, 13].

Rice needs more nitrogen inputs than most other crops, increasing nitrogen use efficiency in rice agriculture has been a key area of scientific interest. Screening for N deprived condition in a controlled hydroponic system, helps to minimize the unpredictable factors of field conditions and allow for a more accurate assessment of genotype performance and trait contributions to nitrogen use efficiency [9]. The thrust area of current research is to examine how different rice genotypes perform and vary under contrasting nitrogen conditions via hydroponics, using a combination of statistical and multivariate methods. By analyzing morphological and biochemical traits, the association studies help to identify key traits that show better adaptation to nitrogen deprived environments.

Materials and Methods

The experiment was conducted under controlled hydroponic conditions for two seasons Kharif 2025 and Rabi 2025 at Karunya Institute of Technology and Sciences, Coimbatore with three replications in a completely randomized design. Thirty rice genotypes comprising of land races and varieties were grown in nutrient solution [14] under two nitrogen treatments namely high nitrogen (HN) of 100 percent and lack of nitrogen (LN) to evaluate their response. The nutrient solution was prepared using standard composition and maintained at optimal pH throughout the experiment period with regular renewal to ensure uniform nutrient availability. Genotypes were grown under controlled environmental conditions with adequate light and temperature in the standard Styrofoam floats, specific tray volume of seven litre, and maintaining single seedling per hole. Three replicates were maintained for morphological studies and three replicates for biochemical estimation for each LN and HN condition.

The plants were carefully taken out of the hydroponic solution on the 26th day, and any particles that had stuck to the roots were gently rinsed with distilled water. Measurements were made of growth parameters like root length, shoot length, root volume, and root shoot ratio. After blotting the plant samples to remove any remaining surface moisture, they were oven-dried at 70°C to determine their dry weight. The dried samples were weighed using an electronic balance, and the results were expressed in grams per plant. Biochemical traits such as proline and enzyme activities were recorded.

Estimation of peroxidase (POD): A total of 0.1 g of leaf tissue was homogenized in ice-cold 0.1 M phosphate buffer (pH 6.0). The homogenate was then centrifuged for ten minutes at 4°C and 10,000 × g. The resulting supernatant was used for enzyme extraction. For the assay, 0.2 mL of O-dianisidine, 0.2 mL of H₂O₂, 2.5 mL of phosphate buffer, and 0.2 mL of enzyme extract were pipetted into a test tube. For the blank, double-distilled water was used instead of H₂O₂. The increase in absorbance at 430 nm was measured every five seconds for two minutes. Under standard conditions, one unit of enzyme activity was defined as a 1.0 rise in OD. The specific activity of the enzyme was expressed as unit's min⁻¹ mg⁻¹ protein or per gram weight of material. The OD difference and the average of the differences (x) were determined for analysis [15].

Estimation of catalase (CAT): The sample (0.1 g) was ground in a cold mortar and pestle using 0.1 M phosphate

buffer (pH 7.0). The homogenate was centrifuged for ten minutes at 10,000 rpm and 4°C, and the supernatant was used as the enzyme extract. Three separate beakers (B1, B2, and B3) were each filled with 1 mL of enzyme extract. To each beaker, either 1.5 mL of phosphate buffer plus 5 mL of 1.5% pyrogallol or 3 mL of phosphate buffer plus 2 mL of H₂O₂ was added. At intervals of 0 minutes (B1), 2 minutes (B2), and 5 minutes (B3), 10 mL of sulfuric acid was added to each beaker [16].

Estimation of Superoxide Dismutase (SOD)

A leaf sample weighing 0.5 g was homogenized in 25 mL of phosphate buffer (pH 7.5). The homogenate was filtered through four layers of cheesecloth and then centrifuged for 30 minutes at 15,000 rpm and 4°C. Three sets of reaction mixtures the test sample, Blank A, and Blank B were prepared. The test sample contained 1.5 mL of phosphate buffer, 0.1 mL of enzyme extract, 0.1 mL of sodium carbonate, 0.1 mL of nitro blue tetrazolium (NBT), 0.2 mL of methionine, 0.1 mL of EDTA, 0.8 mL of distilled water, and 0.1 mL of riboflavin. Blank A contained the same components as the test sample, while Blank B had all components except the enzyme extract, with distilled water adjusted to 0.9 mL. The test sample and Blank B were placed 30 cm below two 15 W fluorescent bulbs for ten minutes, whereas Blank A was kept in the dark. After incubation, the reaction was stopped, and absorbance was measured to calculate SOD activity.

The reaction mixture was titrated against KMnO₄ until a light purple colour persisted for at least 15 seconds. The specific activity of the enzyme was expressed as min⁻¹ mg⁻¹ protein or per gram of sample, and enzyme activity was expressed as min⁻¹. One unit of CAT activity was defined as the amount of enzyme that, under assay conditions, decomposed 1 μM of H₂O₂. The quantity of H₂O₂ was determined using an extinction coefficient of 0.036 μM⁻¹ mL⁻¹. Enzyme activity could also be expressed as nM of H₂O₂ per minute per gram of sample weight [17].

Estimation of proline

A total of 0.5 g of tissue was homogenized with 10 mL of 3% aqueous sulphosalicylic acid using a pestle and mortar. The mixture was then filtered through Whatman No. 2 filter paper. The extraction process was repeated, and the filtrates were pooled. Two milliliters of glacial acetic acid and ninhydrin were mixed with two milliliters of the filtrate. The reaction mixture was incubated in a boiling water bath for one hour and then rapidly cooled in an ice bath to stop the reaction. After adding 4 mL of toluene, the mixture was shaken vigorously for 20 to 30 seconds. The chromophore (toluene) layer was aspirated, warmed to room temperature, and its absorbance at 520 nm was measured against a reagent blank. The amount of proline in the sample was determined using a standard curve prepared from pure proline (molecular weight 115.5 g) and expressed on the basis of the sample's fresh weight (FW) [18].

Data Analysis

The experiment data were analyzed separately under LN and HN conditions. Analysis of variance (ANOVA) was performed under a Completely Randomized Design (CRD), was used to determine the significance among genotypes. Principal Component Analysis (PCA), correlation, and hierarchical clustering were used to evaluate trait

associations and variability. R software and STAR was used for all analysis.

Result and Discussion

Analysis of variance for LN and HN revealed significant differences among the genotypes for both morphological and biochemical traits. A comparative analysis of LN and

HN for the mean values (Fig.1) indicate that morphological traits exhibited lower values in LN whereas the dry weight accumulation remained the same. Proline and enzymes activity were more under LN condition reflecting a general shift in physiological activity, although the way these enzymes levels varied among genotypes didn't always mirror the patterns seen in morphological traits ^[19].

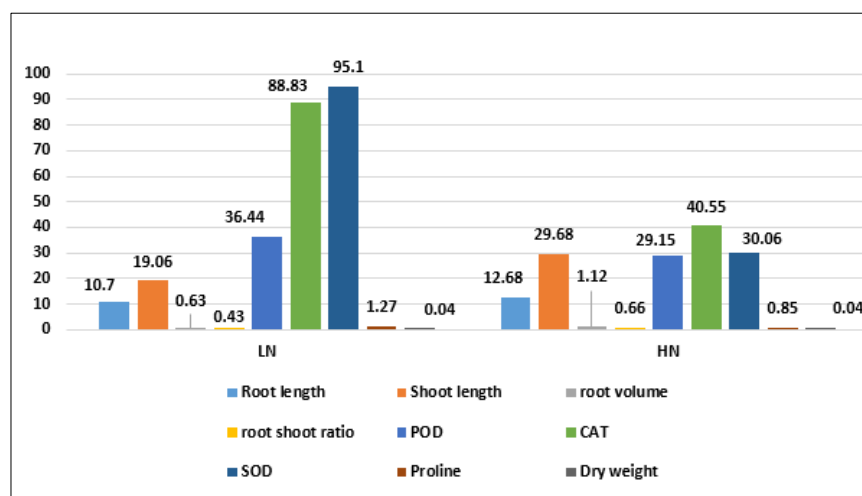


Fig 1: Overall mean comparison of LN and HN for morphological and biochemical traits

However, some genotypes were able to outperform across most traits under LN. Based on the mean performance for dry weight, the genotypes viz., Adukkkan (0.066), Thanga Samba (0.058), Aathur Kichadi Samba (0.056), CO 51 (0.049), and Arai samba (0.046) ranked as top five performers Table 1. These genotypes also recorded among the top five genotypes across multiple traits, indicating their overall superior performance under low nitrogen conditions. Aathur Kichadi Samba has higher mean in root length, shoot length, root shoot ratio, and SOD. Adukkkan had higher mean for root shoot ratio and root volume, Thanga samba had higher mean for POD, CO 51 had higher mean for shoot length, Arai samba for root length and root volume.

Under HN conditions Thanga Samba (0.073), Poongar (0.058), CO 51 (0.058), TPS 5 (0.054), and Adukkkan (0.052) ranked as top five performers for dry weight Table 2. These genotypes consistently ranked within the top five across multiple traits, demonstrating their robust performance under HN conditions ^[20]. Thanga samba has higher mean for dry weight and POD. CO 51 was among the top five performers for shoot length, root shoot ratio, and dry weight. Proline, root volume, and dry weight all have greater means for Poongar. The dry weight and SOD means are greater for TPS 5. The mean dry weight and root volume are greater in Adukkkan. Adukkkan, Thanga Samba, and CO 51 demonstrated stability by performing well in both scenarios. Poongar and TPS 5 fared better under HN, whereas Aathur Kichadi Samba and Arai Samba fared better under LN. This demonstrates that while some genotypes can withstand stress, others react favorably to elevated nitrogen levels ^[21]. Genotypes that maintained larger dry weights under LN did so by combining balanced root systems with moderate shoot growth, making effective use of scarce resources. On the other hand, improved shoot elongation and general plant vigour were the primary causes of increased biomass under HN.

Plants cultivated under LN conditions showed significantly more variability in measured features than those grown under HN. This finding highlights the significant impact of genotypic differentiation and environmental stress under nitrogen shortage. Significantly, root length, root volume, CAT activity, and particularly SOD activity showed increased CV% in LN settings; SOD showed the greatest variability (26.49%), showing its strong potential to differentiate the genotypes under nitrogen deprivation ^[22].

HN circumstances, on the other hand, produced reduced CV% for the majority of characteristics, indicating a more consistent and stable growing environment. However, even in HN circumstances, characteristics including root shoot ratio, POD, CAT, and SOD retained moderate variability. Overall, HN contributed to increased stability across most growth measures, while LN primarily increased variability in physiological and stress-responsive features.

Understanding the relationship between two or more variables without altering or influencing them is possible through correlational research ^[23]. Under LN conditions, correlation analysis showed several important associations among the traits. A positive correlation ($r = 0.46^{**}$) between root length and shoot length indicated simultaneous growth pattern of seedlings. Additionally, there was a moderately favourable connection ($r = 0.27^{*}$) between root length and dry weight. Proline and shoot length showed a strong negative connection ($r = -0.27^{*}$), suggesting that high levels of stress may inhibit overall plant growth. Proline, on the other hand, showed a positive correlation with root volume ($r = 0.31^{*}$), suggesting a possible stress-adaptive mechanism. Shoot length ($r = 0.41^{**}$) and root volume ($r = 0.33^{**}$) were positively linked with dry weight (Fig. 2), highlighting the crucial role these characteristics play in biomass accumulation under LN circumstances ^[24].

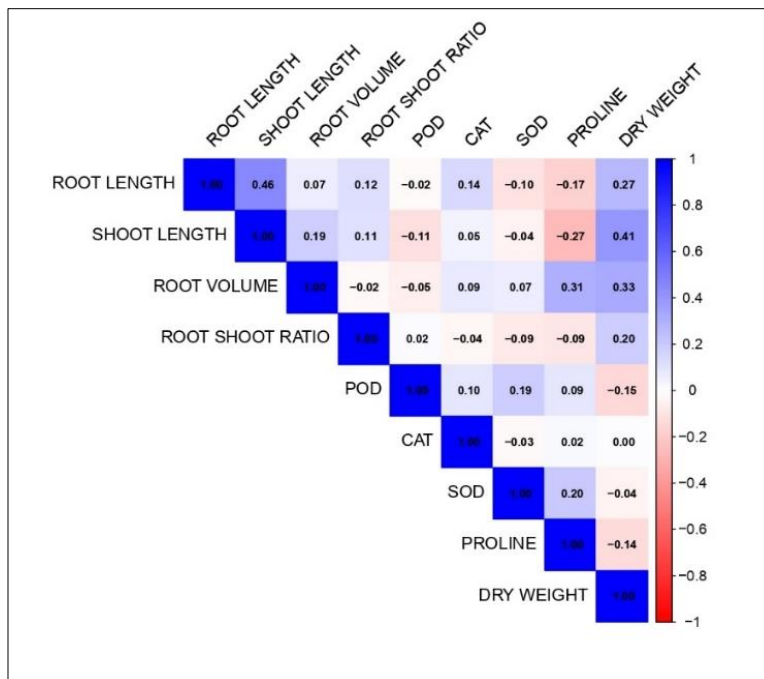


Fig 2: Pearson correlation analysis for all traits under LN conditions

On the other hand, fewer significant relationships were found under HN (Fig. 3). Under sufficient nitrogen availability, root length and shoot length showed a very significant positive correlation ($r = 0.950^{**}$), suggesting synchronized growth. The root shoot ratio and proline had a substantial negative correlation ($r = -0.256^{*}$). Furthermore, there was a positive correlation between dry weight and the

root shoot ratio ($r = 0.250^{*}$), suggesting that when nitrogen is plentiful, biomass formation is facilitated by a balanced distribution between roots and shoots [25]. In tandem, the results exhibited that growth is more balanced and there are fewer significant trait connections under HN environments, plant performance is primarily influenced by root and shoot characteristics, notably root length, under LN conditions.

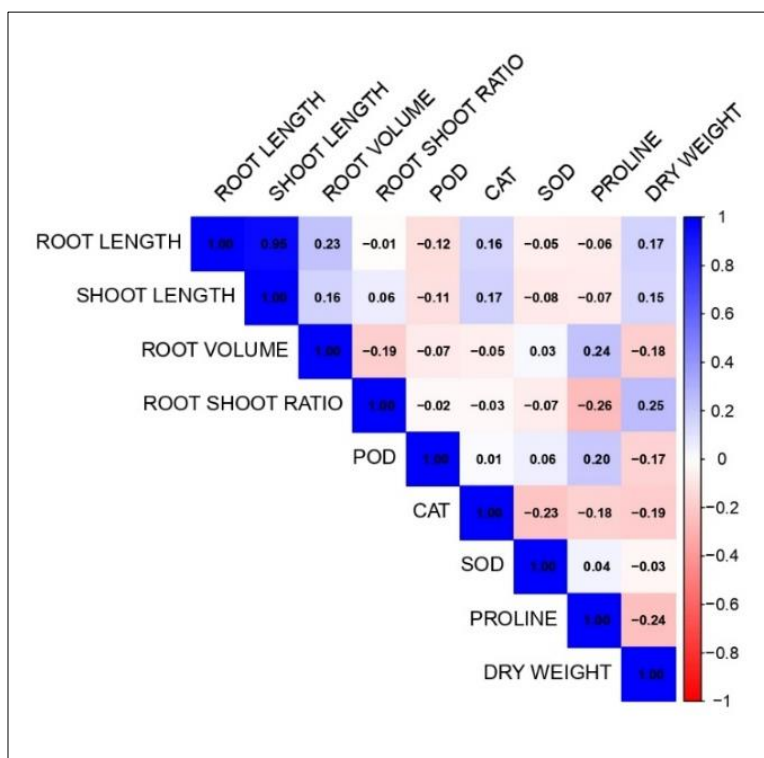


Fig 3: Pearson correlation analysis for all traits under HN conditions.

Genotypes were distinctly distributed across the PCA score plots for LN and HN conditions. A wider distribution of genotypes was found under LN conditions (Fig. 4), particularly along PC1 (-2 to 6), suggesting greater

variability and a range of reactions to nitrogen stress [26]. On the other hand, under HN, genotypes were grouped within a narrower range (PC1: -4 to 2; PC2: -2 to 4), suggesting very consistent performance and minimal variation in non-stress

situations (Fig. 5). Certain genotypes remained around the center, showing less variance, while others, like Kaatuyanam, Maplillai Samba, and Thanga Samba, were positioned far from the origin under LN, indicating distinct adaptive responses. A greater degree of variance under

stress circumstances is implied by the higher dispersion along PC1 under LN. While LN significantly divided genotypes, highlighting stress tolerance variance, overall genotypes under HN circumstances displayed close clustering [27].

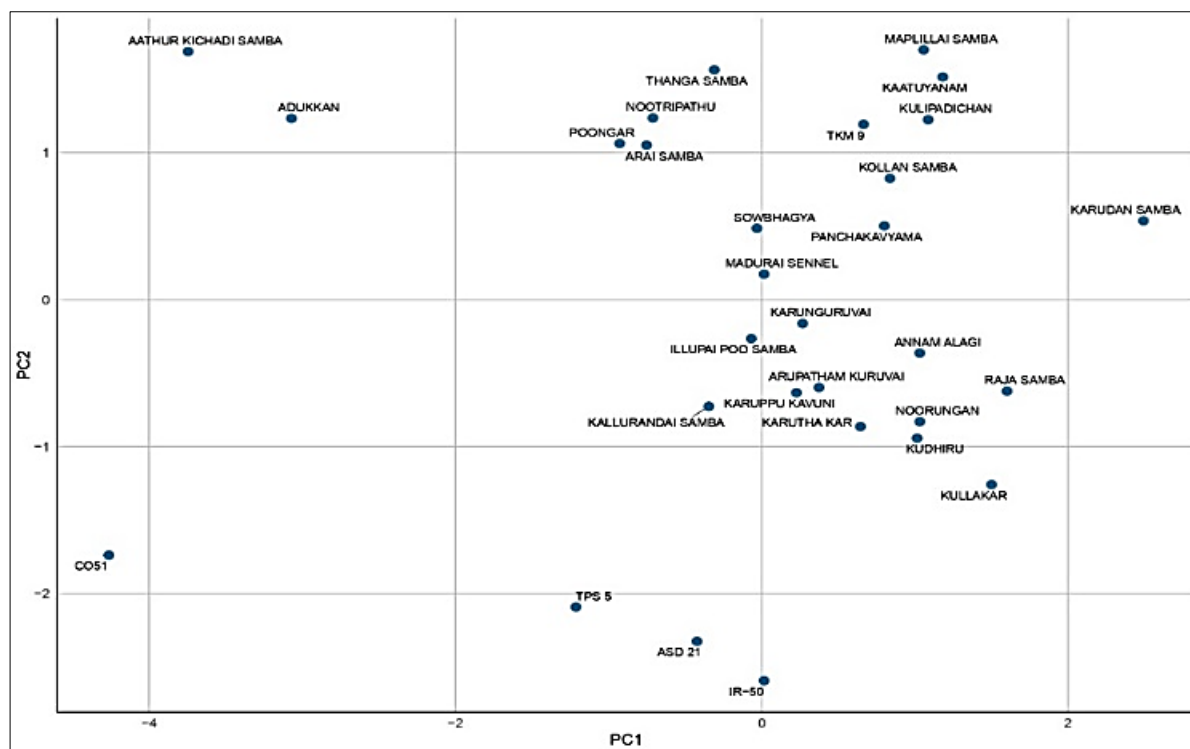


Fig 4: Score plot under LN conditions.

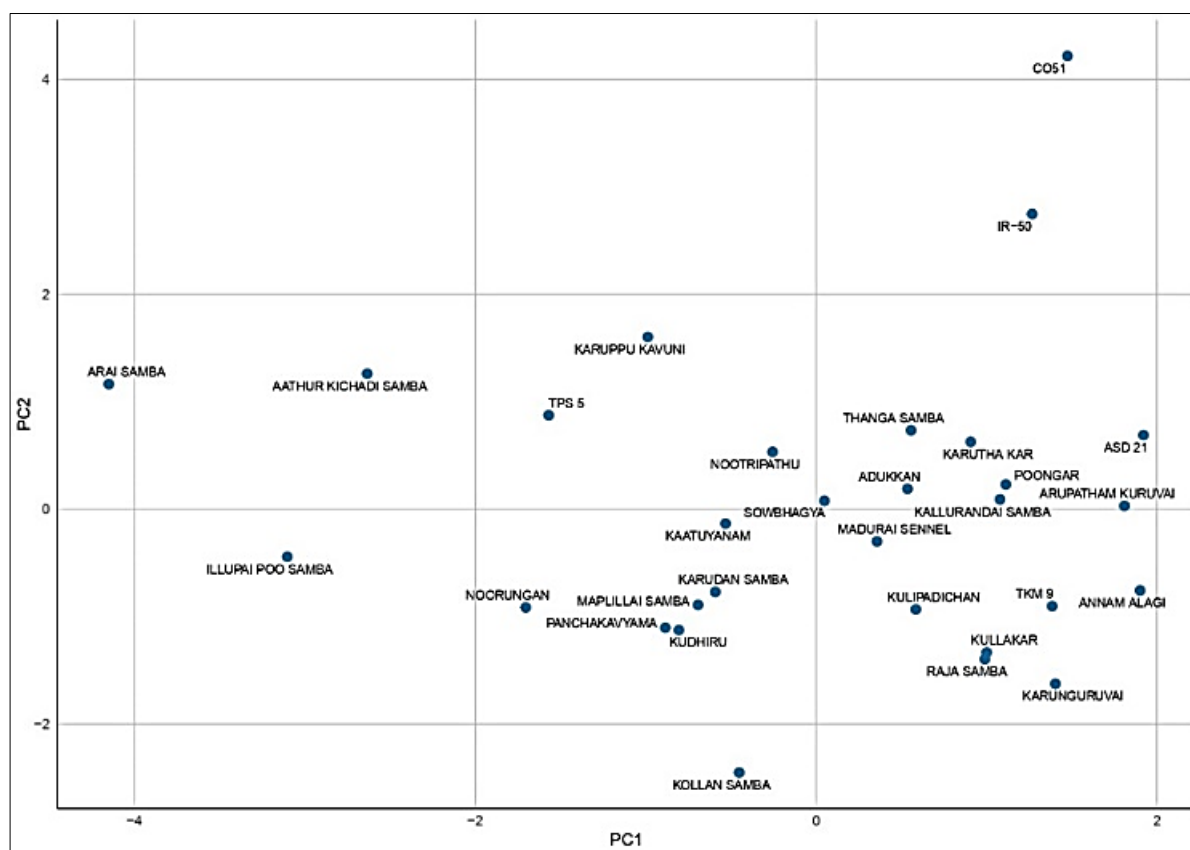


Fig 5: Score plot under HN conditions.

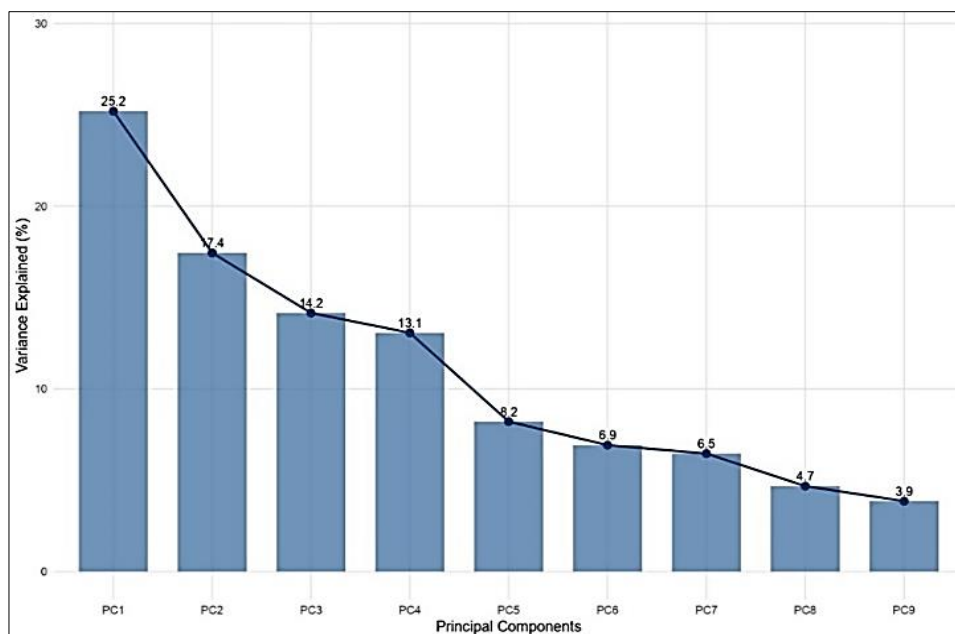


Fig 6: Scree plot under LN conditions

The scree plots showed that the distribution of explained variance among principal components differed between the LN and HN conditions. The first principal component (PC1) under LN explained the largest percentage of variance (25.2%), followed by PC2 (17.4%) and PC3 (14.2%). This suggests that the initial components accounted for a significant amount of overall variance under stress conditions (Fig. 6). The HN scree plot, on the other hand, revealed a more progressive decrease in variance between components, suggesting a more uniformly distributed

variability among features in non-stress conditions (Fig. 7). While HN requires more components because of the relatively uniform trait contribution, LN steeper fall suggests that fewer primary components are required to explain the majority of the variability. Overall, HN conditions showed a more equal variance distribution pattern, indicating stable and less changeable trait expression, while LN conditions concentrated variation in the beginning components, showing more trait differentiation under stress [28].

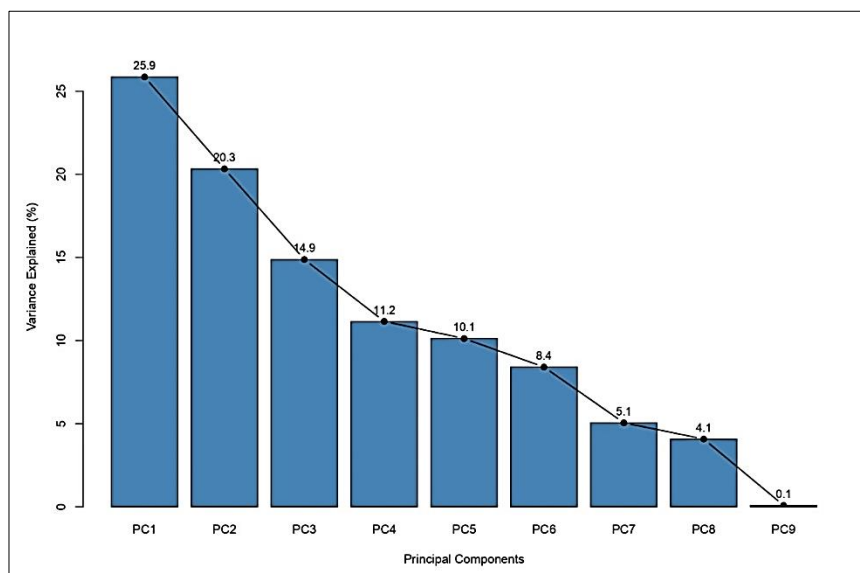


Fig 7: Scree plot under HN conditions.

Different trait contributions to principal components were shown by the PCA loading plots under LN and HN conditions. The studied traits showed more directed loadings and clearer grouping under LN, especially along PC1 (25.2%) and PC2 (17.4%) (Fig. 8). Proline and antioxidant properties were positioned in different directions, indicating their conflicting roles in stress response, growth-related metrics (root length, shoot length, and dry weight) were more closely associated, indicating their considerable

contribution to variation under stress [29]. The majority of features were uniformly distributed throughout PC1 and PC2 under HN, and there was no discernible variable clustering, suggesting that characteristics contribute fairly equally and independently in non-stress scenarios (Fig. 9). While biochemical characteristics like CAT, SOD, and proline were more scattered, suggesting poor coordination, growth variables including root length, shoot length, and dry weight displayed small positive loadings [30].

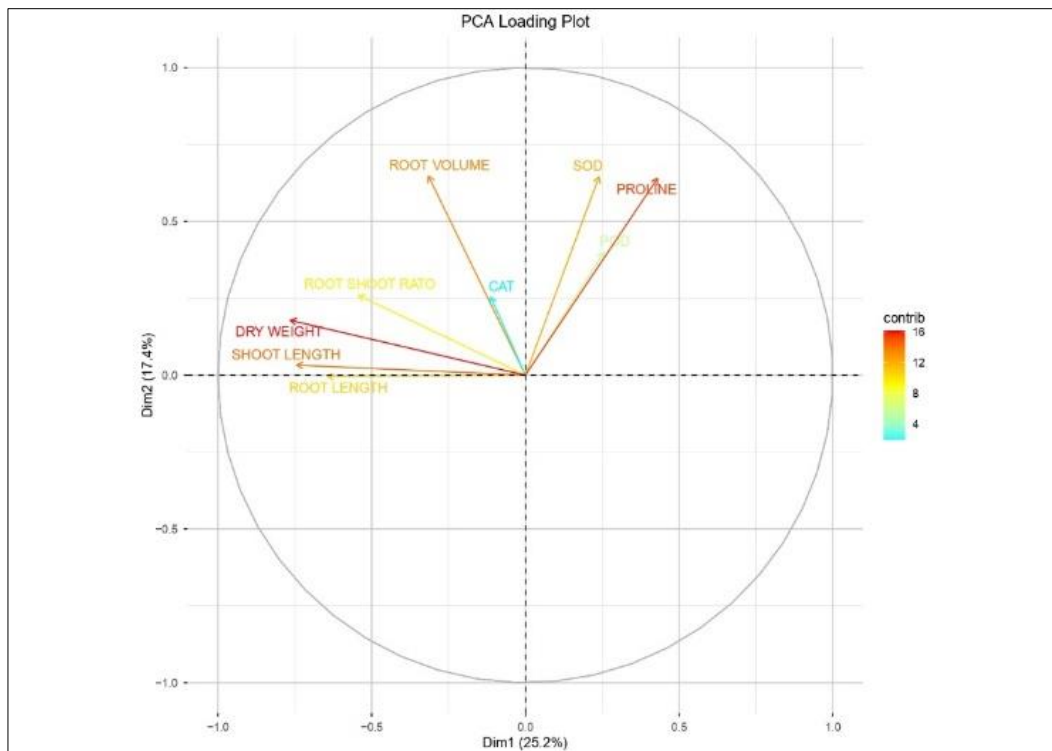


Fig 8: Loading plot under LN conditions.

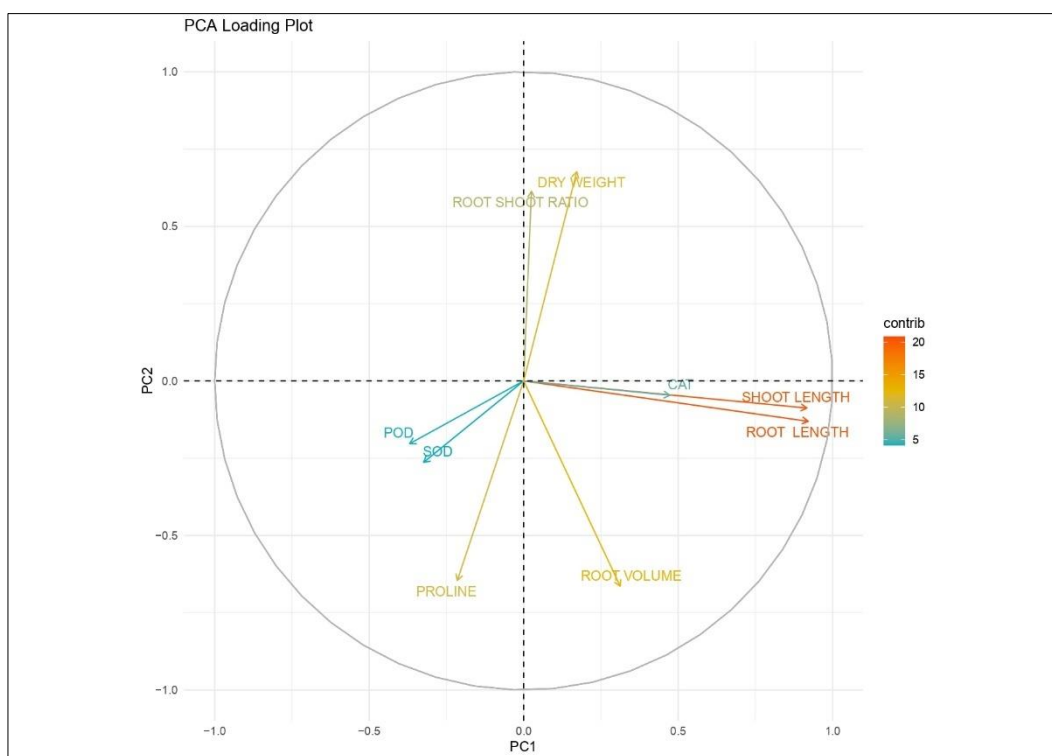


Fig 9: Loading plot under HN conditions.

Under LN circumstances Table 3, inter-cluster distances were much greater, ranging from 32.15 to 68.99, with the greatest difference between clusters C1 and C4 (68.99), showing significant genetic divergence among the genotypes. On the other hand, inter-cluster distances under HN Table 4 were lower, ranging from 11.93 to 19.50, with clusters C3 and C4 having the highest value (19.50), indicating less genetic divergence.

Additionally, intra-cluster distances were comparatively greater under LN than under HN, indicating more variability

within clusters in response to stressful circumstances [31]. More genotype heterogeneity was reflected in the dendrogram produced under LN circumstances (Fig. 10), which showed a broader separation across clusters. On the other hand, the clusters that developed under HN circumstances (Fig. 11) seemed more compact and closely packed, suggesting that the genotypes were more comparable [32].

Overall, dendrogram patterns and inter-cluster distance analysis consistently showed that HN conditions produced

greater similarity and uniformity across genotypes, while LN conditions enhanced genetic divergence and diversity.

This implies that nitrogen stress increases trait expression variability, enabling more distinct genotype differentiation.

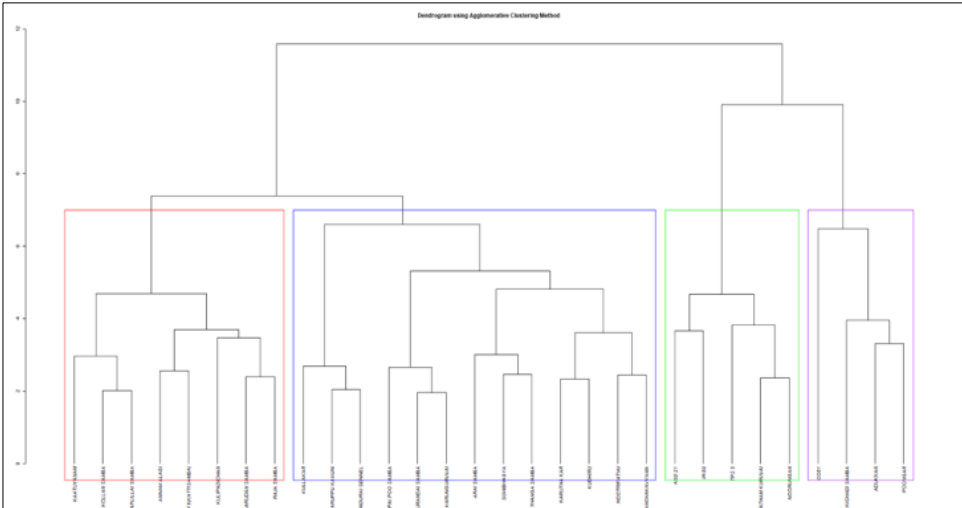


Fig 10: cluster for LN conditions

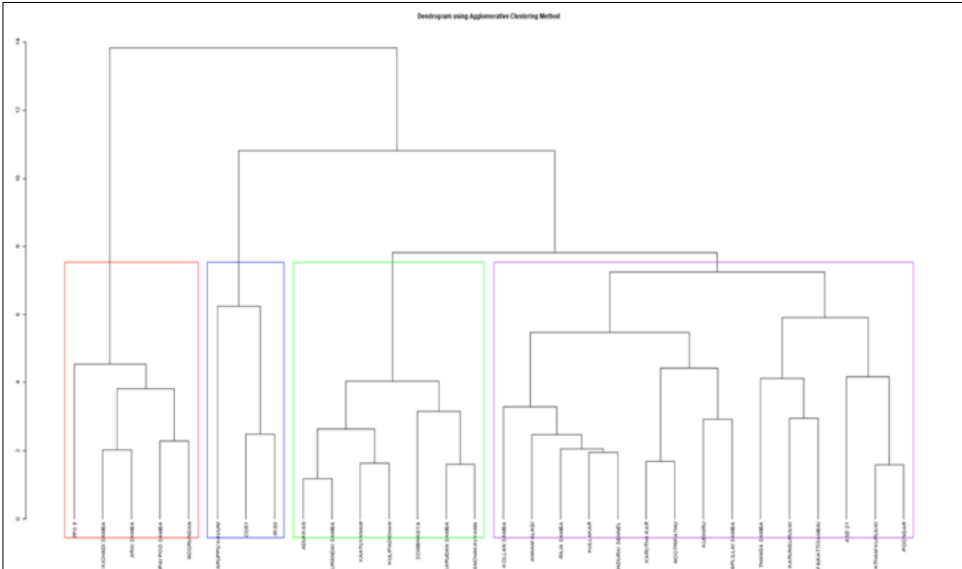


Fig 11: cluster for HN conditions.

Table 1: Overall mean value for morphological and biochemical traits in rice genotypes for Kharif 2025 and Rabi 2025 under LN conditions

Genotypes	Root length (cm)	Shoot length (cm)	Root volume (mL)	Root shoot ratio	POD (U/g FW)	CAT (U/g FW)	SOD (U/g FW)	Proline (μmol g-1 FW)	Dry weight (g)
Aathur kichadi samba	13.23	27.87	0.78	0.48	24.72	88.56	123.10	1.29	0.056
Adukkann	11.77	21.13	0.89	0.47	34.88	95.65	75.90	1.30	0.066
Annam azhagi	12.30	16.07	0.49	0.43	53.84	85.43	103.00	1.19	0.020
Arai samba	12.27	15.37	0.84	0.44	46.80	89.04	94.34	1.25	0.046
Arupatham kuruvai	9.67	15.03	0.56	0.45	16.04	78.28	94.29	1.35	0.041
ASD 21	10.87	15.23	0.42	0.46	9.52	91.67	90.17	0.93	0.032
CO51	14.00	31.13	0.48	0.44	36.68	97.40	83.41	0.76	0.049
Illupai poo samba	11.93	19.57	0.69	0.43	43.80	76.74	72.90	1.38	0.029
IR 50	9.43	18.37	0.38	0.43	40.08	77.61	82.54	0.81	0.040
Kaatuyanam	9.00	20.23	0.62	0.45	55.12	74.28	121.35	1.46	0.032
Kallurandai samba	11.50	18.60	0.40	0.45	42.60	85.68	75.60	1.36	0.041
Karudan samba	9.13	15.50	0.53	0.40	41.60	84.01	127.68	1.40	0.031
Karunguruvai	11.87	16.13	0.55	0.43	36.96	94.27	77.56	1.45	0.034
Karuppu kavuni	10.27	20.93	0.72	0.39	21.56	94.35	68.03	1.44	0.036
Karuthakar	9.53	18.60	0.58	0.40	41.32	101.48	78.28	1.14	0.034
Kollan samba	9.80	21.77	0.66	0.44	41.36	90.66	108.13	1.38	0.017
Kudhiru	10.50	19.00	0.42	0.43	50.72	98.67	83.68	1.18	0.020
Kulipadichan	12.00	17.33	0.62	0.40	37.00	100.61	118.16	1.54	0.031
Kullakar	10.90	19.20	0.53	0.37	29.20	88.83	78.98	1.41	0.022

Madurai sennel	10.30	21.37	0.79	0.40	26.28	89.47	99.09	1.29	0.034
Maplillai samba	9.70	16.93	0.78	0.46	49.76	92.65	114.31	1.35	0.023
Noorungan	10.13	17.83	0.65	0.43	22.12	74.54	97.51	1.27	0.020
Nootripathu	11.50	21.13	0.82	0.44	41.60	105.74	92.85	1.33	0.032
Panchakavyama	9.40	15.50	0.79	0.44	41.60	102.54	79.77	1.28	0.029
Poongar	10.03	21.67	0.84	0.44	18.44	89.73	101.46	1.43	0.046
Raja samba	9.97	15.73	0.34	0.43	32.68	88.89	105.74	1.37	0.029
Sowbhagya	10.20	19.70	0.72	0.42	43.52	92.99	108.29	1.14	0.040
Thanga samba	9.40	18.27	0.66	0.45	50.80	89.63	115.17	1.31	0.058
TKM 9	11.33	16.57	0.69	0.44	43.84	80.89	126.44	1.33	0.032
TPS 5	10.43	19.90	0.74	0.44	18.68	64.67	55.22	1.09	0.043
MIN	9.00	15.03	0.34	0.37	9.52	64.67	55.22	0.76	0.017
MAX	14.00	31.13	0.89	0.48	55.12	105.74	127.68	1.54	0.066
MEAN	10.70	19.06	0.63	0.43	36.44	88.83	95.10	1.27	0.040
Std Dev	1.27	3.57	0.17	0.06	11.91	16.91	27.97	0.18	0.01
CV (%)	2.74	2.58	12.74	16.58	1.11	19.34	26.49	1.22	5.12
CD 5%	0.62	1.03	0.17	0.15	0.85	36.07	52.89	0.03	0.01

POD: Peroxidase, CAT: Catalase, SOD: Superoxide Dismutase, (U/g FW): enzyme activity per gram of formula weight.

Table 2: Overall mean value for morphological and biochemical traits in rice genotypes for Kharif 2025 and Rabi 2025 under HN conditions

Genotypes	Root length (cm)	Shoot length (cm)	Root volume (mL)	Root shoot ratio	POD (U/g FW)	CAT (U/g FW)	SOD (U/g FW)	Proline (μmol g-1 FW)	Dry weight (g)
Aathur kichadi samba	7.47	23.63	0.99	0.64	26.05	38.71	28.29	0.87	0.047
Adukkann	14.57	32.43	1.07	0.58	27.91	37.64	34.09	0.88	0.052
Annam azhagi	15.10	33.77	1.14	0.57	25.63	48.01	33.68	0.79	0.033
Arai samba	6.30	20.53	0.97	0.58	39.14	36.37	29.39	0.84	0.045
Arupatham kuruvai	14.73	33.10	1.15	0.56	12.83	40.08	26.44	0.91	0.047
ASD 21	13.23	30.50	1.17	0.56	7.62	46.46	26.23	0.59	0.036
CO 51	14.10	32.23	0.96	1.31	31.17	42.52	24.83	0.46	0.058
Illupai poo samba	8.07	22.20	1.11	0.70	35.04	39.32	32.65	0.94	0.033
IR 50	13.90	31.50	1.04	0.93	20.64	39.97	27.59	0.50	0.051
Kaatuyanam	12.43	29.47	1.03	0.53	28.00	38.35	29.07	0.99	0.042
Kallurandai samba	14.80	33.30	1.08	0.62	26.88	39.58	30.42	0.91	0.048
Karudan samba	12.87	30.23	1.14	0.60	33.28	34.43	30.33	0.95	0.038
Karunguruvai	15.07	33.77	1.32	0.61	29.57	36.34	27.32	0.97	0.039
Karuppu kavuni	11.90	28.53	1.09	1.64	30.72	36.88	32.28	1.01	0.046
Karuthakar	13.57	31.17	1.03	0.61	34.43	45.00	23.98	0.76	0.038
Kollan samba	12.07	28.13	1.31	0.58	33.09	41.43	31.93	0.94	0.021
Kudhiru	12.33	29.23	1.12	0.62	40.58	43.12	38.93	0.78	0.027
Kulipadichan	14.30	32.40	1.11	0.61	29.60	38.66	28.09	1.03	0.036
Kullakar	13.60	31.43	1.18	0.62	25.12	44.42	27.24	0.96	0.026
Madurai sennel	12.77	29.73	1.12	0.60	21.02	43.16	31.56	0.87	0.038
Maplillai samba	13.07	30.33	0.97	0.40	42.62	41.30	31.12	0.92	0.028
Noorungan	9.33	23.60	1.18	0.55	28.06	42.16	27.46	0.86	0.024
Nootripathu	11.87	28.60	0.99	0.61	33.28	45.20	26.40	0.90	0.042
Panchakavyama	12.90	29.50	1.19	0.60	33.28	33.36	35.18	0.85	0.033
Poongar	13.40	30.40	1.18	0.62	14.75	41.45	27.06	0.98	0.058
Raja samba	14.17	31.33	1.25	0.58	31.58	44.29	32.33	0.90	0.038
Sowbhagya	14.50	32.37	1.08	0.61	34.82	36.46	39.25	0.75	0.047
Thanga samba	13.67	30.77	1.15	0.58	40.64	41.35	29.39	0.89	0.073
TKM 9	14.20	31.60	1.27	0.67	40.45	44.57	23.34	0.90	0.042
TPS 5	10.03	24.70	1.16	0.61	16.71	36.01	35.83	0.73	0.054
MIN	6.3	20.53	0.96	0.4	7.62	33.36	23.34	0.46	0.021
MAX	15.1	33.77	1.32	1.64	42.62	48.01	39.25	1.03	0.073
MEAN	12.68	29.68	1.12	0.66	29.15	40.55	30.06	0.85	0.040
Std Dev	2.27	3.49	0.12	0.25	9.52	5.44	5.86	0.14	0.01
CV (%)	2.29	2.70	8.24	17.69	18.27	12.09	17.40	2.95	10.51
CD 5%	0.61	1.68	0.19	0.25	11.18	10.28	10.98	0.05	0.01

POD: Peroxidase, CAT: Catalase, SOD: Superoxide Dismutase, (U/g FW): enzyme activity per gram of formula weight.

Table 3: Average inter- and intra-cluster distances among rice genotypes under LN condition

	C1	C2	C3	C4
C1	20.58	39.28	34.27	68.99
C2		18.75	32.15	43.43
C3			15.57	46.87
C4				0.00

Diagonal bolded values are intra-cluster values

Table 4: Average inter- and intra-cluster distances among rice genotypes under HN condition

	C1	C2	C3	C4
C1	9.59	13.86	19.27	13.93
C2		8.96	16.90	11.93
C3			11.68	19.50
C4				8.63

Diagonal bolded values are intra-cluster values

Conclusion

The study found substantial differences in genotype performance under both LN and HN circumstances. Adukkann, Thanga samba, CO51 and Aathur kichadi samba were identified as the best performing genotypes based on overall morphological, biochemical traits. Among these 4 genotypes Adukkann, Thanga samba and CO51 showed consistent performance under both LN and HN indicating their stability. Aathur kichadi samba performed well under LN conditions showing strong adaptability to nitrogen stress. Correlation and ANOVA indicated significant trait variability and associations under nitrogen stress. PCA and clustering showed higher variability and divergence under LN, while HN exhibited more uniform performance.

Acknowledgements

The authors express their deepest appreciation to the Chairperson, Advisory Committee members and Dean of Karunya Institute of Technology and Sciences, Coimbatore for their essential support during the entire research process. The authors also gratefully acknowledge the University for providing the necessary facilities for laboratory analysis which supported the successful completion of this research.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper. All authors have approved the manuscript and agree with its submission to the journal.

References

- Lee S. Recent advances on nitrogen use efficiency in rice. *Agronomy*. 2021;11(4).
- FAO. FAOSTAT: Food and agriculture organization corporate statistical database [Internet]. Rome: FAO; 2022 [cited 2022 Nov 1]. Available from: <http://www.fao.org/faostat/en/#data>
- Liu Y, Wang H, Jiang Z, Wang W, Xu R, Wang Q, *et al*. Genomic basis of geographical adaptation to soil nitrogen in rice. *Nature*. 2021;590(7847):600-605.
- Arya RK, Tiwari R, Mishra R, Choudhary M, Namdeo K. Performance of rice (*Oryza sativa*) varieties to applied nitrogen under irrigated condition. *Ann Plant Soil Res*. 2019;21(3):221-225.
- Freschet GT, Roumet C, Comas LH, Weemstra M, Bengough AG, Rewald B, *et al*. Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol*. 2021;232(3):1123-1158.
- Sui B, Feng X, Tian G, Hu X, Shen Q, Guo S. Optimizing nitrogen supply increases rice yield and nitrogen use efficiency by regulating yield formation factors. *Field Crops Res*. 2013;150:99-107.
- Bahrman N, Gouy A, Devienne-Barret F, Hirel B, Vedele F, Le Gouis J. Differential change in root protein patterns of two wheat varieties under high and low nitrogen nutrition levels. *Plant Sci*. 2005;168(1).
- Meng X, Zhu J, Li Y, Mao C. Molecular mechanisms of root development in rice. *Rice*. 2019;12(1):1.
- Zhao X, Wang W, Xie Z, Gao Y, Wang C, Rashid MM, *et al*. Comparative analysis of metabolite changes in two contrasting rice genotypes in response to low-nitrogen stress. *Crop J*. 2018;6(5).
- Wen B, Li C, Fu X, Li D, Li L, Chen X, *et al*. Effects of nitrate deficiency on nitrate assimilation and chlorophyll synthesis of detached apple leaves. *Plant Physiol Biochem*. 2019;142.
- Stitt M, Krapp A. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant Cell Environ*. 1999;22(6).
- McAllister CH, Beatty PH, Good AG. Engineering nitrogen use efficient crop plants: the current status. *Plant Biotechnol J*. 2012;10(9):1011-1025.
- Kissel D, Bock B, Ogles C. Thoughts on acidification of soils by nitrogen and sulfur fertilizers. *Agrosyst Geosci Environ*. 2020;3(1):e20060.
- Singh RK, Redoña E, Refuerzo L. Varietal improvement for abiotic stress tolerance in crop plants: special reference to salinity in rice. In: *Abiotic stress adaptation in plants: physiological, molecular and genomic foundation*. New York: Springer; 2009. p. 387-415.
- Sumner JB, Gjessing EC. A method for the determination of peroxidase activity. *Arch Biochem*. 1943.
- Braber JM. Catalase and peroxidase in primary bean leaves during development and senescence. *Z Pflanzenphysiol*. 1980;97(2):135-144.
- Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem*. 1971;44(1):276-287.
- Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. *Plant Soil*. 1973;39(1):205-207.
- Hawkesford MJ. Genetic variation in traits for nitrogen use efficiency in wheat. *J Exp Bot*. 2017;68(10):2627-2632.
- Kumar N, Mathpal B, Sharma A, Shukla A, Shankhdhar D, Shankhdhar S. Physiological evaluation of nitrogen use efficiency and yield attributes in rice (*Oryza sativa* L.) genotypes under different nitrogen levels. *Cereal Res Commun*. 2015;43(1):166-177.
- Pandey N. Antioxidant defense system in plants exposed to metal toxicity. In: *Plants under metal and metalloids stress: responses, tolerance and remediation*. Singapore: Springer; 2018. p. 107-148.
- Liu L, Cui K, Qi X, Wu Y, Huang J, Peng S. Varietal responses of root characteristics to low nitrogen application explain the differing nitrogen uptake and grain yield in two rice varieties. *Front Plant Sci*. 2023;14:1244281.

23. Putri L, Rezani MR, Hermina D. Correlational research design. *J Riset Multidisiplin Edukasi*. 2025;2(6):306-317.
24. Qi Z, Ling F, Jia D, Cui J, Zhang Z, Xu C, *et al*. Effects of low nitrogen on seedling growth, photosynthetic characteristics and antioxidant system of rice varieties with different nitrogen efficiencies. *Sci Rep*. 2023;13(1):19780.
25. Zhang M, Wang Y, Qi Z, Zhang Q, Wang H, Guan C, *et al*. Screening of germplasm resources with low-phosphorus tolerance during the seedling stage of rice. *Plants*. 2025;14(10):1543.
26. Du F, Liu P, Wang K, Yang Z, Wang L. Ionomic responses of rice plants to the stresses of different arsenic species in hydroponics. *Chemosphere*. 2020;243:125398.
27. Guduru KK, Pandit V, Palakurthi R, Konduru S, Kandi SR, Banavath JN, *et al*. Identification of nitrogen efficient indica rice (*Oryza sativa* L.) genotypes: a physiological and multivariate statistical approach. *Curr Trends Biotechnol Pharm*. 2018;12(1):16-32.
28. Kunta M, Shivani D, Jabeen FCK, Chiranjeevi M, Rao L, Sundaram RM, *et al*. Multivariate analysis and character association for agro-morphological traits in elite rice germplasm. *J Cereal Res*. 2021;13(3):270-278.
29. Wang W, Huang L, Zhu G, Zhang H, Wang Z, *et al*. Screening of rice cultivars for nitrogen use efficiency and yield stability under varying nitrogen levels. *J Plant Growth Regul*. 2022;41(4):1808-1819.
30. Gangadhara K, Singh AK, Krishnan SG, Ellur RK, Pandey R, Kumar S, *et al*. Analysis of root system architecture in rice indicates limited varietal adaptations. *Indian J Genet Plant Breed*. 2024;84(4):515-522.
31. Fageria NK, Moreira A. The role of mineral nutrition on root growth of crop plants. *Adv Agron*. 2011;110:251-331.
32. Pujarula V, Pusuluri M, Bollam S, Das RR, Ratnala R, Adapala G, *et al*. Genetic variation for nitrogen use efficiency traits in global diversity panel and parents of mapping populations in pearl millet. *Front Plant Sci*. 2021;12:625915.