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Genetic Diversity Analysis in Upland Rice (Oryza sativa L.) Germplasm of Nagaland

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Abstract: Rice (Oryza sativa L.) remains a cornerstone of food security for over half of the world's population, with South and Southeast Asia serving as its primary centers of production and consumption. Despite India's significant contribution to global rice cultivation, regions such as Nagaland in the North Eastern Himalayas remain underexplored for their genetic diversity, even though they represent hotspots of traditional landraces. Understanding the extent of diversity within these landraces is crucial for breeding programs aimed at yield stability, pest resistance, and climate resilience. In this study, 18 upland rice genotypes collected from Nagaland were evaluated for genetic divergence using Mahalanobis' D^2 statistics and grouped into four distinct clusters through Tocher's method. Results indicated considerable inter-cluster variation, with maximum divergence observed between Cluster II and Cluster III, suggesting their potential use as parents in hybridization programs. Panicle weight and 100-seed weight contributed the most to total divergence, highlighting their importance in selection. These findings reaffirm the value of local landraces in breeding initiatives, especially in the face of climate variability, disease outbreaks, and rising demand for resilient crops. Incorporating such genetic diversity into breeding pipelines can accelerate the development of rice varieties with enhanced adaptability, productivity, and sustainability.

Key words: Cluster, D^2 values, Genetic divergence, rice

Introduction

Rice (Oryza sativa L.) is one of the world's most critical staple crops, feeding more than 3.5 billion people across Asia, Africa, and Latin America. Globally, it occupies approximately 11% of cultivated land, with Asia accounting for over 90% of production and consumption (Shoukat et al., 2025). In India, rice contributes nearly 40% of total food grain production and is cultivated across diverse agro-ecological zones, from irrigated plains to rainfed uplands. Despite its wide adaptation, the sustainability of rice production faces challenges from climate change, pest outbreaks, and shrinking genetic diversity in modern cultivars.

Nagaland, a state in Northeast India, represents a unique repository of rice landraces due to its agroecological heterogeneity, cultural traditions, and farmer-led seed selection practices. Unlike high-yielding modern varieties, traditional landraces cultivated in Nagaland are adapted to diverse conditions such as upland rainfed fields, jhum (shifting cultivation) systems, and low-input environments. These genotypes often exhibit resilience to abiotic stresses such as drought, low soil fertility, and cold tolerance, alongside tolerance to pests and diseases. Consequently, the region is considered a micro-center of rice diversity within India (Pradhan et al., 2023).

Preserving and utilizing this diversity is not only a matter of cultural heritage but also a strategic necessity for crop improvement programs. The global rice gene pool has narrowed due to the dominance of a few high-yielding cultivars developed during the Green Revolution. This genetic erosion makes rice systems more vulnerable to diseases, pests, and climate extremes. For example, yield stagnation and vulnerability to diseases like bacterial blight and blast have underscored the urgent need for broadening the genetic base (Gadratagi et al., 2025).

Genetic diversity studies provide insight into the extent of variability within a population, which is a prerequisite for designing effective breeding strategies. One of the most widely used methods is Mahalanobis' D^2 statistics, which quantifies divergence among genotypes based on multiple traits. By grouping genotypes into clusters, breeders can identify parents with the widest genetic distances to maximize heterosis and transgressive segregation in breeding populations. Previous studies across India and Asia have consistently shown that selecting parents from divergent clusters enhances the likelihood of developing high-performing hybrids (Yaacob et al., 2023).

Moreover, in the context of climate change, resilience is emerging as an equally important breeding goal as productivity. Traits such as panicle weight, seed weight, tillering ability, and flowering time not only determine yield but also influence adaptability to changing environments. Identifying which traits contribute most to genetic divergence provides a roadmap for breeders to prioritize selection. For instance, panicle weight has been reported as a major contributor to diversity in multiple studies, reflecting its complex genetic basis and strong association with yield stability (Pradhan et al., 2023).

Nagaland's rice landraces are especially valuable for climate-smart breeding because of their inherent adaptability to low-input and stress-prone environments. Integrating these genotypes into mainstream breeding programs could generate varieties that thrive under water-limited conditions, resist emerging pests, and contribute to sustainable agricultural systems. Modern genomic tools such as genome-wide association studies (GWAS) and marker-assisted selection are increasingly being applied to identify functional alleles from traditional varieties (Kannababu et al., 2025). However, the first step remains careful phenotypic and statistical evaluation of diversity, as performed in this study.

Therefore, the present research aimed to assess the magnitude of genetic divergence among 18 upland rice genotypes from Nagaland using multivariate statistical methods. The objectives were: (i) to classify the genotypes into clusters based on phenotypic traits, (ii) to identify clusters and traits contributing most to divergence, and (iii) to suggest potential parent combinations for breeding programs targeting yield improvement and adaptability. By linking local diversity with modern breeding needs, this study contributes toward sustainable rice improvement strategies in India and beyond.

Materials and Methods

Experimental Site and Design

The experiment was conducted during the 2019 growing season at the experimental farm of the School of Agricultural Sciences and Rural Development (SASRD), Nagaland University, Medziphema campus. The site lies in the subtropical hill region with annual rainfall exceeding 2000 mm, predominantly during the kharif season, and temperatures ranging between 20–32 °C. The soil was sandy loam, moderately acidic, and representative of upland conditions in the region.

The trial was laid out in a randomized block design (RBD) with two replications. Each plot consisted of 30 hills, spaced 20 cm between rows and 10 cm between plants, ensuring adequate representation of genotypic performance. Standard agronomic practices for upland rice were followed, including three rounds of hand weeding at 25, 40, and 60 days after sowing. Fertilizer was applied at the recommended rate of 120:60:60 kg ha^{-1} ($N:P_2O_5:K_2O$). Nitrogen was split-applied, with half as basal and half as top-dress at the booting stage.

Plant Material

Eighteen traditional upland rice genotypes were used, sourced from different villages of Nagaland. The genotypes included SHENGYA, CHAUNYAK, KEREBE, TENGAKADU, TOILANG, REUDINE, ONGSHO, ONGPANG, AWONGLU ASHUH, KILU SHUH, ONGCHANG, TANGHA, MYNRI, JWAIN, TENGAKEZE, ONGMEI, and TANGHA-1. These genotypes represent landraces maintained by farmers under rainfed and low-input systems, with potential for breeding due to their adaptation and variability.

Data Collection

The data were recorded on five randomly sampled plants in each plot for 9 characters viz., Days to 50% flowering , Effective tillers/plant, Days to 80% maturity, plant height, Panicle length, Panicle weight, number of seeds per panicle, 100 seed weight and yield per plant.

Statistical Analysis

Analysis of variance (ANOVA) was conducted to test the significance of genotypic differences for each trait. Genetic divergence was estimated using Mahalanobis' generalized distance (D^2) statistic (Mahalanobis, 1936). Clustering of genotypes was performed following Tocher's method (Rao, 1952), which groups genotypes based on minimum average inter-cluster distance. Intra- and inter-cluster distances were calculated to assess divergence levels. The relative contribution of each trait to total divergence was estimated to identify the most influential characters.

Results and Discussion

Analysis of Variance

The ANOVA revealed significant variation among the 18 genotypes for all traits studied, confirming the presence of ample genetic variability. This aligns with recent findings that rice landraces in India, particularly in the northeast, harbor untapped diversity in yield and adaptability traits (Pradhan et al., 2023; Kumar et al., 2024). Variability for flowering and maturity periods indicates potential for selecting genotypes suited to diverse cropping systems, from short-duration upland varieties to longer-duration lowland types.

Cluster Analysis

Based on D^2 statistics, the 18 genotypes were grouped into four clusters: Cluster I: 9 genotypes (largest group), Cluster II: 2 genotypes, Cluster III: 4 genotypes and Cluster IV: 3 genotypes (Table 1). The clustering pattern suggests considerable divergence among the genotypes, with most landraces concentrated in Cluster I, while smaller clusters indicate unique combinations of traits. Similar clustering of Indian landraces into 3–6 groups has been reported in other diversity studies, reaffirming the heterogeneity of traditional varieties (Yaacob et al., 2023).

Inter- and Intra-Cluster Distances

The maximum inter-cluster distance was observed between Cluster II and Cluster III (D^2 = 1413.22) (Table 2.) indicating that crossing genotypes from these clusters could yield high heterosis and broader segregating populations. Minimum divergence occurred between Cluster I and Cluster III, suggesting shared ancestry or similar selection pressures. The intra-cluster distances ranged from 39.89 in Cluster II to 190.22 in Cluster IV, reflecting varying degrees of homogeneity.

Such findings are consistent with the principle that genetically distant parents are more likely to produce heterotic combinations. Recent genome-wide studies in rice have also validated that inter-cluster distances correspond to molecular divergence, underscoring the reliability of phenotypic clustering for breeding applications (Gadratagi et al., 2025; Kannababu et al., 2025).

Cluster Means and Trait Performance

Cluster-wise mean performance revealed valuable insights: Cluster I: High plant height (table 3.), indicating tall landraces often linked to biomass and lodging issues but useful for straw yield. Cluster II: High seed number per panicle, a key determinant of yield potential. Cluster III: High panicle weight and grain yield per plant, suggesting suitability for direct yield improvement. Cluster IV: High values for days to flowering, effective tillers, maturity, panicle length, and 100-seed weight, representing balanced yield components. These differences can guide breeders in choosing complementary parents. For instance, genotypes from Cluster II (many seeds per panicle) and Cluster III (high panicle weight) could be combined to enhance both seed number and seed weight, thereby maximizing yield.

Trait Contributions to Divergence

Panicle weight contributed the most (62.75%) to total divergence, followed by 100-seed weight (33.99%) (table 4). Traits like flowering and maturity had minor contributions, while effective tillers and yield per plant contributed minimally. These results echo recent studies emphasizing panicle architecture and seed size as major determinants of variability in landraces (Pradhan et al., 2023; Shoukat et al., 2025). This suggests that breeders should prioritize panicle and seed traits when exploiting Nagaland landraces for yield improvement. Furthermore, with climate change threatening grain filling and seed quality, selection for panicle weight stability under stress conditions becomes even more critical (Yaacob et al., 2023).

The high divergence observed between Clusters II and III highlights opportunities for hybridization programs. Using parents from these clusters may generate novel recombinants with enhanced heterosis. Moreover, incorporating traits like heavier panicles and larger seeds into modern cultivars could improve yield stability under stress-prone environments.

Climate-resilient breeding requires tapping into traditional varieties that already thrive under marginal conditions. Landraces in Nagaland, cultivated under rainfed uplands without external inputs, are pre-adapted to drought and nutrient limitations. Integrating these into breeding programs can contribute to sustainable intensification, aligning with recent calls for climate-smart rice systems (Kumar et al., 2024).

Additionally, with emerging pests like gall midge and blast becoming more destructive, genetic diversity provides insurance against widespread crop failures. Recent molecular studies have identified resistance alleles in landraces, reinforcing the role of traditional varieties as reservoirs of useful genes (Gadratagi et al., 2025).

The findings underscore the need for systematic conservation and characterization of Nagaland's rice diversity. While this study focused on phenotypic traits, integrating molecular marker data through SSRs, SNPs, or

GWAS would provide deeper insights into allelic richness. Recent advances in genomic selection can accelerate the use of such landraces in breeding pipelines (Kannababu et al., 2025).

Furthermore, participatory breeding involving local farmers could ensure that new varieties retain farmer-preferred traits like taste, aroma, and adaptability, alongside yield. This holistic approach would preserve cultural heritage while addressing food security.

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Table 1: Clustering pattern of 18 genotypes of rice on the basis of Genetic Divergence

Cluster no	Number of Genotypes	Genotypes
Cluster I	**9**	Mynri, Tengakeze, Tangha, Ongchang, Awonglu Ashuh, Jwain, Tangha-1, Shengya, Ongsho
Cluster II	**2**	Chaunyak Toilang
Cluster III	**4**	Kerebe , Ongmei, Kerebe, Tengakadu
Cluster IV	**3**	Ongpang, Kilu Shuh , Reudine

Table 2: Average Inter and intra cluster of 18 rice genotypes

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	91.05 (9.54)	459.53 (21.43)	430.87 (20.75)	475.03 (21.79)
Cluster II		39.89 (6.31)	1413.11 (37.59)	645.52 (25.40)
Cluster III			84.94 (9.21)	578.22 (23.39)
Cluster IV				190.22 (13.79)

Table 3: Cluster wise mean values of 18 rice genotypes

Characters	Days to 50% flowering	Effective Tiller per plant	Days to 80 % maturity	Plant height (cm)	Panicle Length (cm)	Panicle weight (g)	No. of seed per panicle	100 seed wt (g)	Yield per plant(g)
Cluster I	132.07	4.69	159.62	128.59	25.79	2.21	148.56	1.81	10.34
Cluster II	129.90	4.10	158.30	120.95	26.20	1.17	160.25	2.27	4.66
Cluster III	132.08	4.43	158.90	115.28	25.35	3.22	159.50	2.25	13.70
Cluster IV	135.93	4.87	161.67	123.05	26.31	2.25	157.43	3.06	10.82

Table 4: Contribution of each character towards Divergence

Sl. Number	Characters	Contribution %
1	Days to 50% flowering	6%
2	Effective tillers/plant	-
3	Days to 80% maturity	-
4	Plant height (cm)	-
5	Panicle length (cm)	-
6	Panicle weight (g)	62.75%
7	No. seed/panicle	2.61%
8	100 seed wt (g)	33.99%
9	yield per plant(g)	-