

Multivariate Analysis of North East Indian Rice Landraces

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ABSTRACT: North East India is renowned for its rich biodiversity, cultural diversity, and remarkable agricultural heritage. Within this region, a multitude of rice landraces—traditional rice varieties developed over generations—flourish, each possessing distinct characteristics and adaptations to their local environments. The study aims to comprehensively understand the genetic and phenotypic diversity present in these landraces. By employing multivariate analysis techniques, researchers can extract valuable insights from large datasets comprising various agronomic, morphological, and molecular traits associated with rice landraces. In a ground breaking experiment researchers conducted an evaluation of 52 genotypes during Kharif-2021 RBD, with five replications at the esteemed Agricultural Research Station (ARS) in Bapatla, affiliated with ANGRAU. Data was meticulously recorded and analyzed, focusing on nine quantitative characters to assess the genetic diversity of 52 rice landraces. To achieve this, the researchers employed the K-means cluster analysis and PCA methodologies. The non-hierarchical cluster analysis (k-mean) technique was utilized to divide the rice landraces into five distinct clusters, while the Elbow method was used to determine the optimal number of clusters. The analysis revealed a diverse clustering pattern, with Cluster I, II, III, IV, and V comprising of 21, 5, 6, 15 and 5 accessions, respectively. Moreover, the PCV unveiled the existence of three principal components, contributing to more than 54.58% of the cumulative variance in rice landraces for yield-contributing characters. PC1 demonstrated a significant variation of 25.48% in the study, while PC2 contributed 15.55% to the total variability, and PC3 exhibited 13.55% of the variance not explained by PC1 and PC2. Overall, the study revealed a plethora of valuable insights into the genetic diversity of rice landraces, paving the way for future research in the field.

Keywords: Rice, Landraces, K-means, principal component analysis, Multivariate analysis.

INTRODUCTION

Rice is an ancient crop that has been cultivated for a very long time. It has been grown since 2500 B.C in China and nearby regions (Fuller *et al.*, 2016). Rice belongs to the *Poaceae* family and the *Oryzaceae* tribe. There are 24 different species of rice, but two domesticated species are well-known (Xuet *et al.*, 2010). The first is Asian rice, known as *Oryza sativa*, which originated in southern and eastern Asia and is now grown worldwide. The second is African rice, called *Oryza glaberrima*, which is only found in western Africa (Nourollah *et al.*, 2016). Rice is a versatile crop with a C3 photosynthetic pathway and strong genetic traits, making it resilient and adaptable. It has become an important food source for people all around the world (Fahad *et al.*, 2021).

In the vast fields of rice, there are special types of rice called landraces. These landraces are strong and adaptable, able to survive in different tough conditions (Liu *et al.*, 2022). They have unique combinations of genes that can help improve other rice varieties. However, landraces are at risk of disappearing because

farmers prefer modern hybrid varieties (Rana *et al.*, 2007). When modern farming methods were introduced, they increased productivity but also caused the loss of genetic diversity in crops like rice (Bailey-Serres *et al.*, 2010). Many old and traditional rice varieties were replaced, resulting in the loss of special genetic traits. Despite this, landraces give us hope (Zeng *et al.*, 2007). They hold a large amount of untapped genetic diversity that can be explored through special breeding programs. It is crucial to protect and use landraces to ensure the future resilience and diversity of rice, and to make sure that agriculture can continue sustainably (Latha *et al.*, 2013).

The art of genetic improvement is a dance between nature and human intervention, as we select and cultivate populations of plants that can thrive in diverse environments and accumulate a rich tapestry of genetic diversity (Thi *et al.*, 2018). This diversity is often hidden in plain sight, waiting to be discovered in the form of landraces, ancient cultivars, or improved germplasm. To unlock these hidden gems, we rely on the power of statistical tools that can help us trace the genetic divergence between different populations and

identify the specific traits that contribute to this divergence (Rashid *et al.*, 2018). With these insights, we can analyze the evolutionary patterns of crops and strategically choose the most promising parents for our hybridization programs, ushering in a new era of genetic innovation and agricultural prosperity (Anyaoa *et al.*, 2018).

Multivariate analysis helps identify hidden patterns and relationships among the diverse traits present in the landraces. It allows researchers to uncover complex associations between different variables and provides a holistic understanding of the interplay between genetic and phenotypic factors (Chandra *et al.*, 2007). Through multivariate analysis, distinct groups or clusters within the rice landraces can be identified based on shared traits. This clustering helps in classifying and categorizing the landraces, aiding in the conservation and management of their unique characteristics (Sanni *et al.*, 2012). Understanding the genetic and phenotypic diversity of rice landraces is vital for their conservation. Multivariate analysis provides insights into the extent of diversity present within the landraces, facilitating informed conservation strategies to preserve these valuable genetic resources (Akhtar *et al.*, 2012). Multivariate analysis enables researchers to identify landraces with specific adaptive traits suited to local environments. This knowledge can be utilized to develop sustainable agricultural practices that promote the cultivation of landraces adapted to specific agroecological conditions (Maione *et al.*, 2019).

As we learn more about data analysis, we discover an interesting method that could change the way we understand information. It's called cluster analysis, and it helps us group data based on similarities. This technique is really useful for understanding complex data sets. One important tool in cluster analysis is the K-means clustering algorithm (Worede *et al.*, 2014). It puts objects into clusters by finding the mean closest to each object. This helps us see how different data points are connected. But it's not always easy to know how many clusters we should have. That's where the elbow approach comes in. It's a clever algorithm that helps us find the best number of clusters. By studying the data and finding the elbow point, we can figure out the perfect number of clusters and make the most of this technique. Join us on this amazing journey as we explore cluster analysis and change the way we understand data (Gyani *et al.*, 2021).

Imagine you have a giant puzzle with many different pieces, and each piece represents a variable related to a particular agricultural sector. It would be very difficult to see how all the pieces fit together and to identify any patterns or relationships between them. Principal Component Analysis (PCA) is like a magic tool that can take all those puzzle pieces and transform them into a smaller set of uncorrelated pieces that still contain all the important information. It's like solving a Rubik's Cube, where each move affects the others and the ultimate goal is to get all the colors aligned. The result is a clear representation of the data that can be used to assess genetic diversity in a population, and can help identify the most important variables to focus on in agricultural research.

The study aims to understand the genetic and physical diversity of these rice varieties in a comprehensive way. However, previous research has shown that there is a lack of exploration using multivariate analysis techniques for this particular group of rice landraces. Firstly, while some studies have acknowledged the diversity of rice landraces in North East India, they have not conducted a thorough analysis using multivariate techniques. Multivariate analysis considers many different traits at the same time, which helps us better understand the overall diversity of the landraces. This research gap shows the need for a detailed investigation using multivariate analysis to uncover the complex relationships and patterns among various agronomic, morphological, and molecular traits related to these rice varieties. Secondly, North East India has a unique ecological and cultural setting that requires special attention. This region is known for its rich biodiversity and diverse agricultural practices, leading to a wide variety of rice landraces. However, there is limited research specifically focusing on the genetic and physical diversity of these landraces using multivariate analysis techniques. As a result, we need more information about the specific characteristics, adaptations, and potential uses of these landraces within the context of North East India. Additionally, we have a knowledge gap when it comes to conserving and sustainably utilizing these rice varieties in North East India. With the increasing popularity of modern hybrid varieties, traditional landraces are at risk of being lost. It is crucial to explore and document the genetic and physical diversity of these traditional rice varieties. Multivariate analysis can provide valuable insights for conservation strategies, crop improvement efforts, and the development of sustainable farming practices specific to North East Indian rice landraces.

In terms of future scope, there are several avenues for further exploration in the study of North East Indian rice landraces. This would contribute to a deeper understanding of their diversity and potential applications. Furthermore, exploring functional traits such as disease resistance and nutritional quality would enable researchers to identify landraces with desirable characteristics, offering valuable resources for crop improvement programs and the development of sustainable agriculture practices. By considering these future directions and addressing conflicts of interest, the study can further contribute to our knowledge of North East Indian rice landraces and their implications for agricultural development and sustainability.

MATERIALS AND METHODS

Behold, the stage was set for an extraordinary experiment that would push the boundaries of agricultural research. In the verdant fields of ANGRAU's Agricultural Research Station in Bapatla, 52 North East Indian land race accessions (Table 1) were brought to life and evaluated with the utmost care during the *Kharif-2021* season. Using a Randomized Block Design with five replications, each accession was cultivated in a single, three-meter-long row, with a meticulous 20-cm-apart-row and 15-cm-apart-plant spacing. As the days went by, the recommended

agronomic packages of practices were strictly followed, ensuring that the experiment was carried out with the utmost precision. With nine quantitative characters at the forefront of our minds, five randomly desired plants of each genotype were carefully selected and subjected to a rigorous morphological evaluation, following the standard protocol of *Oryza sativa* (IIRR). These characters included the Days to 50 per cent flowering (DF50%), Plant height (PH), Number of tillers per hill (NTPH), Number of effective tillers per hill (NETPH),

Panicle length (PL), Number of grains per panicle (NGPP), Panicle fertility percentage (PFP), Test Weight (TW) and Grain yield per plant (GYPP). As the data began to pour in, we knew that it was time for a multivariate statistical analysis. The phenotypic diversity was illustrated by principal component analysis (PCA) using the R package FactoMineR (Le *et al.*, 2008), factoextra (Kassambara and Mundt 2017) and clustering analysis based on the algorithm of *K-means* method (Khan, and Ahmad 2004).

Table 1: List of landraces used in the study.

Sr. No.	Designation	S. no	Designation
G1	Ambemohar	G27	Kudral-7
G2	Apputhakal	G28	Kulakar
G3	Arakuloya	G29	Machakanta
G4	Asandi	G30	Madhumurangi
G5	Bahurupi	G31	Madhuraj-55
G6	Badshabhog	G32	Mangala Mahsuri
G7	Burmablack	G33	Mappilai samba
G8	Chattisgarh local	G34	Mathatriveni
G9	China biceinee	G35	Mysore malliga
G10	Chinni krushnudu	G36	Narayanakamini
G11	Chintalurisannalu	G37	Navara
G12	Chittiga	G38	Pancharatna
G13	Chittimuthyalu	G39	Parimalasanna
G14	Doddiga	G40	Pathariya
G15	Halla Bhatta	G41	Poongar
G16	Illappu samba	G42	Ramyagali
G17	Kakirekkalu	G43	Ranikanaka
G18	Kalabhatt	G44	Ranikanda
G19	Kalachampa	G45	Samuelbhog
G20	Kalajeera	G46	Seeraga samba
G21	Kalanamak	G47	Selamsanna
G22	Kandasagar	G48	Sonakshika
G23	Karuppukavuni	G49	Kalasur
G24	Karigajavalli	G50	Kuzipatali
G25	Karisala	G51	Raktasali
G26	Kudral-6	G52	Krishna Vreehi

RESULTS AND DISCUSSION

In the quest for understanding the genetic diversity among rice landraces, a non-hierarchical cluster analysis known as k-mean was employed to group 52 genotypes into five distinct clusters (Table 2) mean values represented at (Table 3). To determine the optimal number of clusters, the Elbow method was used, resulting in the identification of five clusters with unique characteristics. Cluster I comprised of 21 accessions that exhibited low mean values for most traits, while Cluster II consisted of only five genotypes with high mean values for panicle length, number of grains per panicle, and grain yield per plant, while the rest of the traits displayed average mean values. Cluster

III, on the other hand, contained six accessions with low mean values for all traits. Cluster IV comprised of 15 genotypes with high mean values for the number of tillers and effective tillers, with the rest of the traits showing average mean values. Lastly, Cluster V included five accessions with high values for days to 50% flowering, plant height, panicle fertility percentage, and test weight, while the number of tillers, effective tillers, and panicle length displayed low mean values. Overall, this clustering pattern indicates the presence of sufficient genetic diversity among the rice landraces.

As the experiment unfolded, it was revealed that the maximum inter-cluster distance was observed between Cluster II and III (Table 4, Fig 1. and Fig 3(b)). The

former cluster comprised of five exceptional genotypes with the highest mean values for panicle length, number of grains per panicle, and grain yield per plant. Interestingly, the remaining traits displayed average mean values. Therefore, the genotypes originating from this cluster are highly desirable and selection for them may prove fruitful in future hybridization programs. Similar results reported by Nagamani *et al.*, (2022) in rice, K-means cluster analysis resulted in the formation of nine optimum clusters. Among these clusters, cluster III had the highest number of genotypes (15), followed by cluster IV with 12 genotypes. Bozchaloyi *et al.*, (2017) reported K-Means clustering revealed populations, genetic stratification in 15 geographical populations of *Geranium purpureum* Vill. According to Kanavi *et al.*, (2020), using K-means clustering, all 205 germplasm accessions were sorted into seven different groups. In another study by Rao *et al.*, (2008), K-means clustering showed that trees from different geographic

regions were placed together in one group, while trees from the same area were placed in different groups. This suggests that there was no direct relationship between geographical diversity and genetic diversity. Lastly, Hussain *et al.*, (2017) found that cluster analysis divided 28 sunflower hybrids into three distinct groups, highlighting their diverse characteristics.

In this study, Principal Component Analysis (PCA) was carried out on 52 rice landraces and nine quantitative traits related to yield and yield attributing characteristics. Three principal components were identified, each exhibiting more than one Eigen value (Table 5&6, Fig 2. And Fig 3(a)) and contributing to more than 54.58% of the cumulative variance in rice landraces for yield contributing characters. These components are responsible for a high magnitude of variance in the population and are useful for selecting elite genotypes from a diverse genetic population.

Table 2: Cluster membership summary.

Cluster	Members	Genotypes
1	21	G2, G10, G13, G15, G17, G18, G24, G27, G28, G29, G30, G32, G34, G35, G39, G40, G44, G47, G48, G49& G51.
2	5	G12, G31, G33, G37 & G41.
3	6	G3, G7, G8, G9, G20 & G38.
4	15	G1, G6, G11, G14, G16, G19, G21, G22, G23, G25, G36, G42, G43, G50 & G52.
5	5	G4, G5, G26, G45 & G46.

Table 3: Cluster means.

Cluster No	Means								
	DF50%	PH	NTPH	NETPH	PL	NGPP	PPF	TW	GYPP
1	86.733	116	12.838	10.771	22.562	236.467	82.243	26.913	21.743
2	87.104	124.2	13.48	11.56	22.84	275.16	82.774	22.472	26.88
3	83.96	114.233	13.033	11	22.267	162.467	81.412	21.803	23.533
4	87.501	117.96	14.453	12.493	22.48	202.107	82.535	26.035	23.787
5	89.888	142.56	12.28	10.56	22.36	199.8	83.002	27.604	22.52

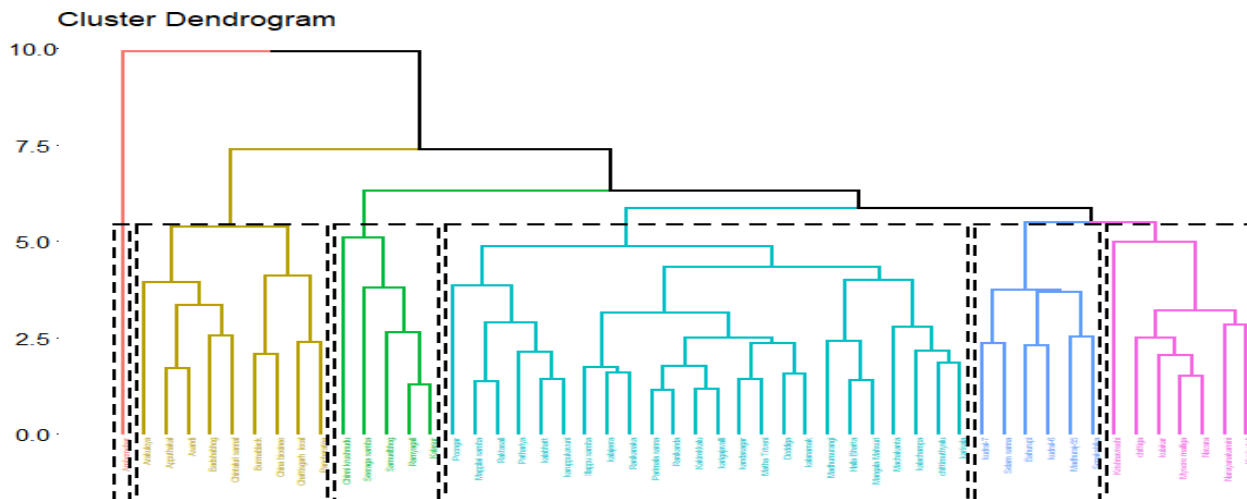
Days to 50 per cent flowering (DF50%), Plant height (PH), Number of tillers per hill (NTPH), Number of effective tillers per hill (NETPH), Panicle length (PL), Number of grains per panicle (NGPP), Panicle fertility percentage (PPF), Test Weight (TW) and Grain yield per plant (GYPP).

Table 4: Inter Cluster Distances.

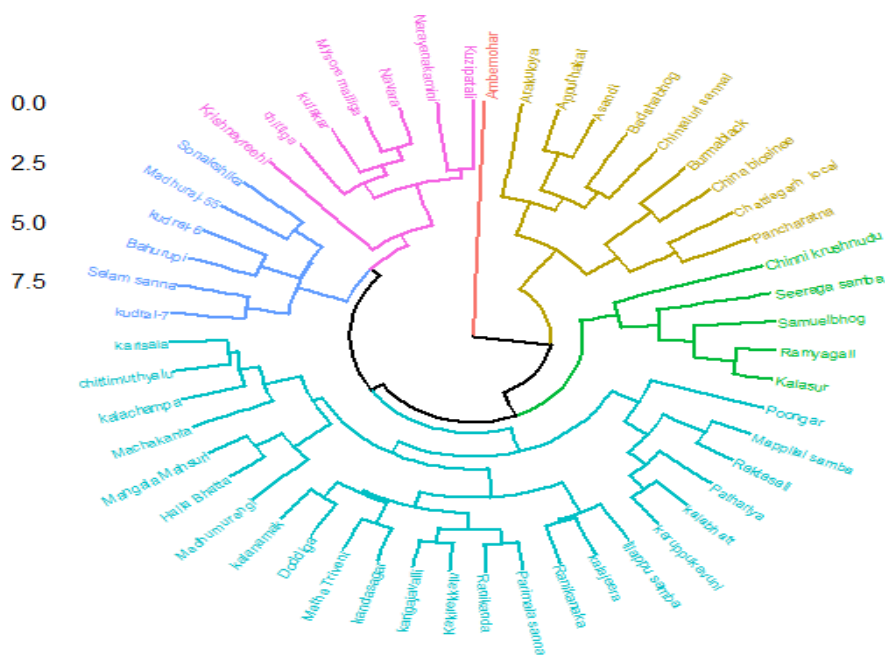
Cluster No	1	2	3	4	5
1	0	40.151	74.276	34.578	45.408
2	40.151	0	113.24	73.486	77.923
3	74.276	113.24	0	40.265	47.637
4	34.578	73.486	40.265	0	25.079
5	45.408	77.923	47.637	25.079	0

Table 5: Eigenvalues of Correlation Matrix.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalues	2.293	1.4	1.22	0.999	0.978	0.817	0.66	0.629	0.004
Proportion	0.255	0.156	0.136	0.111	0.109	0.091	0.073	0.07	0
Cumulative Proportion	0.255	0.41	0.546	0.657	0.765	0.856	0.93	1	1

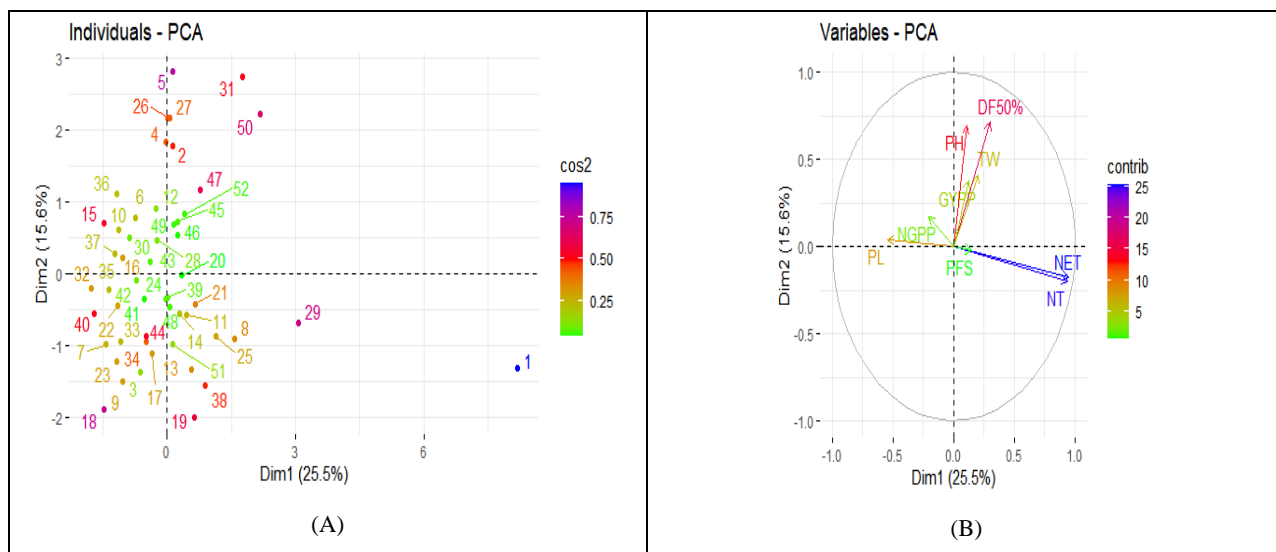


(A)



(B)

Fig. 1. A) Dendrogram showing clustering pattern of 52 rice landraces in *Kharif*-2021 using Hierarchical method; B) Phylogenetic tree showing clustering pattern of 52 rice landraces in *Kharif*-2021 using Hierarchical method.



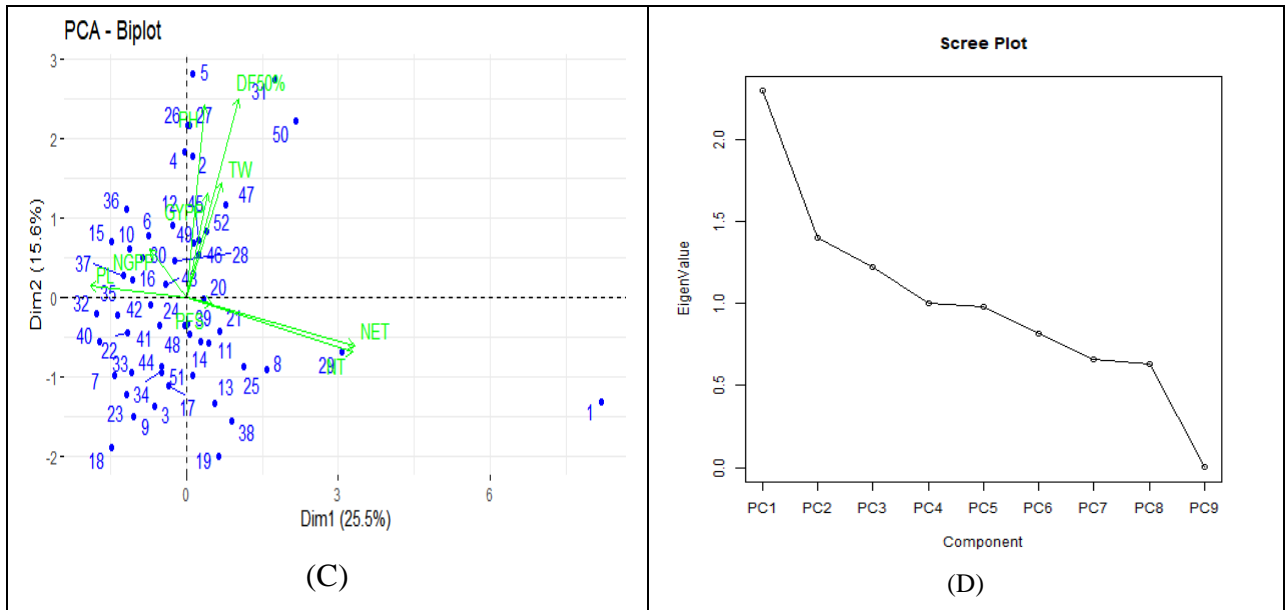


Fig. 2. (A, C) Distribution and grouping of germplasm lines across first two principal components (B)PCA graph of different traits for first two principal components(D)Screen plot based on eigenvectors.

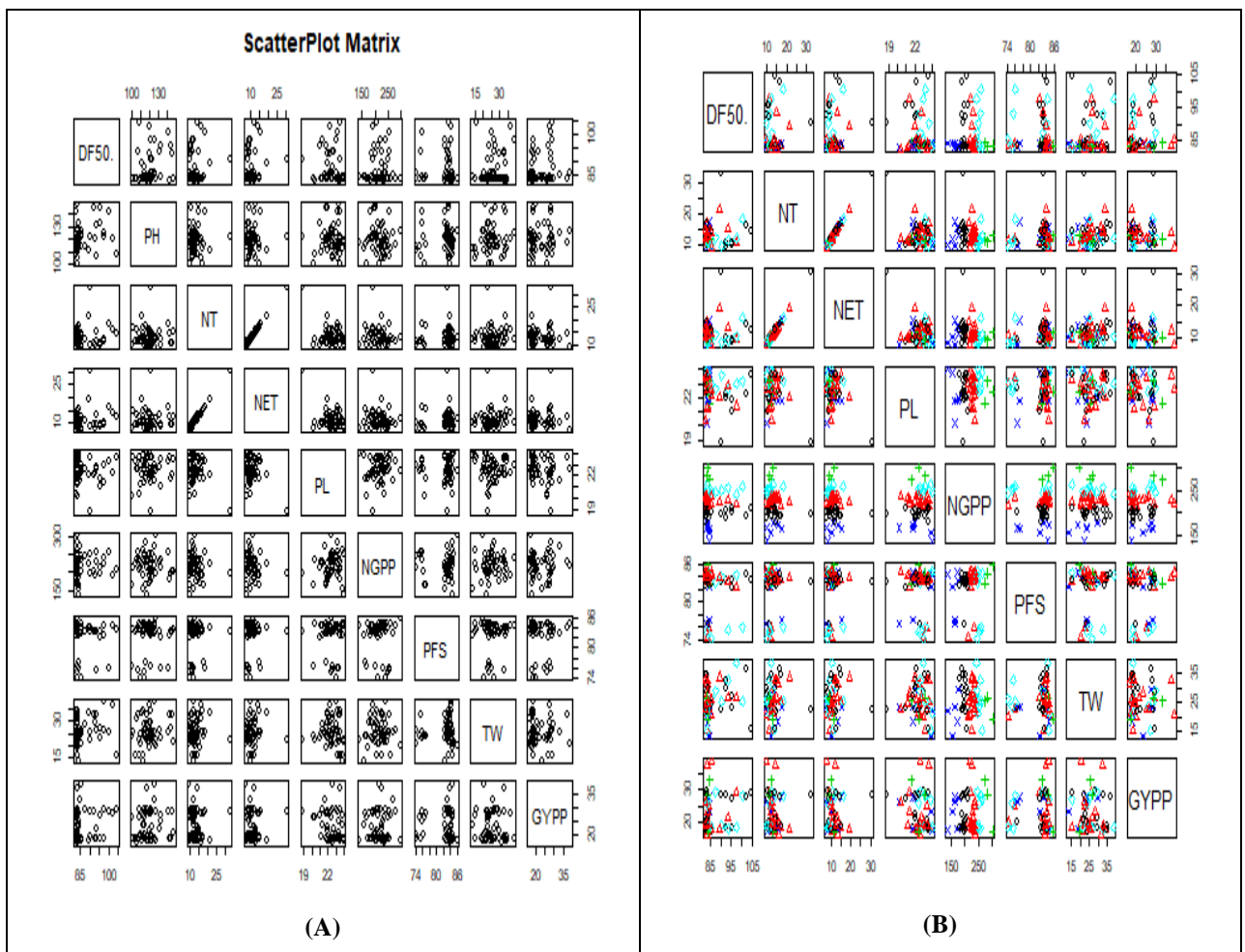


Fig. 3. A) Screen plot of principal compound analysis B) Screen plot of K-means.

Table 6: Loadings (Eigenvectors) of Correlation Matrix.

	PC1	PC2	PC3
DF50%	0.196	-0.604	-0.049
PH	0.07	-0.584	0.107
NT	0.62	0.168	0.093
NET	0.626	0.148	0.086
PL	-0.358	-0.033	0.415
NGPP	-0.136	-0.146	0.103
PFS	0.096	0.02	0.402
TW	0.133	-0.348	0.491
GYPP	0.081	-0.316	-0.62

PC1 explains 25.48% of the variation in the study, with number of tillers per hill and number of effective tillers per hill positively correlated to PC1, while panicle length shows negative correlation. PC2 explains 15.55% of the next largest portion of the variation not explained by PC1, and day to 50% flowering, plant height, test weight, and grain yield per plant are significantly negatively correlated with PC2. PC3 explains 13.55% of the next largest portion of the variation not explained by PC1 and PC2, with panicle length, panicle fertility percentage, and test weight positively correlated to PC3, while grain yield per plant is negatively correlated. The results of this analysis can aid plant breeders in selecting desirable traits for use in plant selection.

As per the analysis conducted in this study, PC1 is primarily associated with the number of tillers and number of effective tillers, while PC2 is associated with day to 50% flowering, plant height, test weight, and grain yield per plant. PC3 is associated with panicle length, panicle fertility percentage, test weight, and grain yield per plant. These traits have more weightage in their respective principal compounds, indicating their significant contribution towards total divergence. Therefore, selecting genotypes based on these traits could be useful in breeding programs.

Similar findings were reported by Nachimuthu *et al.*, (2014) who discovered that the first Principal Component Analysis (PCA) explained 80.56% of the total variation among 12 different characteristics. Shoba *et al.*, (2019) also found that four out of nine Principal Components (PCs) had eigen values greater than 1.0 and accounted for 70.14% of the total variability. PC1 explained the highest proportion of total variation (25.9%), while PC2, PC3, and PC4 contributed 16.96%, 15.06%, and 12.22% of the total variance, respectively. Another study conducted by Pachauri *et al.*, (2017) revealed that the first four principal components explained approximately 37.12% of the total variability, with PC1 being the most influential. Seetharam *et al.*, (2009) reported that the PCA components explained 41.6% of the overall variation. Furthermore, Pathak *et al.*, (2018) found that in PCA, Component 1 was influenced by traits such as days to maturity and harvest index, accounting for 25.34% of the total variability, while plant height, biological yield per plant, and grain yield per plant contributed 19.30% to the total variability in Component 2.

CONCLUSION

The rice landraces studied here exhibit a remarkable level of genetic diversity, as revealed by both clustering pattern and principal component analysis. The three principal components with eigen values greater than one contribute significantly to the variance in the population, making them attractive targets for plant selection. Among the clusters, Cluster II contains five genotypes with particularly desirable mean values for all traits, making them excellent candidates for use in breeding and crossing programs. By leveraging these insights, plant breeders can effectively harness the natural diversity present in these landraces to develop improved rice varieties.

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