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An Investigation into the Nature and Magnitude of Genetic Diversity for Anaerobic Germination Traits in Rice using Principal Component Analysis

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ABSTRACT: Anaerobic germination (AG) is a major limiting factor for the successful adoption of direct seeding in rice cultivation. Anaerobic germination in rice poses challenges such as lack of oxygen, toxicity, disease, and limited nutrient availability, but rice plants have adapted specialized mechanisms to overcome them. The development of rice cultivars tolerant to AG and early seedling vigor is an important objective under direct seeding. Multivariate analysis tools such as principal component analysis (PCA) and cluster analysis are effective for evaluating phenotypic diversity and identifying genetically distant clusters of genotypes. This study aimed to estimate the genetic diversity of anaerobic germination traits in rice using PCA. A total of 103 rice genotypes were evaluated for eight anaerobic germination traits. The PCA results revealed four principal components that accounted for 83.63% of the total variation. The first principal component (PC1) explained 32.62% of the total variation and was positively correlated with germination percentage, seedling vigour index, shoot length and length of first internode. The second principal component (PC2) explained 20.29% of the total variation and was positively correlated with shoot length, root length, and number of leaves. The third principal component (PC3) explained 17.56% of the total variation and was positively correlated with shoot length, length of first Internode, root length, number of leaves, shoot dry weight, root dry weight. The fourth principal component (PC4) explained 13.16% of the total variation and was positively correlated with shoot length and length of first internode. The PCA analysis provided valuable information on the genetic diversity of anaerobic germination traits in rice and can aid in the selection of parental genotypes for breeding programs aimed at developing rice varieties tolerant to anaerobic germination (AG).

Keywords: Anaerobic germination, principal component, eigen values.

INTRODUCTION

Rice, which serves as a major staple food for one-third of the global population, has seen a shift in production methods in recent years. Specifically, rice production is increasingly shifting from transplanting to direct seeding, due to reductions in cultivation costs and early maturity of direct-sown crops (Pandey and Valesco 2002). The poor seedling establishment under direct seeding in standing water is a major obstacle for the large-scale adoption of this rice cultivation method. This problem is attributed to the lack of tolerance to anaerobic germination (AG) caused by submergence, which is identified as the main limiting factor for popularizing direct seeding in rice according to Yang *et al*. (2019). However, Ismail *et al*. (2009) observed varietal differences for anaerobic germination, indicating that development of rice cultivars tolerant to anaerobic conditions during germination coupled with early seedling vigor is an important objective under direct seeding, as reported by Joshi *et al*. (2013); Miro and Ismail (2013); Vijayan *et al*. (2018). Therefore, studies on genetic diversity for anaerobic germination traits are essential for devising effective breeding strategies aimed at developing rice varieties tolerant to anaerobic conditions during germination for wet direct seeding under puddled conditions.

Multivariate analysis tools, such as principal component analysis (PCA) and cluster analysis, have been reported to be effective for evaluating phenotypic diversity, identifying genetically distant clusters of genotypes, and selecting important traits contributing to the total variation in genotypes. In particular, PCA allows the natural grouping of genotypes and is a precise indicator of differences among genotypes (Mohammadi, 2000). On the other hand, cluster analysis is useful for identifying and separating a core subset of genotypes with distinct phenotypic traits. Previous studies conducted by Sharma *et al*. (2018); Barik *et al*. (2019)

have used PCA to study the genetic diversity for anaerobic germination traits, and the present investigation was undertaken to study the nature and magnitude of genetic diversity for anaerobic germination traits using PCA towards the development of anaerobic germination tolerant varieties. These statistical programs aid in the selection of elite genotypes by identifying redundancy of genotypes with similar characters and their elimination. This information is useful for better selection of parental genotypes with specific traits and devising breeding strategies for trait improvement.

MATERIAL AND METHODS

The experimental material for this study comprised 103 germplasm accessions of the BAAP population, and Odisha landraces (Table 1). The aim of the study was to screen these accessions for anaerobic germination tolerance. The experiment was conducted at ICAR-NRRI (National Rice Research Institute) Cuttack during Kharif 2019, under net house by simulating anaerobic conditions for germination. A seedling tray with 156 wells (13 columns × 12 rows) was filled with fine soil, and three seeds per well (a total of 36 dry seeds per entry) were sown in each column, covered with 1cm of fine soil. The tray was then placed in a large container filled with water to a height of 10cm above the seedling tray for a duration of 21 days. Each seedling tray was sown with tolerant and susceptible checks AG 387 and Cr Dhan 801, respectively. Observations were recorded after 21 days of submergence, and data on the percentage of anaerobic germination (%), as well as various anaerobic germination traits such as length of first internode (cm), shoot length (cm), number of leaves, root length (cm), shoot biomass (g), root biomass (g), and seedling vigour index (SVI), were collected.

Principal component analysis was conducted in accordance with the procedure outlined by Banfield (1978) and detailed by Gomez and Gomez (1984). PCA can be performed on two types of data matrices: variance-covariance matrix and correlation matrix. A correlation matrix standardizing the original data set is preferred when the variables are on different scales. Conversely, a variance-covariance matrix can be used when the variables are on the same scale. In this study, PCA was conducted on the correlation matrix of the anaerobic germination tolerance traits, which removed the effects of scale (Jackson, 1991). A data matrix consisting of 107 rice genotypes across six variables was prepared for analysis. A covariance matrix derived from the data matrix was then converted to a correlation matrix.

Eigenvalue and eigenvector pairs created from the data matrix were used to identify the principal components. Eigenvalues represent the total amount of variation displayed on the principal components, with the proportion of variation accounted for by each principal component expressed as the eigenvalue divided by the sum of the eigenvalues. The analysis was carried out using the web-based softwarehttps://www.kaugrapes.com/home.

Percent variance explained for PCI = Eigen values (PCI)/Sum of eigen values

RESULT AND DISCUSSION

Principal Component Analysis (PCA) is a statistical method used to identify a small number of factors that explain most of the variability in a dataset. In this analysis, eight principal components found, but only four principal components were with Eigen values more with one and remaining four were with less than one eigen value were considered non-significant and excluded from the analysis since they are unlikely to have any practical significance. Table 2 presents the Eigen values, percentage of variance, cumulative percentage of variance, and factor loadings of different variables.

In present study, it was found that the first four principal components contributed 83.63 percent out of the total variability. The first principal component (PC1) accounted for 32.62% which is maximum toward the total variability (Fig. 1). The characteristics germination percentage (0.54), seedling vigour Index (0.55), shoot length (0.23), and length of first Internode (0.27) were positively loaded. while, root length (-0.18), shoot dry weight (-0.31), root dry weight (-0.35), number of leaves (-0.04) were negatively loaded. The second principal component (PC2) was characterized by 20.29% contribution to the total variation. PC2 was positively influenced by traits like shoot length (0.15), root length (0.43), and number of leaves (0.46). while traits root dry weight (-0.47), shoot dry weight (-0.47), length of first Internode (-0.22), germination percentage (-0.18), and seedling vigour Index (-0.17) were negatively influenced.

The third principal component (PC3) has described 17.56% of variation of the total variation. Six Characters *viz*., shoot length (0.62), length of first Internode (0.53), root length (0.40), number of leaves (0.15), shoot dry weight (0.26), root dry weight (0.230) was contributed positively to the total variation. While PC3 was negatively influenced by germination percentage (-0.09) and seedling vigour index (-0.002). The fourth Principal component (PC4) accounted for 13.16% of variability of the total variability. The characters contributing most positively were shoot length (0.14) and length of first internode (0.33). whereas it was negatively influenced by germination percentage (-0.35), root length (-0.12), number of leaves (-0.65), shoot dry weight (-0.34), root dry weight (-0.26), seedling vigour index (-0.33). The prominent traits that were coming together in different principal components and which contributed towards explaining the total variability have the tendency to remain together Girijarani *et al.* (2014); Sudeepthi *et al.* (2020). The hybridization of genetically diverse genotypes is anticipated to generate transgressive segregants characterized by superior yield and enhanced tolerance to anaerobic germination. Hence, such segregants can be effectively utilized in breeding programs aimed at producing cultivars that exhibit both high yield potential and tolerance to anaerobic germination under dry direct seeded rice (DSR).

Table 1: List of the germplasm entries utilized in screening for various anaerobic germination tolerance traits.

Sr. No.	Name of the Genotype	Sr. No.	Name of the Genotype
1.	ARC 10958	53.	AUSMERI
2.	ARC 11600	54.	Banda
3.	ARC 14855	55.	Bankoi
4.	ARC 14965	56.	Basantibhog
5.	ARC 5959	57.	Basapatri
6.	ARC 5977	58.	BJ 1
7.	ARC 6000	59.	BORO
8.	ARC 6240	60.	BOWALIA
9.	ARC 7098	61.	BOWALIA 2
10.	ARC 7325	62.	CHAMKA
11.	AS 2	63.	Champa
12.	ASSAM 4(BORO)	64.	Chholaboro
13.	AUS 125	65.	Chinamali
14.	AUS 127	66.	DA 12
15.	AUS 131	67.	Dhala basmati
16.	AUS 151	68.	DHINGHA
17.	AUS 169	69.	Gadakati
18.	AUS 175	70.	Gahamphulla
19.	AUS 204	71.	Goria
20.	AUS 22	72.	IR 64-21
21.	AUS 267	73.	Jabaful
22.	AUS 268	74.	Jangalijata
23.	AUS 273	75.	Jubaphul
24.	AUS 283	76.	Kala kadamba
25.	AUS 29	77.	Kalachampa
26.	AUS 294	78.	Kalajeera
27.	AUS 298	79.	Kalakataki
28.	AUS 309	80.	Kalamkati
29.	AUS 314	81.	Khajara
30.	AUS 317	82.	Krishnabhog
31.	AUS 321	83.	Lajakuli
32.	AUS 335	84.	Lajakuli
33.	AUS 350	85.	Mahakamati
34.	AUS 353	86.	Mukta kiari
35.	AUS 354	87.	MUNSHISHAIL
36.	AUS 361	88.	Nababi
37.	AUS 362	89.	NaliBaunsaGaja
38.	AUS 366	90.	Panirohi
39.	AUS 369	91.	Parbat jeera
40.	AUS 37	92.	Pimpudibasa
41.	AUS 382	93.	RAJ MUNDO
42.	AUS 385	94.	Samudrabali
43.	AUS 391	95.	SANHUANGZHAN NO 2
44.	AUS 417	96.	SATHA
45.	AUS 435	97.	Solari
46.	AUS 46	98.	Swarna
47.	AUS 60	99.	T 1
48.	AUS 62	100.	Tulasiphula
49.	AUS 63	101.	Tupa
50.	AUS 77	102.	**AG 387 (Tolerant check)**
51.	AUS 93	103.	**Cr Dhan 801 (Suceptible check)**
52.	AUS PADDY(WHITE)		

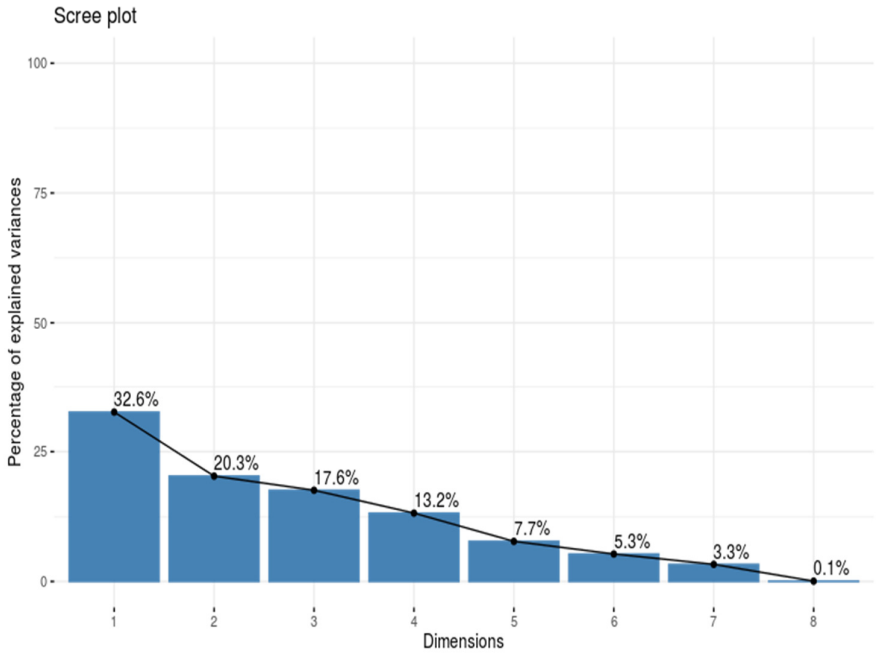

Fig. 1. Scree plot graphical representation.

Table 2: Eigen values, proportion of the total variance represented by first four principal components, cumulative per cent variance and component loading of different characters in rice for anaerobic germination traits.

variables	PC1	PC2	PC3	PC4
Germination percentage (%)	0.544	-0.182	-0.092	-0.35
Shoot length (cm)	0.237	0.15	0.623	0.147
Length of first internode (cm)	0.277	-0.229	0.538	0.335
Root length (cm)	-0.18	0.435	0.408	-0.122
Number of leaves	-0.04	0.465	0.156	-0.655
Shoot dry weight (g)	-0.315	-0.478	0.264	-0.34
Root dry weight (g)	-0.357	-0.477	0.233	-0.268
Seedling Vigor Index	0.557	-0.173	-0.002	-0.337
Eigen Value	2.61	1.62	1.40	1.05
Percentage of Variance	32.621	20.29	17.56	13.16
Cumulative % of variance	32.621	52.91	70.47	83.63

The biplot diagram is a useful tool that provides insight into the interaction between traits and genotypes, highlighting the superior performing genotypes for specific traits. The vector length of each trait on the biplot reflects its contribution to the overall variation, with longer vectors indicating a greater contribution. In the present study, the biplot diagram (Fig. 2) was utilized to depict the distribution and nature of diversity among genotypes and quantitative traits, using PC1 and PC2 as the principal components. The seedling vigour index showed maximum vector length followed by germination percentage indicating the contribution towards total divergence. The orientation of trait vectors in the biplot diagram also conveys information about the association between traits. Specifically, the angle between trait vectors indicates the degree and direction of correlation between them. When the angle is acute (less than 90 degrees), it signifies a positive correlation between traits. In contrast, an obtuse angle (greater than 90 degrees) indicates a negative correlation, while a right angle (90 degrees) denotes no correlation between the traits (Fig. 2). The traits germination percentage and seedling vigour index has showed positive correlation with length of first internode, shoot dry weight and root dry weight and negatively correlated with root length and number of leaves (Girijarani *et al*., 2014; Sudeepthi *et al.*, 2020).

The biplot diagram facilitates the identification of high-performing genotypes for specific traits by examining their proximity to the corresponding trait vectors within the same quadrant. For a given set of phenotypic traits, genotypes that are in proximity to the corresponding quadrant of the trait vector are expected to exhibit superior performance for those traits. The genotype AG

387 (tolerant check), BJ – 1 were the best performing genotypes for germination percentage and seedling vigour index. While, the genotypes T-1 and Lajakuli were found superior genotypes for length of first internode. The genotype ARC 600 and AUS435 were best performing genotypes for shoot and root length respectively. The shoot and root biomass trait were found superior in genotypes AUS 354, AUS 366, whereas the genotypes AUS PADDY (WHITE) and munshilal were found best for number of leaves.

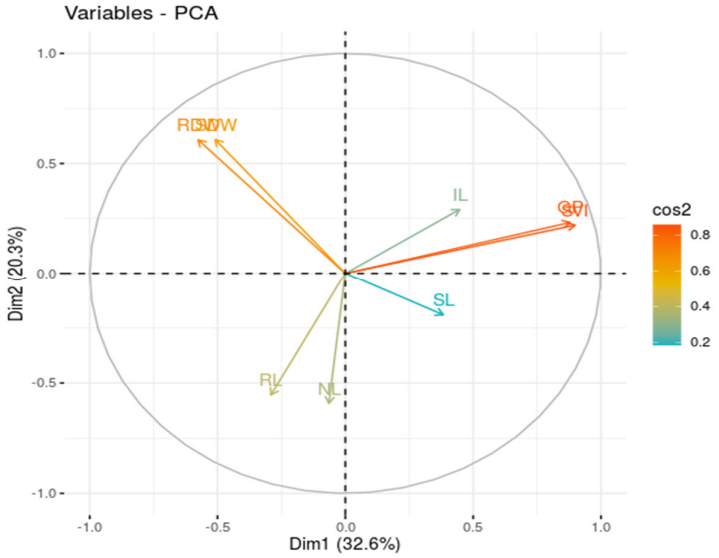

Fig. 2. Variables of Principal component analysis for anaerobic germination tolerance traits.

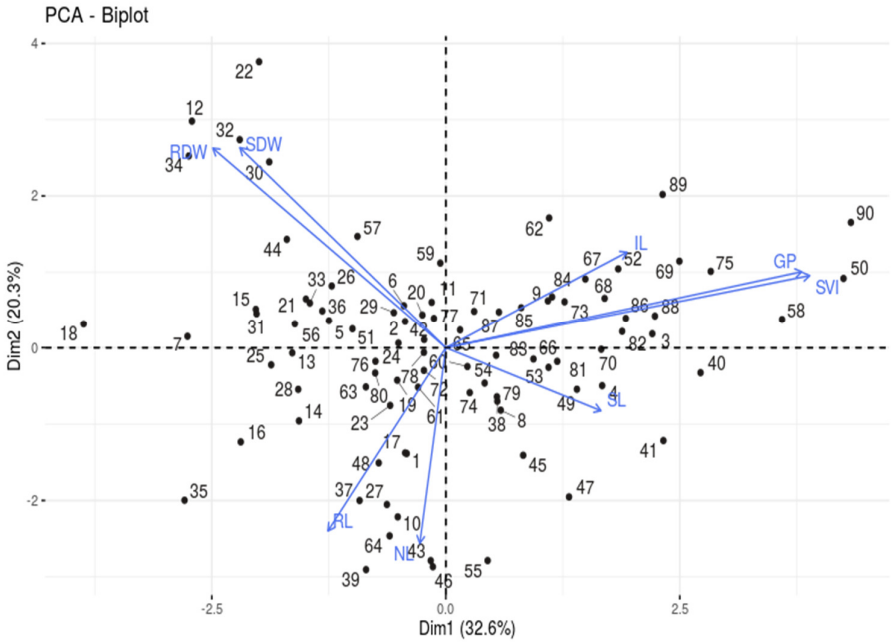

Fig. 3. Bi plot of Principal components 1 and 2 (Dimensions).

CONCLUSIONS

Principal component analysis (PCA) is a powerful technique for analysing complex datasets related to anaerobic germination tolerance. By identifying patterns and relationships among the variables involved in the germination process, PCA can provide valuable insights into the mechanisms of anaerobic germination and help to develop effective strategies for improving crop yields under low-oxygen conditions. Some key findings that have emerged from studies using PCA to analyse anaerobic germination tolerance include the identification of specific genetic traits that are associated with tolerance, such as germination percentage, length of first internode, shoot length and root length. Overall, PCA has proven to be an essential tool for understanding the complex interactions that influence anaerobic germination tolerance, and its use is likely to continue to

drive advances in crop breeding and agricultural practices in the future.

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Conflict of Interest. None.

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