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RESEARCH ARTICLE

Exploring rice genotypes for anaerobic germination: Towards sustainable direct seeding

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Abstract

Direct-seeded rice (DSR) production is the primary focus of this study, which explores the diversity of rice genotypes concerning their ability to withstand anaerobic germination conditions. Using advanced statistical analysis, a total of 250 rice cultivars were assessed for several anaerobic germination attributes. Significant variability was observed among genotypes for key traits including germination % (ARC14855, AG 387), shoot length and seedling dry weight, indicating potential donors for anaerobic germination tolerance. Trait association analysis, variability analysis, principal component analysis and cluster analysis were employed to uncover the genetic and phenotypic factors influencing anaerobic germination tolerance. The genotypes of cluster III could be used as donors for anaerobic germination studies, as the genotypes have revealed high average values for all the traits. The findings underscore the importance of genetic variability and heritability in breeding resilient rice varieties suited for DSR cultivation, offering valuable insights for enhancing rice cultivation practices in the face of challenges such as water scarcity and labour shortages. To improve the sustainability and productivity of paddy production systems, this research advances rice breeding procedures that aim to create varieties tolerant to anaerobic conditions.

Keywords

anaerobic germination; cluster analysis; correlation; principal component analysis; variability

Introduction

Rice, the second largest cereal crop globally, dominates Asian agriculture with an annual production of 510 million tonnes (1, 2). The lack of water, manpower and rising input costs make paddy cultivation unsustainable, more expensive and less lucrative (3). Farmers in India are using dry seed broadcasting technology to implement direct-seeded rice (DSR). This technique, however, seriously jeopardizes lowland rice farming by harming yield, growth and germination (4). According to a study, rice producers sometimes face a range of issues resulting from flooding stress, ranging from chronic waterlogging to germination inundation (5).

Direct-seeded rice (DSR) adoption is hindered greatly by anaerobic germination (AG) (6). Under DSR conditions, AG is essential for maintaining yield stability and adaptation, followed by shoot and root development (7). Genotypes that are AG tolerant grow their shoots quickly, which allows them to quickly get to the water's surface to obtain oxygen. Increased coleoptile development under submergence is a crucial morphological change for these tolerant cultivars (8). Nonetheless, regular germination and growth of seedlings are significantly hampered by the decrease in ATP generation in anaerobic environments. However, to sustain the development of embryos and coleoptile elongation in low-oxygen environments, some rice cultivars have evolved distinctive strategies (9), such as enhanced starch metabolism.

Present day rice cultivars are not developed with direct seeding conditions in consideration. A small number of investigations have addressed this element of rice farming and earlier cultivars showed lower yields when exposed to direct sowing. Anaerobic germination has additionally received little focus in research. Interestingly, a study examined around 2000 *Oryza glaberrima* cultivars at a water level of 10 cm and found 6 breeding reservoirs that showed noteworthy anaerobic % of survival (10). Another study contributed to this region by selecting 5 cultivars from a set of rice cultivars that exhibited a minimum of 70 % survival in a screening carried out under comparable conditions (11).

Despite the limited literature on anaerobic germination, it's imperative to acknowledge that this field has garnered global attention. To produce rice cultivars that are tolerant of anaerobic (hypoxic) circumstances during germination, especially in the context of direct seeding methods, research into genetic variation concerning anaerobic germination features is essential. To accomplish this, applying multivariate analysis tools like Principal Component Analysis (PCA) and Cluster Analysis has proven to be a powerful method for analyzing phenotypic diversity and pinpointing critical features that influence overall genetic variance. The exact selection of parental genotypes with desired qualities and the development of breeding plans aimed at improving these traits are made possible by these analyses, which offer significant insights.

Materials and Methods

Plant Materials

The population under the experiment had 356 accessions, including the majority of *Aus* landraces (12, 13), landraces of Odisha and a few released cultivars and breeding lines.

Anaerobic Germination Screening

A total of 356 lines were screened for anaerobic tolerance conditions in Net House No. 07 at ICAR-National Rice Research Institute (NRRI), Cuttack. For this trial, a seedling tray comprising 156 wells (arranged in 13 columns by 12 rows) was utilized. Every genotype was depicted by planting 12 wells with 72 healthy, seeds that were dry in each, with 3.5 cm of good soil below the seeds and 1 cm of well-dried field soil on top of each well. After being seeded, the trays were placed in a bigger plastic container that was filled with water until it reached a depth of 10 cm above the seedling tray. This level of water was kept constant for the entire 21 days of the experiment.

Phenotyping of the lines was conducted 21 days after sowing (DAS). After the experiment, 12 seedlings were randomly selected from each genotype for data observation. The experiment was replicated 2 times to ensure robustness. The anaerobic germination rate was determined by counting the number of emerged plants above the water surface relative to the total number of seeds sown. Phenotyping and subsequent data analysis were performed for 250 lines, (Supplementary Table 1) while the 106 genotypes that failed to germinate were excluded from the analysis. Shoot length was measured from the plant base to the tip, while the length of the first internode was calculated from the base to the first node. Root length was measured from the crown root to the tip. Before phenotyping, uprooted seedlings were meticulously rinsed with running tap water to remove soil residues. After being completely dried for 5-6 days at 60 °C in an air-forced oven, the seedlings were weighed to determine their dry weight in micrograms. The seedling vigour index-1 and -2 were calculated using the formula (14). The statistical analyses were performed using the ggplot package of R software for the development of violin plots and https://www.kaugrapes.com/ website was used to perform correlation and principal component analysis. The cluster analysis was performed by JMP software (trial version) through the wards method.

Seedling vigour index – 1 (SVI - 1): Seedling germination percentage × Seedling length (cm)

Seedling vigour index – 2 (SVI - 2): Seedling germination percentage × Seedling dry weight (mg)

Results and Discussion

In this research, every statistical analysis carried out was aimed at analyzing the mean performance of the genotypes that germinated in both the replications and which included the whole genotype pool that was employed in the investigation. Significant differences were found across the 250 genotypes in the analysis of variance results for the 8 characters related to anaerobic germination tolerance (15) (Supplementary Table 2). These results emphasize the diversity seen across all investigated characteristics. Table 1 presents the results of the computation of descriptive statistics.

This study found a wide range of responses among entries to various anaerobic germination tolerant traits. Another study also reported that seedlings showed specific reactions to oxygen deficiency during the germination stage, emphasizing the significance of diverse responses in selecting tolerant lines (16). The trait of germination % under anaerobic conditions exhibited a mean value of 28.04 %, with values ranging from 2.76 % to 94.4 %. Poten-

Table 1. Descriptive statistics for anaerobic germination tolerance traits among 250 genotypes.

Character	Minimum	Maximum	Range	Mean	SE (m)	CD at 5 %
Germination %	2.76	94.4	91.64	28.04	2.42	6.74
Length of the first internode	2.50	27.1	24.60	7.34	0.78	2.17
Shoot length	12.1	37.8	25.70	27.35	1.99	5.55
Root length	5.80	32.4	26.60	12.02	1.68	4.70
Number of leaves	2.00	3.50	1.50	2.49	0.21	0.59
Seedling dry weight	2.00	15.00	13.00	6.78	1.07	2.98
SVI - 1	65.0	3948.0	3883.0	1090.0	116.76	325.23
SVI - 2	6.00	1228.0	1222.0	191.75	38.14.0	106.25

tial donors for hypoxic germination tolerance are cultivars that show viability in low-oxygen environments. Most of the genotypes exhibited poor germination falling under the susceptible category and only a few genotypes displayed higher germination %. The other most important trait under anaerobic conditions length of the first internode exhibited a mean length of 7.34 cm with a range of 2.5 cm to 27.1 cm. The mean for shoot length was observed with a mean value of 25.7 cm, spanning from 12.1 cm to 37.8 cm. The root growth that is considered a critical trait for good anchorage of germinated seedlings under anaerobic conditions was observed with a mean value of 26.6 cm, ranging from 5.8 cm to 32.4 cm. The trait seedling dry weight has exhibited a mean value of 6.78 mg, ranging from 2 mg to 15 mg (17).

The average performance, as depicted in (Supplementary Table 3), underscored significant variability among the rice genotypes under anaerobic conditions. The landraces Tulasi phula, BJ1, AG 387, Chinamali, pani rohi and ARC14855 exhibited the highest germination % higher than 75 % indicating tolerance to anaerobic conditions during germination. The genotype 'Dhiniksali'(12.5 cm) exhibited the highest length of the first internode, followed by white dubhi (12.15 cm) and the lowest was exhibited by Mugei (3.3 cm). The genotype White dubhi exhibited the highest shoot length of 35.4 cm, whereas the genotype Cr Dhan 801 showed the lowest shoot length (18.2 cm). The landrace Sorishaphulla exhibited the highest root growth 23.6 cm and the lowest (7.05 cm) was observed in Kalikati. Most of the entries were in the 2-3 leaf stage at 21 days of observation. The highest seedling dry weight of (14 mg) was observed on DM59, AUS175, Kalamkati and Kakudi manji ARC 7325 and the lowest (2 mg) was observed in many entries. The landraces AG 387 and BJ1 displayed a higher seedling vigour index in both calculated methods. The violin plot (Fig. 1) depicts the variation present among the genotypes for the various anaerobic germination tolerant traits.

Trait association analysis

The computed correlation coefficients are illustrated in (Fig. 2) (Supplementary Table 4) for all the traits under study. The findings revealed significant relationships among various traits. The trait germination % under anaerobic conditions displayed a strong positive correlation with SVI -1 (0.98***) and SVI – 2 (0.77***) and moderate positive association with length of first internode (0.20**), emphasizes the pivotal role of early seedling emergence in alleviating anaerobic stress and fostering successful establishment, while a negative correlation was observed with root length (-0.33***) and number of leaves (-0.12) after

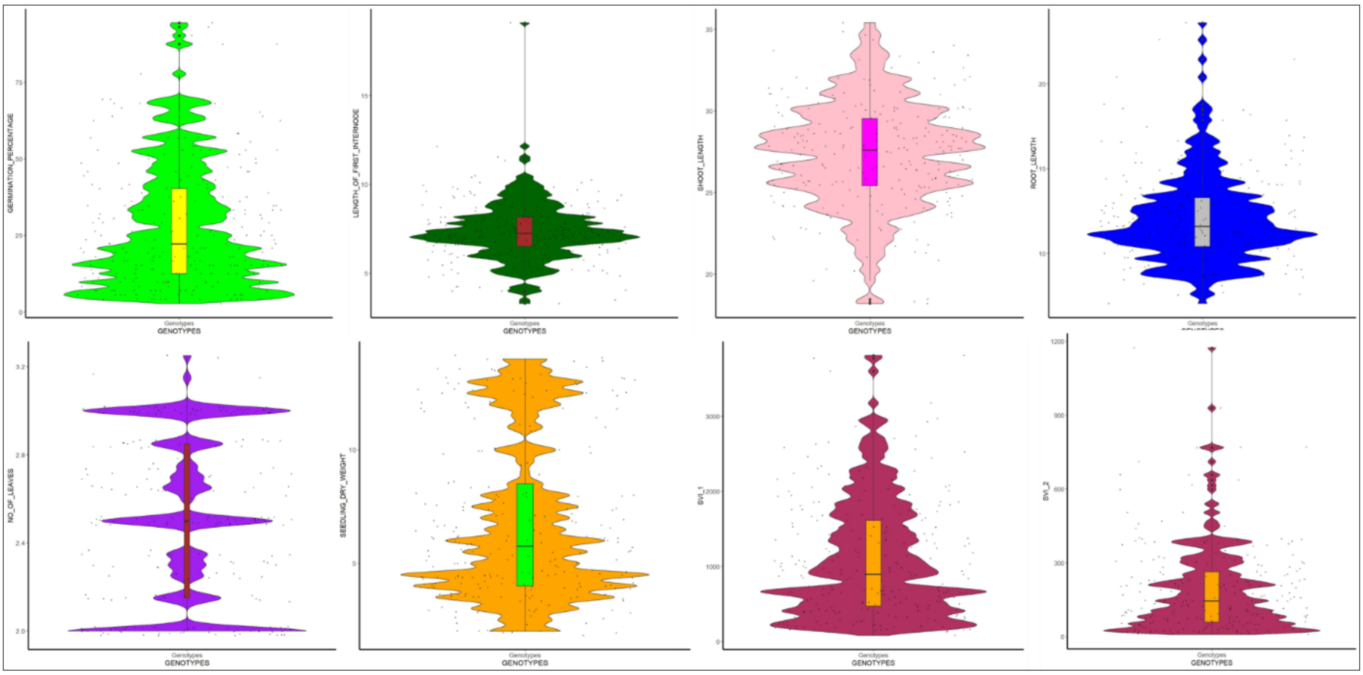
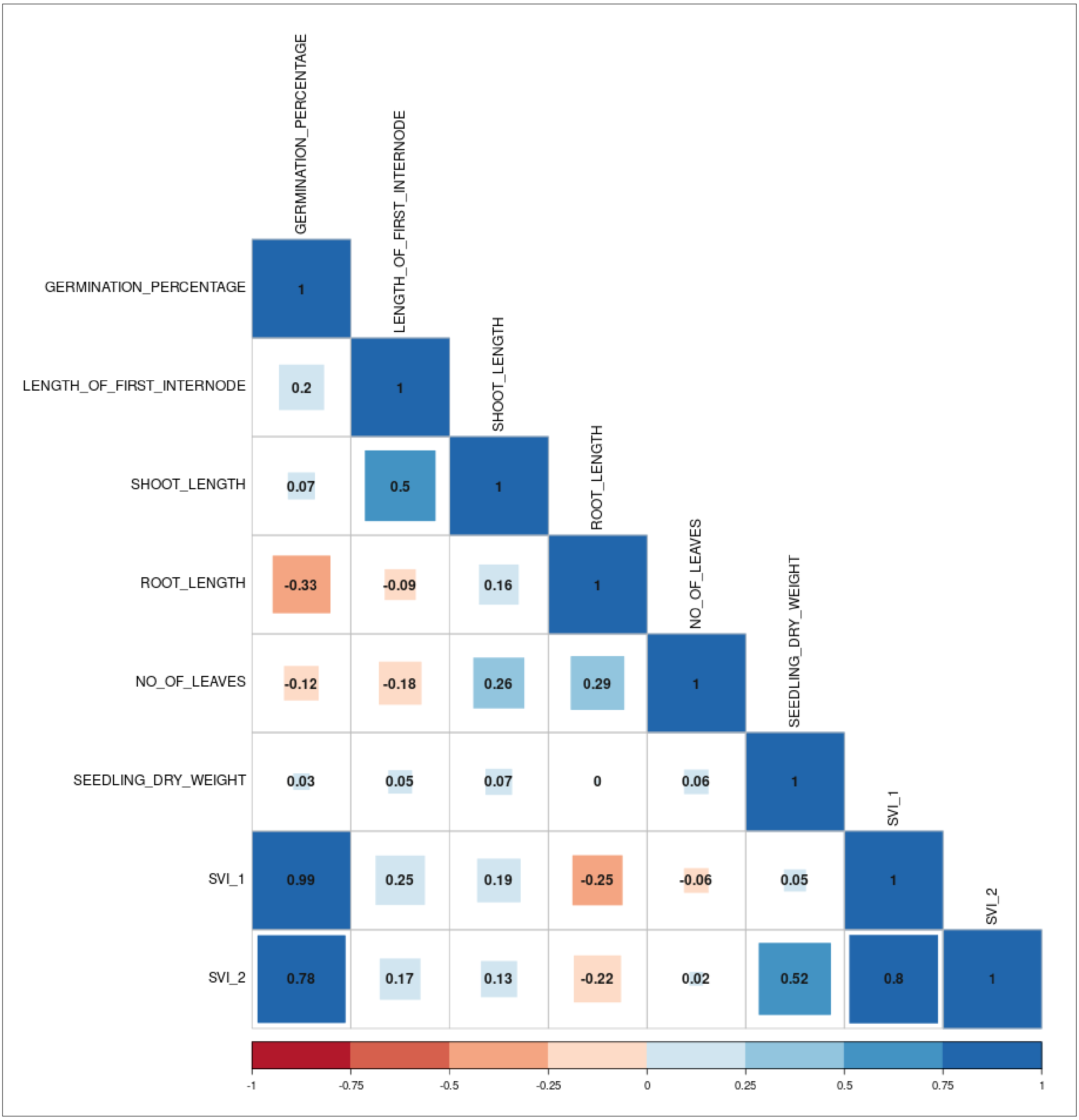


Fig. 1. Distribution pattern of 250 genotypes for anaerobic germination tolerance traits.



	GERMINATION_PERCENTAGE	LENGTH_OF_FIRST_INTERNODE	SHOOT_LENGTH	ROOT_LENGTH	NO_OF_LEAVES	SEEDLING_DRY_WEIGHT	SVI_1	SVI_2
GERMINATION_PERCENTAGE	1							
LENGTH_OF_FIRST_INTERNODE	0.2	1						
SHOOT_LENGTH	0.07	0.5	1					
ROOT_LENGTH	-0.33	-0.09	0.16	1				
NO_OF_LEAVES	-0.12	-0.18	0.26	0.29	1			
SEEDLING_DRY_WEIGHT	0.03	0.05	0.07	0	0.06	1		
SVI_1	0.99	0.25	0.19	-0.25	-0.06	0.05	1	
SVI_2	0.78	0.17	0.13	-0.22	0.02	0.52	0.8	1

Fig. 2. Corrplot of anaerobic germination tolerant traits of 250 genotypes.

21 days post-sowing (DAS) (18). This is consistent with the observation that during floods, the development of roots is severely inhibited as the plant diverts its energy toward seedling emergence at the level of the water's surface, where it can more effectively gain oxygen. At the expense of the growth of roots, starch breakdown is prioritized for coleoptile elongation, which facilitates oxygen uptake (9, 19, 20). The other trait length of the first internode has displayed a strong positive association with shoot length (0.50***), moderately positive association with SVI - 1 (0.24***) and SVI – 2 (0.16***) and negative association with several leaves (-0.18**). The shoot length has exhibited a positive association with all the traits under the study. Root length displayed Number of leaves exhibited a significant positive association with number of leaves (0.28***) and a significant negative association with SVI -1 (0.25***) and SVI - 2 (0.22***), seedling dry weight exhibited positive association with all the traits. SVI -1 displayed a significant positive association with SVI - 2 (0.79***).

Variability analysis

For the 8 traits included in the evaluation of variability findings (Table 2), phenotypic variance (PV) was greater than genotypic variance (GV) for every parameter that was evaluated. Comparably, the % of PCV exceeded the % of GCV, which suggests that environmental factors had only a little impact on the characteristics under investigation. Notably, traits such as anaerobic germination %, length of first internode, root length, seedling dry weight, SVI – 1 and SVI - exhibited substantial PCV and GCV values. However, despite the high PCV and GCV values observed for characters like seedling dry weight and SVI -2, environ-

Table 2. Variability parameters of anaerobic germination tolerance traits for 250 genotypes.

Character	PV	GV	EV	PCV %	GCV %	ECV %	H^2 (Bs)	GA	GA % Mean
Germination %	417.84	406.1	11.73	72.88	71.85	12.21	0.97	40.92	145.93
Length of the first internode	3.28	2.06	1.21	24.69	19.59	15.03	0.62	2.35	32.02
Shoot length	14.06	6.1	7.95	13.7	9.03	10.31	0.43	3.35	12.26
Root length	9.75	4.04	5.7	25.96	16.72	19.86	0.41	2.66	22.18
Number of leaves	0.19	0.1	0.09	17.51	12.72	12.03	0.52	0.47	19.04
Seedling dry weight	13.32	11.02	2.3	53.83	48.96	22.37	0.82	6.22	91.73
SVI – 1	617732	590463	27268	72.05	70.44	15.13	0.95	1547.6	141.87
SVI – 2	32306	29395	2910	93.73	89.41	28.13	0.9	336.9	175.69

mental component variance (ECV) values of 22.37 % and 28.13 % respectively, were noted, suggesting significant environmental influence rendering them less reliable. Additionally, traits such as shoot length and number of leaves demonstrated moderate PCV and GCV values. The seedling vigour index - 2 exhibited the highest PCV % and GCV %, reaching 93.73 % and 89.41 % respectively, while the lowest (moderate) values were observed in shoot length, amounting to 13.7 % and 9.03 %.

Heritability becomes crucial and demands consideration to progress breeding efforts (21). The extent to which a characteristic can be passed down to offspring is measured by its heritability (22). Breeding plans that are effective and efficient take heritability into account in addition to genetic advances and variances (23). Thus, prior knowledge of trait heritability is essential for this study. Every characteristic under investigation showed strong broad-sense heritability. Germination % had the highest heritability (0.97), while root length had the lowest (0.41). SVI – 1 displayed higher heritability (0.95) and genetic advance (1547.6), followed by SVI – 2, which exhibited higher heritability (0.90) and genetic advance (336). These results imply that additive gene effects are most likely responsible for the observed heritability, suggesting that traits-based selection might be advantageous. As highlighted in a study, traits with higher heritability significantly contribute to determining the potential for population enhancement (24). The GAM % ranges from 12.26 % (lowest observed in shoot length) to 175.69 % (highest observed in SVI - 2).

Principal component analysis (PCA)

To determine the relative contribution of each trait to overall variability and to direct the selection of characteristics, principal component analysis (PCA) was utilized. Condensing the whole variation attributed to the variables under study into a small number of factors was the main goal of PCA. Subsequently, the component characteristics that were mainly accountable for the obtained principal components (PCs) were determined (Fig. 3). The estimated values of the Eigenvectors, the variance % and the cumulative % are presented in (Table 3). Around 86.1 % of the variation was recorded by the first 4 PCs. Consequently,

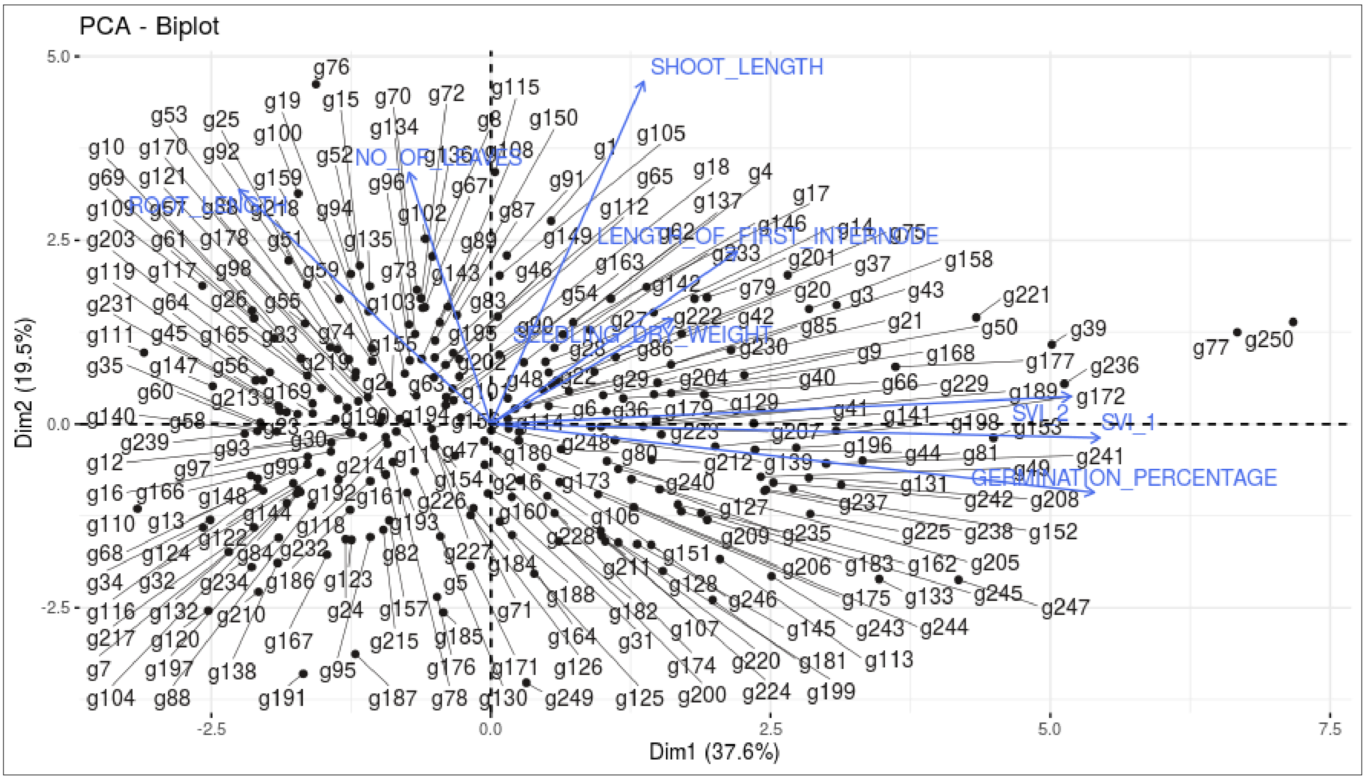


Fig. 3. PCA – Biplot between principal components 1 and 2.

Table 3. Eigenvalues, % of variance, loading of variables in each principal component for anaerobic germination tolerance traits.

variables	PC1	PC2	PC3	PC4
Eigenvalue	3.007	1.56	1.28	1.041
% of variance	37.585	19.501	16.003	13.01
Germination %	0.538	-0.129	0.008	-0.283
Length of the first internode	0.22	0.325	-0.599	0.299
Shoot length	0.136	0.645	-0.309	-0.018
Root length	-0.225	0.441	0.193	-0.183
Number of leaves	-0.073	0.474	0.418	-0.418
Seedling dry weight	0.162	0.198	0.485	0.723
SVI - 1	0.543	-0.027	0.005	-0.294
SVI - 2	0.517	0.051	0.313	0.116

traits loaded onto these four PCs were deemed more significant. PC1, with an eigenvalue of 3.00, made the most substantial contribution to variability, accounting for 37.58 %. Characters, viz. SVI – 1 (0.54), germination percentage (0.53), SVI – 2 (0.51), length of first internode (0.22), seedling dry weight (0.16) and shoot length (0.13) were positively loaded while root length (-0.22), number of leaves (-0.07) was negatively loaded. The maximum % of variance towards PC1 was contributed by the following traits SVI -1 (29.46 %), germination % (28.91 %) and SVI – 2 (26.73 %) (Supplementary Table 5). The second principal component PC2 with an eigenvalue of 1.56 accounted for 19.50 % of the variability. The traits shoot length (0.64), number of leaves (0.47), root length (0.44) and length of first internode (0.32) were positively loaded while germination % (-0.12) was negatively loaded. The maximum % of variance towards PC2 was explained by shoot length (41.64 %) and number of leaves (22.42 %).

Table 4. Cluster and its membership of 250 genotypes.

Cluster No	Members
1	14,17,20,21,36,42,43,114,137,141,142,146,151,160,164,174,188,206,211,220,224,229,230,233,235,244,248
2	2,18,31,33,38,47,51,56,67,70,73,78,83,94,95,102,103,115,119,121,130,115,119,121,130,134,135,138,155,156,161,163,176,187,190,193,195,202,215,216, 226,227
3	39,77,133,153,177,236,247,250
4	37,50,79,85,86,91,107,113,127,128,129,139,145,158,162,168,172,175,181,182,189,199,201,204,205,207,209,212,225,237,245,246
5	5,13,15,16,19,25,57,59,71,82,87,88,92,97,98,99,100,109,117,122,143,144,167,169,170,178,186,228,231
6	1,27,29,48,52,54,65,90,101,106,112,125,126,149,159,171,173,179,184,223,240
7	7,10,12,24,26,30,32,35,55,60,84,93,104,110,124,147,148,166,197,210,217,219,232,234,239
8	6,8,22,28,46,53,62,63,72,80,89,96,105,108,118,123,136,150,154,157,180,185,194,200,218,222,249
9	3,9,40,41,44,49,66,75,81,131,152,183,196,198,208,221,238,241,242,243
10	11,23,34,45,58,61,64,68,69,74,76,111,116,120,132,140,165,191,192,203,213,214

The variation is explained by the third principal component, PC3, which had an eigenvalue of 1.28, accounting for 16.00 %. The trait length of the first internode (-0.59) and the length of the shoot (-0.30) were negatively loaded, while the trait seedlings' dried weight (0.48) along with the number of leaves (0.41) were positively loaded. The maximum % of variance towards PC3 was explained by the length of the first internode (35.91 %) and seedling dry weight (23.55 %). The fourth principal component PC4 with eigenvalue 1.04 accounted for 13.01 % of total variability. The traits seedling dry weight (0.72) length of first internode (0.29) were positively loaded and several leaves (-0.41), SVI – 1 (-0.29) and germination % (-0.28) were negatively loaded. The maximum % of variance towards PC4 was explained by seedling dry weight (52.26 %). The results were in alignment with the previous finding (25).

Cluster analysis

More genetic variation among the parent lines is associated with higher levels of variation in the subsequent segregating generation. Therefore, parental genetic diversity is crucial for promoting variability. The 250 cultivars in this study were divided into ten clusters, with cluster II having the greatest number of genotypes (38), subsequently followed by clusters IV and V, which had 32 and 29 genotypes respectively. The distribution further included clusters with 28, 27, 25, 22, 21 and 20 genotypes in clusters I, VIII, VII, V, X and VI respectively and 8 genotypes in cluster III. Most genotypes were predominantly clustered within clusters II, IV and V (Table 4).

The cluster averages for different characteristics are outlined in Table 5. Notably, cluster III displayed the highest average values for 6 characters. viz., anaerobic germination % (83.05), length of first internode (8.54), shoot length (28.96), seedling dry weight (8.56), SVI – 1 (3261.18) and SVI – 2 (705.06) followed by cluster V and VIII for root length (13.13) and number of leaves (2.63). Previous studies have produced findings that are comparable to these (25-27). Thus, it shows that genotypes in cluster III (ARC14855, BJ1, Chinamli, Panirohi, Mrunalini, Baspatri, Tulasiphula and AG 387) were best performing for traits germination %, length of first internode, shoot length, seedling dry weight and seedling vigour index – I and II.

The mean inter-cluster distances were computed for the 10 clusters (Table 6). Cluster III and Cluster VII had the longest inter-cluster distance (3148.9), which was closely followed by Clusters III and X (3027.4) and Cluster III and V (2869.7). On the other hand, clusters VII and X had

Table 5. Final cluster centers for anaerobic germination tolerance traits.

Clusters	Germination %	Length of the first internode	Shoot length	Root length	Number of leaves	Seedling dry weight	SVI - 1	SVI - 2
1	37.04	7.87	27.53	11.92	2.42	7.55	1449.07	271.42
2	16.88	7.68	27.21	12.33	2.5	6.94	660.77	117.13
3	83.5	8.54	28.96	10.36	2.46	8.56	3261.18	705.06
4	48.56	7.47	27.49	11.08	2.39	6.46	1854.7	312.39
5	11.54	7.03	27.56	13.13	2.53	7.03	460.81	85.13
6	28.89	6.92	27.56	12.4	2.59	7.5	1142.64	214.19
7	5.05	6.62	25.38	11.71	2.44	6.34	185.96	32.14
8	23.44	7.09	27.88	12.19	2.63	7.42	922.88	174.25
9	61.66	8.06	28.38	10.65	2.46	5.75	2397.5	351.92
10	7.96	6.68	26.73	13.21	2.52	4.95	309.25	37.47

Table 6. Intercluster distance values of 10 clusters by wards method.

Clusters	1	2	3	4	5	6	7	8	9	10
1	0									
2	803.5	0								
3	1863.8	2666.8	0							
4	404.8	1207.2	1463.5	0						
5	1006.5	203	2869.7	1410.3	0					
6	311.8	491.6	2175.3	716.1	694.7	0				
7	1285.9	482.5	3148.9	1689.7	279.4	974.1	0			
8	535.2	268.3	2398.5	939.3	471.3	223.4	750.7	0		
9	952.1	1753	933.3	547.3	1956	1262.8	2235.2	1485.7	0	
10	1163.9	360.5	3027.4	1567.2	158	852.1	123.4	628.8	2112.4	0

the shortest inter-cluster distance (123.4). As inter-cluster distances increase, genotype diversity also tends to rise. Cluster analysis, utilized to explore diversity between groups, serves as a valuable tool for classifying genotypes in plant improvement programs. Similar investigations have been conducted (28-31), highlighting its significance in planning hybridization programs for rice improvement.

Conclusion

In summary, the study delved into understanding the diversity of rice genotypes in terms of their tolerance to anaerobic germination conditions. Through rigorous statistical analyses, significant variability among the genotypes across various traits was identified, shedding light on potential donors for anaerobic germination tolerance. Characteristics such as germination percentage, shoot elongation and seedling dry biomass have emerged as critical parameters indicative of tolerance. Additionally, the study underscores the importance of trait association analysis, variability assessment and principal component analysis in elucidating the underlying genetic and phenotypic determinants governing anaerobic germination tolerance. These findings underscore the significance of genetic variability and heritability in breeding programs directed toward the development of rice cultivars resilient to anaerobic conditions, thus contributing to advancements in rice cultivation.

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Authors' contributions

AA contributed to the study's conception and design. Material preparation, data collection and analysis were performed by VKRC and AA. The first draft of the manuscript was written by VKRC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The author (s) declares no conflict of interest.

Ethical issues: None.

Supplementary data

Supplementary Table 1**.** List of the cultivars used for anaerobic germination tolerance screening.

Supplementary Table 2**.** Anova for anaerobic germination tolerant characteristics among 250 genotypes.

Supplementary Table 3**.** Average performance of 250 genotypes for anaerobic germination tolerant characters.

Supplementary Table 4**.** Correlation matrix for anaerobic germination tolerance traits if 250 genotypes.

Supplementary Table 5**.** % contribution of variable on PCs

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