Received: 15 Aug 2023 | Revised: 28 Aug 2023 | Accepted: 20 Sep 2023

Electronic Journal of Plant Breeding

Research Article

Elucidating the genetic potential of rice germplasm for anaerobic germination tolerance in rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) serves as the staple food for over 50% of the global population. Puddled transplanted rice (PTR) remains the most prevalent method of rice cultivation. However, challenges like declining water tables, escalating water scarcity, higher labor costs and elevated methane emissions are diminishing its productivity and profitability. These factors have spurred the quest for an alternative crop establishment approach. Direct-seeded rice (DSR) is gaining traction due to its lower input requirements in comparison to PTR. Although DSR is a profitable and effective alternative method of rice cultivation to PTR, flooding during sowing which hinders seed germination is a major limiting factor in DSR. This study aimed to elucidate the genetic potential of anaerobic germination tolerance (AGT) of 121 rice genotypes which consists of 100 land races, twenty commercial varieties and a pipe line culture. All the genotypes were screened artificially in glass house condition using seedling box cup method. AGT of the genotypes were assessed based on two important morphological traits *viz*., Anaerobic Germination Percentage (AGP) and Anaerobic Vigour Index (AVI). Five land races with accession numbers RL 79, RL 4088, RL 141, RL 3849 and RL 639 showed more than 90 % AGP under anoxic condition coupled with high AVI values ranging from 2872.9 to 4702.6. These genotypes also recorded high values for Shoot length and it ranged from 23.86 to 43.28 cm. The genotypes *viz*., RL 79, RL 4088, RL 141, RL 3849 and RL 639 having ability to germinate under anaerobic condition may be used as a potential donor for developing high yielding rice varieties with AGT, which is required for the area expansion of DSR method of rice cultivation in future.

Keywords: Rice, Anaerobic germination tolerance, genetic diversity

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population (Zhang *et al*., 2019) which is the major dietary and food security source of many Asian countries. Globally, irrigated rice ecosystems encompass 55% of the total rice cultivation area and contribute to 75% of the world's rice production (Mahender *et al*., 2015). Nonetheless, current evidence indicates a diminishing trend in grain yield productivity as a result of depleting natural resources, declining water tables, escalating labor scarcities, energy constraints, rising input costs and shifting climatic patterns. Direct sown rice preferably consumes relatively less water and energy as compared to transplanted flooded rice. Particularly in Asia, vast areas once covered by puddled transplant systems have adopted DSR systems (Ghosal *et al*., 2020). Deep-water and rain-fed lowland ecosystems cover roughly 50 million hectares of land globally, constituting about one-third of the total area dedicated to rice production worldwide (Oladosu *et al*., 2020). In India, DSR is becoming a preferable method of rice cultivation in the states of Punjab, Haryana. In these states vast area traditionally under PTR system are now transformed into DSR due to crippled labour scarcity and scope for mechanized rice cultivation. Similarly in the states of Andhra Pradesh, Tamil Nadu, Telangana and Karnataka this method of rice cultivation is expanding year after year. For instant in Tamil Nadu, roughly 1.5 lakh hectare was traditionally under DSR. But in recent years DSR method is expanding to a tune of 2.5 to 3.0 lakh hectares. This shift of rice cultivation to direct seeding method is much more needed in the changing climate era to overcome aforesaid problems.

Direct seeded rice (DSR) has several advantages when compared to PTR. However, it has few disadvantages such as sensitivity to flooding during seed germination and high weed competitiveness in the early phase of crop growth. Submergence during seed germination and seedling emergence leads to no germination or very poor germination and seedling establishment due to the failure of adequate shoot and root formation. This phenomenon frequently results in complete crop failure (Ismail *et al*., 2009; Miro *et al*., 2017; Lal *et al*., 2018). This condition mainly arises due to unpredicted rainfall occurrence and inadequate land leveling in irrigated regions (Kirk *et al.,* 2014). In either case severe reductions in seed germination or failure of crop establishment can be experienced (Ismail *et al.,* 2012). Tolerance to anaerobic conditions is a prerequisite for practicing effective direct-seeded rice in rainfed and flood-affected areas. However, rice is extremely sensitive to anoxia during germination and early seedling growth (Ismail *et al*., 2009; Yang *et al*., 2019). Although certain rice genotypes initiate germination while submerged, only the tolerant genotypes manage to persist by elevating themselves above the water level under oxygen-deprived situation. Rapid coleoptile elongation after germination is the major escape mechanism found in tolerant genotypes (Kretzschmar *et al*., 2015). Adaptive mechanisms and pivotal traits linked to anaerobic stress tolerance includes anaerobic germination percentage (AGP) (Septiningsih *et al*., 2013; Barik *et al*., 2019), Anaerobic Vigor Index (AVI) (Barik *et al*., 2019), Response Index (RI) (Islam *et al*., 2022), accelerated coleoptile elongation (Kretzschmar *et al*., 2015), and carbohydrate reserves (Ella *et al*., 2011; Ismail *et al*., 2012). These mechanisms are widely present in several native land races, which needs to be tapped for breeding AGT rice varieties.

Historically, many diverse rice varieties commonly known as traditional varieties or land races were grown in Tamil Nadu and elsewhere in the country, but many of them are not in cultivation as a result of the widespread cultivation of high yielding cultivars (Dhanuja *et al*., 2021). Landraces are considered as the library of novel genes that provides breeders with enormous genetic variations to develop pre-breeding materials. Recent investigations into anaerobic germination (AG) have unveiled numerous native landraces displaying anaerobic germination tolerance (AGT). The gentoypes *viz*., MTU 1140 (Reddy *et al*., 2015), *Bausaganthi, Patadhan, and Basantichudi* (Barik *et al*., 2019), *BarkheTauli*, 498-2 A BR 8, *JagliBoro, ParaNellu*, and Improved Blue Rose (Rauf *et al*., 2019), *Vellaikavuni, Varappu kudaichan, NRungan* and *Karuppukavuni* (Mohanapriya *et al*.,2022) and *Karuthakar, Poovan samba, Mattaikkar, Edakkal,* and *Manvilayan* (Shanmugam *et al*., 2023) were reported to have high level of AGT. Landraces are known to have a wide genetic base composed of various useful genes. This emphasizes the importance of breeders tapping into the rich diversity of existing rice varieties to pinpoint novel and promising candidates for AGT. Hence, this study entails the examination of a panel consisting of 121 genotypes that were exposed to hypoxic conditions. The objective is to identify novel genotypes showcasing AGT through effective phenotyping. These identified genotypes can subsequently be integrated into forthcoming breeding programmes aimed at development of rice cultivars suitable for direct seeded conditions.

MATERIALS AND METHODS

The experimental material consisted of 121 rice genotypes (**Table 1**) consisting of 100 landraces and 20 released varieties and a pre-release culture. Screening of the rice varieties for their tolerance to anaerobic conditions was undertaken at Tamil Nadu Rice Research Institute, Aduthurai during Summer 2023 (March – June) using seedling box cup method under glass house condition. The experiment was conducted following completely randomized design with two replications.

Screening experiment for AGT: Ten uniform sized and well filled seeds were selected from each entry and sown in fine clay soil-filled cups (measuring 10 × 8 cm) at a depth of about 1 cm and arranged in seedling box measuring 60 × 30 × 40 cm and replicated twice. Then the seedling boxes were filled with water to a height of 15 cm so as to submerge the cups (**Fig.1**). Observations were recorded after 14 days of submergence. A control batch under normal conditions, where the soil surface was appropriately moistened instead of being fully submerged was also maintained to compare the performance of each genotype under normal and anoxic conditions. Both in treatment and control, data on 9 AGT related traits viz., AGP (%), Shoot Length (SL), Root Length (RL), Total seedling Length (TL), Shoot to Root Ratio (SRR), Number of Roots (NR), Number of Leaves (NL), Fresh Weight (FW) and Dry Weight (DW) were recorded in five seedlings in each replication. AVI and Response index (RI) were calculated using the formula given by Barik *et al*. (2019) and Islam *et al*. (2012) respectively.

AVI = AGP × (shoot length + root length)
RI = shoot length (submerged) – shoot length (control)

Statistical analysis was performed using the software ‘Rstudio’ for variability, correlation, Principal component analysis and cluster analysis

Table 1. List of genotypes evaluated for anaerobic germination tolerance

S. No .	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1	RL 500	42	RL 3041	82	IC 115775
2	RL 1199	43	RL 2339	83	IC 379307
3	RL 2285	44	RL 1116	84	IC 86337
4	RL 79	45	RL 3052	85	IC 540621
5	RL 4382	46	RL 197	86	IC 252219
6	RL 703	47	RL 141	87	IC 379436
7	RL 857	48	RL 10099	88	IC 463873
8	RL 5703	49	RL 3849	89	IC 435142
9	RL 6390	50	RL 106	90	IC 135873
10	RL 4140	51	IC 387012	91	IC 389548
11	RL 4149	52	IC 114574	92	IC 457996
12	RL 5553	53	IC 114210	93	IC 426090
13	RL 4278	54	IC 378565	94	IC 386334
14	RL 688	55	IC 205953	95	IC 460491
15	RL 319	56	IC 461311	96	IC 376499
16	RL 1591	57	IC 218643	97	IC 377829
17	RL 4088	58	IC 126210	98	IC 114017
18	RL 10064	59	IC 458608	99	IC 301077
19	RL 3883	60	IC 459860	100	IC 114751
20	RL 199	61	IC 133690	101	ADT 36
21	RL 3877	62	IC 594031	102	ADT 37
22	RL 2196	63	IC 389714	103	ADT 38
23	RL 195	64	IC 213779	104	ADT 39
24	RL 25	65	IC 114260	105	ADT 41
25	RL 3900	66	IC 125800	106	ADT 42
26	RL 4078	67	IC 378152	107	ADT 43
27	RL 4114	68	IC 390841	108	ADT 45
28	RL 639	69	IC 388692	109	ADT 47
29	RL 1720	70	IC 463986	110	ADT 48
30	RL 8811	71	IC 380623	111	ADT 51
31	RL 550	72	IC 206332	112	ADT 52
32	RL 111	73	IC 115551	113	ADT 53
33	RL 4415	74	IC 213779	114	ADT 54
34	RL 8675	75	IC 114501	115	ADT 55
35	RL 1062	76	IC 218157	116	ADT 56
36	RL 4966	77	IC 133584	117	ADT 57
37	RL 10039	78	IC 218843	118	ADT 58
38	RL 2583	79	IC 207802	119	TPS 5
39	RL 4513	80	IC 114971	120	ASD 16
40	RL 27	81	IC 215125	121	AD 17152
41	RL 1650				

RL and IC are the accession numbers provided by NBPGR, New Delhi

RESULTS AND DISCUSSION

Estimates of variability: Present study was carried out to explore the genetic diversity for AGT related traits from a set of 121 rice genotypes. Mean performance of genotypes for AGT traits, unveiled a substantial and extensive spectrum of variation for all the traits associated

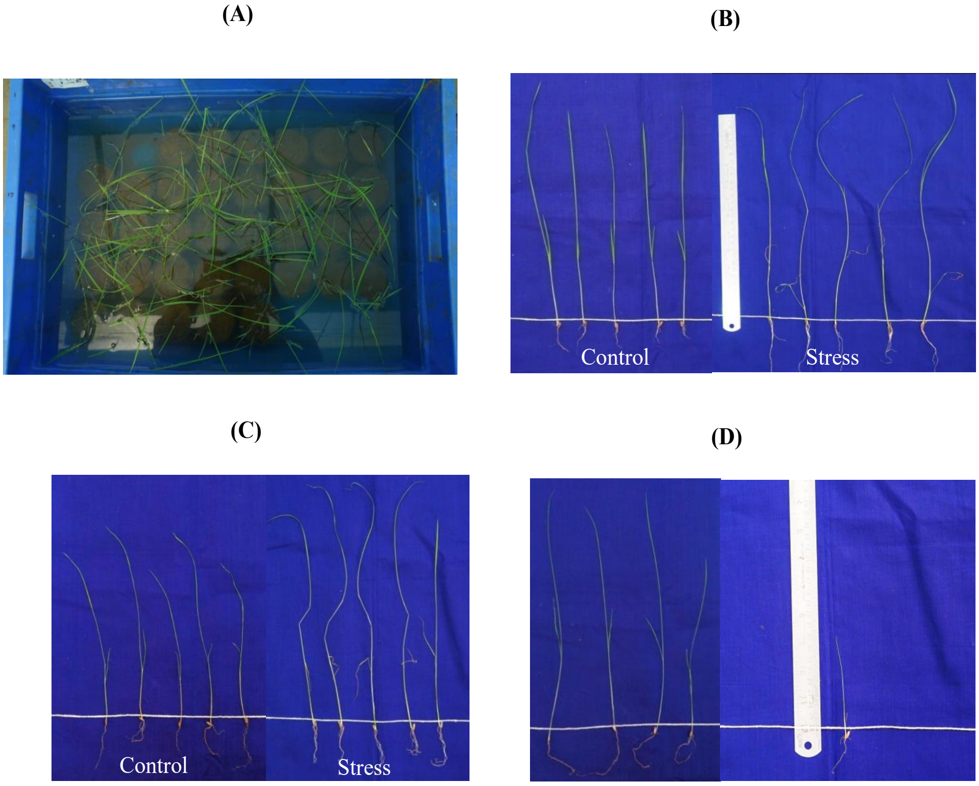

Fig. 1. Anaerobic germination screening experiment (A), RL 4088 (B), RL 79 (C), IC 114751 (D) under control and stress condition

with anaerobic germination potential. A significant variation ($p< 0.01$) was found for AGP, ranging from 5% (IC-206332) to 100% (RL 79) and AVI ranged from 71 (IC 115551) to 4702.6 (RL 639) (**Table 2**). Shoot lengths of the germinating seedlings varied from 4.80 cm (IC 115551) to 43.28 cm (RL 639) and the root length varied from 1.2 cm (IC 213779) to 12.08 cm (RL 1720). Total seedling length varied from 7.10 cm (IC 115551) to 54.71 cm (RL 639). The RI ranged from –14.17 (RL 5703) to 13.81 (RL 1591). The traits SRR (0.41–10.61), NL (0.5–2.4) and NR (0.5–8.12) showed considerable variation among the genotypes studied.

Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV), Broad-sense heritability (h^2_{bs}), Genetic Advance (GA) and Genetic Advance as percentage of Mean (GAM%) were assessed and presented in **Table 2**. The PCV values ranged from 47.88% (NL) to 957.01% (RI), whereas GCV ranged from 28.25% (NL) to 628.11% (RI). For all the AGT related traits under investigation, PCV values were higher than GCV. This observation underscores the significant role of environmental factors in influencing the manifestation of these traits, indicating a considerable level of interaction between the genotype and the environment. Traits linked to AGT, including AGP, AVI, SL, and RL, demonstrated high PCV coupled with high GCV values. Broad-sense heritability varied widely from 18.30% to 73.09%. Traits majorly associated with AG potential, AGP and AVI, had high heritability. GAM% values varied from 25.97 % (SRR) to 139.93 % (AVI). AGT traits like AGP and AVI exhibited high GAM% along with a high level of heritability.

AGT-associated traits such as AGP and AVI were found to be highly heritable while other traits were moderately heritable except for SRR which had low heritability. Earlier studies also found that traits associated with anaerobic germination potential had moderate-to-high heritability (Darko Asante *et al*., 2021). Of the 11 traits studied, the traits AGP and AVI had high $h^2> 0.60$, and high GAM % indicating that phenotypic selection for these traits would be effective under anaerobic stress. High heritability plus high genetic advance would be the best genotype selection indicators (Afrin *et al*., 2017). Thus, selection for these two traits in improving AGT should be given the foremost priority during breeding programs.

The selection of rice genotypes with high seedling vigor is of prime importance for successful germination under anaerobic conditions in a DSR system. Among the 121 genotypes evaluated, RL 141, RL 3849, RL 79, RL 4088 were found to have > 90% AGP, and regarded as AG tolerant genotypes. Darko Asante *et al.* (2021) and Shanmugam *et al.* (2023) also examined rice genotypes and reported that those with AGP > 90% as AG tolerant genotypes. The current investigation

Table 2. Genetic variability parameters of studied traits of 121 rice genotypes germinating under anaerobic conditions

Trait	Range	Mean ± SE	GCV (%)	PCV (%)	h^2_{bs}	GA	GAM (%)
AGP (%)	5 to 100	40.89 ± 2.74	67.33	78.76	73.09	45.83	118.59
SL (cm)	4.8 to 43.28	21.63 ± 0.98	43.41	60.96	50.70	13.08	63.67
RL (cm)	1.2 to 12.08	5.48 ± 0.21	34.25	61.54	30.96	1.98	39.25
TL (cm)	7.1 to 54.71	27.31 ± 1.11	41.53	58.23	50.87	15.62	61.02
SRR	0.41 to 10.61	3.92 ± 1.77	29.47	68.88	18.30	0.97	25.97
AVI	71 to 4702.6	1334.54 ±116.57	82.16	99.37	68.36	1780.27	139.93
RI	14.17 to 13.881	0.66 ± 0.72	628.11	957.01	43.08	7.15	849.23
FW (mg)	0.01 to 1.1	0.22 ± 0.01	65.07	102.88	40.00	0.176	84.79
DW (mg)	0.01 to 0.13	0.06 ± 0.003	56.33	75.58	55.56	0.04	86.58
NR	0.5 to 8.12	4.52 ± 0.17	40.35	55.07	53.70	2.55	60.91
NL	0.5 to 2.4	1.69 ± 0.04	28.25	47.88	34.83	0.53	34.34

GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; h2bs, broad-sense heritability; GA, genetic advance; GAM%, genetic advance as a percentage of mean; SED, standard error deviation. AGP, anaerobic germination percentage; SL, shoot length; RL, root length; RI, response index; SRR, shoot-to-root ratio; NL, number of leaves; NR, number of roots; FW, fresh weight; DW, dry weight; AVI, anaerobic vigor index.

revealed considerable variations in AVI values (71 to 4702.6) among the genotypes, which is higher than previous studies that reported the range of 150 to 4433 (Shanmugam *et al.*, 2023), 0 to 870 (Mohanapriya *et al.*, 2022) and 81 to 1720 (Barik *et al.*, 2019). High AGP in combination with maximum AVI are the best indicators for AGT in rice (Mohanapriya *et al.*, 2022). Considerable variations were found between control and treatment groups among the genotypes in growth traits under hypoxic conditions. Susceptible landraces showed substantial decreases in shoot and root length under hypoxic condition compared to tolerant ones. Previous studies have also stated that genotypes with longer shoots had higher AGP values (Shanmugam *et al.*, 2023). This outcome is mainly attributed to rapid shoot elongation during submergence, a characteristic recognized as a marker of AGT (Ismail *et al.*, 2009; Barik *et al.*, 2019; Darko Asante *et al.*, 2021). The elevated mean response index values within the tolerant and moderately tolerant groups further implied the significance of shoot elongation within the context of AGT genotypes. Under hypoxic conditions, susceptible landraces displayed diminished root length, whereas tolerant (with AGP > 90%) and moderately tolerant landraces (with AGP > 70%) exhibited notably greater root length compared to the control group. In the present study five genotypes *viz*., RL 639, RL 4088, RL 79, RL 3849 and RL 141 were found to have high AGP coupled with high AVI. Hence these genotypes can be given importance in breeding programmes aiming to develop AGT rice varieties and development of mapping population to dissect the QTLs / QTNs / genes harboring AGT in rice. The study also revealed 21 genotypes are highly susceptible to AGT which are mainly high yielding commercial cultivars. This may be due to selection pressure on yield and its contributing traits over a period of more than 50 years.

Correlation: Association of AGT traits was estimated through simple correlation analysis using the package ‘corrplot’. The AGP and AVI (r=0.94***, p< 0.001) exhibited a highly significant and positive association with the following traits: RI, SL and RL, NL and NR. Under hypoxic conditions, there was a positive correlation observed between SL and NR (r = 0.88***, p< 0.001) (**Fig. 2**). SL, RL and SRR exhibited significant and positive correlation among each other. RI, a major trait indicating the response of the genotypes to anaerobic stress exhibited positive and significant correlation with all the traits except for NL for which positive but non-significant association was observed. Shanmugam *et al.* (2023) reported highest association between AGP and AVI under anoxic conditions. Similarly, Barik *et al.* (2019) and Darko Asante *et al.* (2021) documented a direct correlation between SL and AGP. All the 11 traits showed positive association among each other. As expected, the derived values or traits such as AVI and RI also showed positive association among themselves and also with other traits. Therefore, AGT of a genotype is governed by all the dependable traits. However, AGP and AVI are two major traits contributing to AGT with high levels of association. Therefore, due importance should be given to these two traits to identify AGT rice genotypes.

Principal component analysis (PCA): Principal component analysis measures the contribution of each component to total variance (Sinha and Mishra, 2013). In present study, PCA was performed in ‘Rstudio’ using ‘FactoMineR’ package for 11 AGT associated traits with an aim to quantify individual trait contributions to overall variability and explore genetic diversity within the evaluated genotypes. The first two axes of the principal components (PCs) with an eigenvalue of > 1 accounted for 86.5% of the total variation (**Table 3**). Among them,

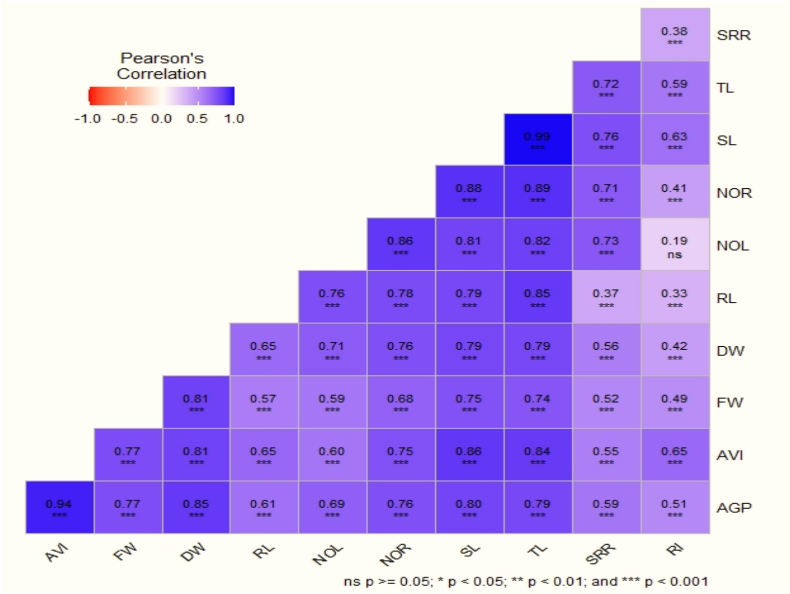

Fig. 2. Correlogram showing association between traits and traits associated with anaerobic germination potential

AGP, anaerobic germination percentage; SL, shoot length; RL, root length; SRR, shoot to root ratio; NL, number of leaves; NR, number of roots; FW, fresh weight; DW, dry weight; AVI, anaerobic vigor index; RI, response index.

Table 3. Principal components of traits associated with AGT

Parameters	PC1	PC2
Eigen values	8.44	1.08
Variance (%)	76.69	9.81
Cumulative (%)	76.69	86.50
AGP (%)	**10.23**	0.39
SL (cm)	**12.18**	0.49
RL (cm)	6.82	**33.68**
TL (cm)	12.06	0.11
SRR	5.39	**20.13**
AVI	**11.04**	1.29
RI	5.81	**28.06**
FW (g)	8.62	0.47
DW (g)	9.50	0.43
NR	**10.29**	2.53
NL	8.06	12.41

PC, principal component; AGP, anaerobic germination percentage; AVI, anaerobic vigor index; SL, shoot length; RL, root length; RI, response index; SRR, shoot to root ratio; NL, number of leaves; NR, number of roots; FW, fresh weight. Bold values indicates the major contribution of corresponding variables to the particular principal component

PC1, with an eigenvalue of 8.44, accounted for 76.69% of the variation followed by PC2, which accounted for 9.81% of the variation. In PC1, SL exhibited the highest positive contribution value with loading of (12.17), followed by TL (12.06), AVI (11.04), NR (10.29) and AGP (10.23). Traits linked to anoxic tolerance, including AVI, AGP, RI, SL and RL played a pivotal role in contributing to the overall variation observed in PC1. In PC2, RL, RI and SRR were the traits with high contribution for variation with values of 33.68, 28.05 and 20.12 respectively. Screeplot also shows that PC1 contributes higher percentage of variation followed by PC2 towards the total variation present among the genotypes (**Fig. 3**).

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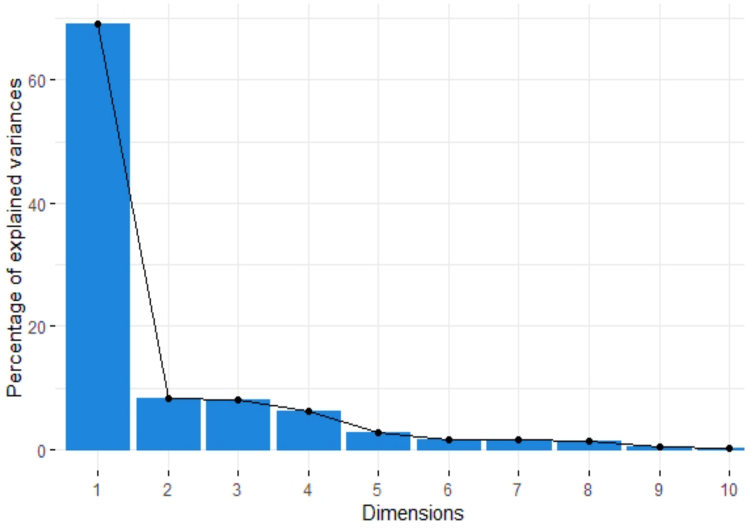

Fig. 3. Screeplot showing the degree of variation contributed by the two principal components towards overall variation

A biplot analysis between PC1 and PC2 explains the distribution and nature of diversity in relation to both variables and genotypes. This analysis provides insights into how individual traits (variables) contribute to the overall variability observed among different genotypes (**Fig. 4**). The genotypes analyzed in this study were effectively plotted across four distinct quadrants. The position of each genotype plotted across the two principal components is based on the coordinates values which represents the degree of variation it contributes towards the PC1 and PC2. The biplot in turn separated the tolerant genotypes from other genotypes into distinct quadrants. Three out of four highly tolerant genotypes such as RL 79, RL 4088, RL 141 were plotted on the first quadrant (bottom left) and the fourth genotype RL 3849 was plotted on third quadrant (top right). One of the highly tolerant genotypes, RL 141 was plotted far away from the axis in the first quadrant and the other two tolerant genotypes *viz*., RL 79 and RL 4088 were positioned in the centre and near to the origin respectively in the same quadrant, indicating the degree of variation among the three resistant genotypes and also contribution of these genotypes towards the total variation, which clearly shows the distinctive nature of the genotypes studied.

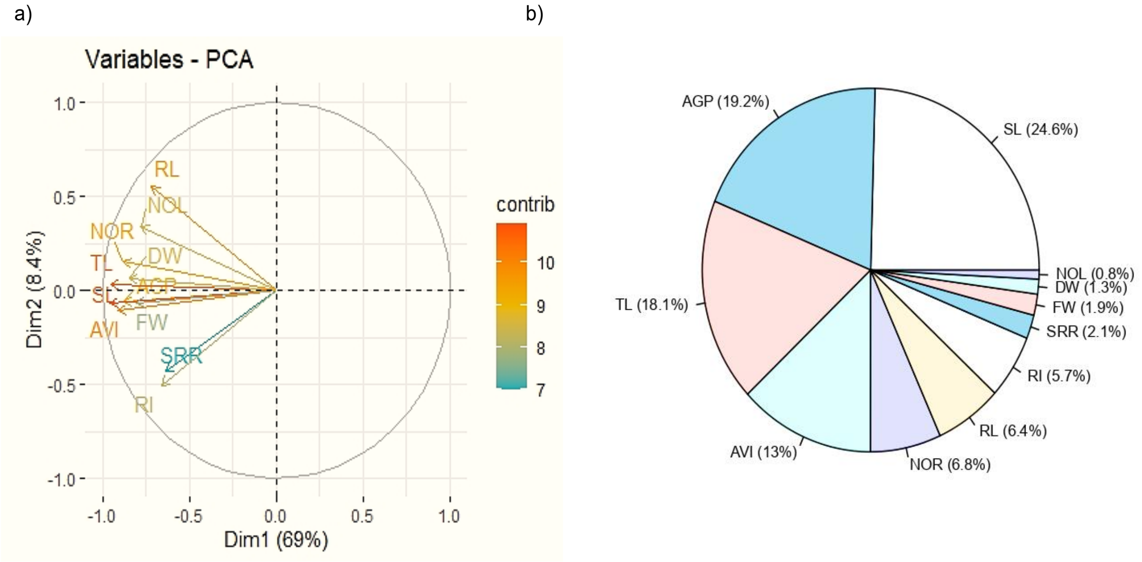

Fig. 4. a) PCA analysis plotting variation on two principal components contributed by individual traits associated with AG potential based on contribution values. b) Pie chart depicting percentage of variation by individual AG traits towards overall variation

Most of the moderately tolerant and moderately susceptible genotypes were plotted on the second quadrant (top left). One of the significant moderately tolerant genotype, RL 639 with high anoxic tolerance traits, AVI (4,702.60) and RI (13.44) was observed to be positioned distinctively apart from the other genotypes in the second quadrant indicating the genotypic contribution towards overall variation. The susceptible genotypes were dispersed across third (top right) and fourth quadrants (bottom right). Notable susceptible genotypes such as RL 2339, IC 218157, IC 218843, IC 115551 and IC 213779 formed a small cluster of plots on top of third quadrant indicating its distinctiveness from other genotypes (**Fig. 5**). Therefore, the PCA biplot effectively differentiates the highly tolerant genotypes from susceptible ones, highlighting the extent of variation present among the examined genotypes for AGT. The individual contribution of each trait to the overall variation were; SL (24.6%), AGP (19.2%), TL (18.1%), AVI (13%). Earlier studies also revealed that shoot length, AGP contributed majorly towards total variation (Sudeepthi *et al*., 2020). Principal component analysis also revealed that the most discriminatory AGT traits were SL, TL, AVI and AGP. Earlier studies also reported that traits such as AVI, AGP, SL, RI (Shanmugam *et al.,* 2023) and FW and DW (Barik *et al*., 2019; Sudeepthi *et al*., 2020) to be discriminating traits. In addition to per se performance, the principal component analysis also indicates that, the traits such as SL, TL, AVI and AGP are to be considered while breeding for AGT in rice.

Cluster analysis: WardD^2 cluster analysis method was employed to categorize the 121 rice genotypes based on the traits linked to AGT potential. It grouped the genotypes into five distinct clusters with range between five (cluster V) and 77 (cluster I) genotypes per cluster. In the present study 21 genotypes were identified as tolerant (> 90 %) and moderately tolerant (> 70 to 89 %). Out of these 21 genotypes 80.9 % (17 genotypes) were grouped in clusters III and V. Highly tolerant genotypes *viz.,* RL 79, RL 4088, RL 141 and RL 3849 along with most of the moderately tolerant genotypes were all grouped together in cluster III with highest mean values of AGP (88%), AVI (3336.60) and SRR (5.16); SL (31.33), RL (6.62), RI (7.96). Some of the moderately tolerant genotypes were grouped in cluster V with mean values of AGP (74%), AVI (2908.19), SL (35.40), RL (7.67) and RI (10.88). The genotypes with high values for AG specific traits such as AGP, AVI and RI were in cluster III and V (**Table 4**). These genotypes shown high values for AGP (> 74%), AVI (2908.19 – 3336.60), SL (31.33 – 35.4) and RI (7.96 – 10.88). Some of the moderately tolerant (RL 10099, IC 115775, RL 10039, RL 4278) and moderately susceptible genotypes were grouped together in cluster II with mean AGP (55.21%) and AVI (1844.77). Cluster I with 77 genotypes, forming the largest cluster, grouped mostly with susceptible genotypes (eg. RL 4140, IC 114574, IC 133690, IC 389714) with the lowest mean values for every traits; AGP (16.75%), AVI (438.83) , RI (-0.91), SL (11.73), RL (3.30) and SRR (2.31). Cluster I and cluster IV contained all the susceptible and some of moderately susceptible genotypes. Mean values for each cluster exhibited significant differences across all traits. In the present study distinct clustering pattern was observed, which clearly groups tolerant and susceptible genotypes into separate clusters. No duplicate genotypes were discovered in our study as most of the landraces show high level of variation in regards with AG potential (Barik *et al*., 2019)

Table 4. Cluster mean for AG associated traits among 121 rice genotypes

Traits	Cluster I (n= 77)	Cluster II (n=23)	Cluster III (n=10)	Cluster IV (n=6)	Cluster V (n=5)
AGP (%)	16.75	55.21739	88.00	24.17	74.00
SL (cm)	11.73	26.19	31.33	16.52	35.40
RL (cm)	3.30	6.30	6.62	4.66	7.67
TL (cm)	15.26	32.51	37.95	21.32	43.07
SRR	2.31	4.38	5.16	4.18	4.84
AVI	438.81	1844.77	3336.60	787.46	2908.19
RI	0.91	0.23	7.96	1.54	10.88
FW (g)	0.10	0.29	0.32	0.11	0.53
DW (g)	0.03	0.08	0.09	0.05	0.11
NR	2.65	5.09	6.32	3.64	6.46
NL	1.06	1.92	1.97	1.50	1.98

Values in parenthesis represent the number of landraces present in each cluster. AGP, anaerobic germination percentage; SL, shoot length; RL, root length; RI, response index; SRR, shoot to root ratio; NL, number of leaves; NR, number of roots; FW, fresh weight; DW, dry weight; AVI, anaerobic vigor index.

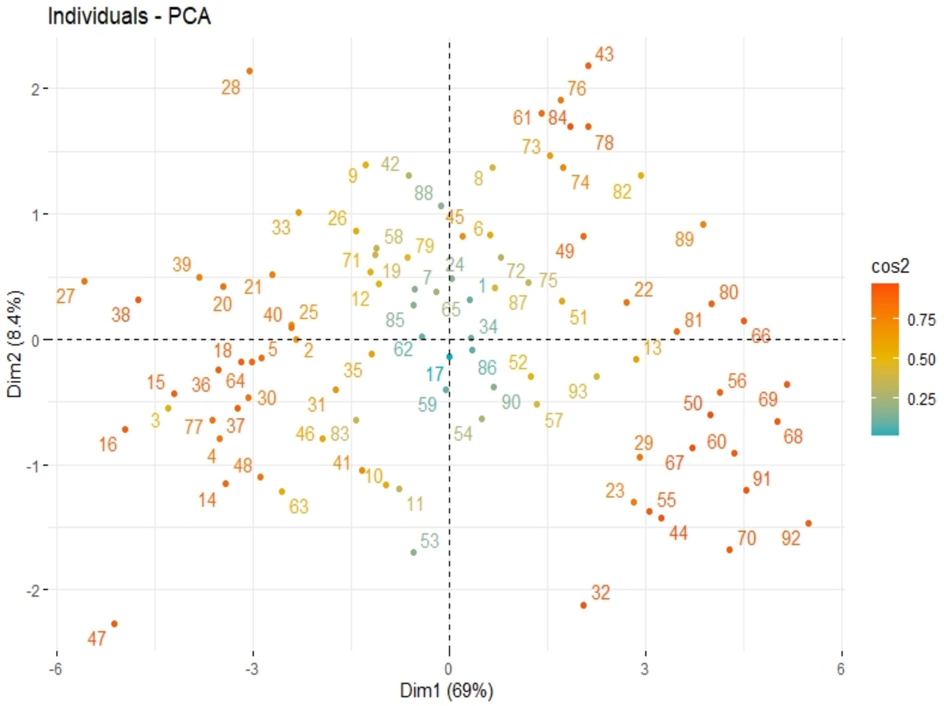

Fig. 5. Principal component analysis biplot plotting all the 121 genotypes in four quadrants based on the variation contributed towards two principal components named as Dim 1 and Dim 2 on the graph

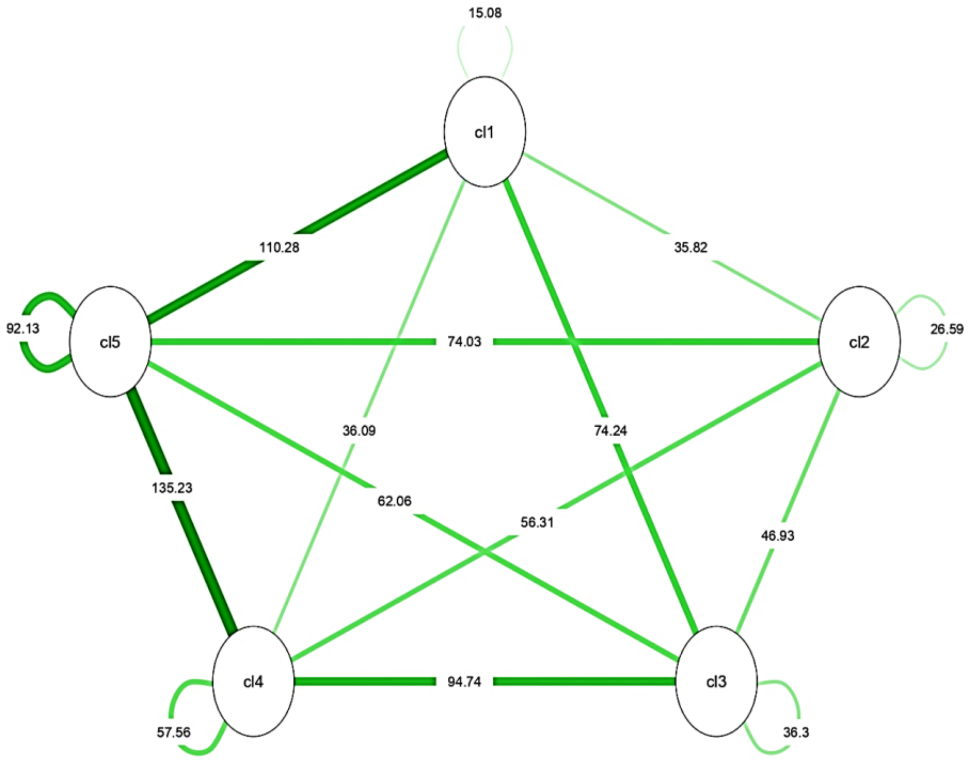

Fig. 6. Cluster diagram of the studied 121 genotypes divided into five different clusters based on traits associated with anaerobic germination along with intra and inter cluster distances between the five clusters.

The average intra-cluster distance was found to be highest in cluster V followed by cluster IV and III with the smallest being cluster I. Inter-cluster distance was found to be highest between cluster V and cluster IV followed by cluster V and I and lowest was between cluster I and II (**Fig 6**). Diverse clusters provide breeders with distinct genotypes which can be effectively used in breeding programmes in many ways. These discoveries can be harnessed to generate innovative recombinants, unveiling the underlying genetic mechanisms, and facilitating the mapping of novel quantitative trait loci (QTLs) related to AGT traits.

The present study, revealed considerable variation among the genotypes for AGT related traits, it also found out that two traits viz., AGP and AVI are the key traits contributing to AGT. Four landraces *viz*., RL 79, RL 4088, RL 141 and RL 3849 were identified as tolerant genotypes for AGT based on high AGP and AVI values. As AGT genotypes exhibit strong suitability for direct seeded rice (DSR) cultivation the tolerant genotypes identified in this study are of greater use in future breeding programme to develop AGT rice varieties and development of mapping population to dissect the QTLs / QTNs / genes harboring AGT in rice. This study also revealed a notable information that, all the 20 released varieties were susceptible to AGT, which is primarily due to their narrow genetic base as they are released with a targeted focus on high yield Hence, the above-mentioned AG tolerant landraces with broad genetic base and novel genes can be included in subsequent future breeding programs as a donor parent to facilitate the transfer of AG capabilities to improve the genetic architecture of desired high yielding varieties.

ACKNOWLEDGEMENT

We acknowledge Department of Biotechnology for financial support to this work through IRLR Project “Mainstreaming rice landraces diversity in varietal development through genome wide association studies: A model for large scale utilization of gene bank collections of rice”

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