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Research Note

Genetic divergence among indigenous rice (*Oryza sativa* L.) genotypes of Kerala based on anaerobic germination and early seedling growth

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Abstract
Flooding during germination severely hinders rice establishment in direct-seeded rice. One hundred and fifty rice genotypes indigenous to Kerala were evaluated for key traits including anaerobic germination potential under submerged conditions and its early seedling growth. Significant variability was observed among the traits under study, with germination per cent, shoot length and vigour indices registering high heritability and thereby indicating strong genetic control on expression of these traits. Genetic divergence studies by Mahalanobis D^2 analysis grouped the genotypes into 11 distinct clusters, with notable inter and intra-cluster distances, indicating considerable diversity. The study identified cluster III with 22 genotypes and cluster VI with three genotypes as clusters with promising genotypes. The results thus provide a reliable basis for parental genotype selection in breeding programs focusing on developing genotypes suitable for direct seeded rice systems through improvement of anaerobic germination tolerance. A hybridization program involving parents from these identified clusters is likely to produce a higher frequency of promising segregants or favourable combinations, which can be utilized for developing valuable genetic stocks or new varieties.

Keywords: Anaerobic germination, traditional rice, DSR, Genetic divergence, submergence tolerance

Direct Seeded Rice (DSR) systems face unique challenges, particularly during the germination and early seedling stages. The rice fields are often subjected to temporary or prolonged water logging due to incessant rainfall or poor drainage, following seeding. This frequently leads to partial or complete crop failure owing to the high sensitivity of rice to anaerobic conditions during germination (Yamauchi *et al*., 1993). Under such circumstances, *anaerobic germination* (AG) becomes a vital trait, enabling rice seeds to germinate, seedlings to emerge and continue growth unhindered under low-oxygen or submerged environments. According to Verma and Sandhu (2024), anaerobic germination contributes significantly to better crop stand establishment. The rising incidence of biotic and abiotic stresses, intensified by climate change necessitates the development of resilient rice varieties (Khatun *et al*., 2025). Identifying genetically diverse and tolerant genotypes is crucial for breeding rice varieties capable of withstanding early submergence, suitable for direct seeding. Considering the above, the present study aimed to evaluate the variability and genetic divergence among indigenous rice genotypes of Kerala for traits associated with anaerobic germination tolerance.

An in-depth analysis of the genetic divergence among 150 traditional rice genotypes in Kerala based on their ability to withstand flooding during germination and traits associated with early seedling growth was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellanikkara, Kerala Agricultural University during 2023-24. The genotypes collected from the Seed Gene Bank, Department of Seed Science and Technology, Kerala Agricultural University, Vellanikkara, Thrissur, were evaluated along with varieties CR 1009 *Sub1* (Department of Rice, TNAU) and IR 42 (ICAR-NRRI, Cuttack) that served as the submergence tolerant check and submergence susceptible check, respectively, following a Completely Randomised Design with two replications. The seeds sown in protrays and maintained under submerged condition under a water level of 25 cm in cement tanks for 14 days. The germination and early seedling growth characters were taken five days after de-submergence. Observations on germination (%) under submergence, Days to germination, Shoot length (cm), Root length (cm), Seedling fresh weight (mg) and Seedling dry weight (mg) were recorded. Seedling vigour indices I and II were computed in accordance with Abdul-Baki and Anderson (1973) and Bewley and Black (1994), respectively. Genotypic and Phenotypic coefficients of variations were estimated. The genetic divergence among the genotypes was determined using Mahalanobis' D^2 (1936) statistic, and grouping of genotypes into clusters was done by Tocher's method (Rao, 1952), using R software version 4.4.3.

Estimates of the variance components, genetic variability, and heritability: The analysis of variance (ANOVA) for anaerobic germination-associated traits is presented in **Table 1.** The results revealed significant differences among the genotypes for all the traits studied. Significant variation was recorded for Anaerobic Germination Per cent (AGP), ranging from 3.57% (TGC 135 and UN 109) to 100% (TGC 191).Days to Germination (DG) varied from 3.5 to 5.0. Shoot length of the germinating seedlings ranged from 8.47 cm (TGC 247) to 41.08 cm (TGC 88), while the root length varied between 4.31 cm (TGC 14) and 11.58 cm (UN14). For Anaerobic Vigour Index-I (AVI-I), the range was 67.16 (UN 109) to 4,153 (TGC 58), whereas Anaerobic Vigour Index-II (AVI-II) varied from 23.21 (UN 109) to 2,933.93 (TGC 364) (**Table 2**). The study by Muvendhan ***et al***., (2023) also revealed considerable variation among the genotypes for anaerobic germination tolerance related traits. Out that two traits viz., AGP and AV-I were the key traits contributing to anaerobic germination tolerance. The major traits, such as shoot length and root length, fresh weight, dry weight and anaerobic vigour index are the major determinants of phenotypic diversity under anaerobic conditions (Barik *et al*., 2019).

Table 1. Analysis of variance for anaerobic germination-related traits in indigenous rice (*Oryza sativa* L.) genotypes

Source of variation	d.f.	AGP	DG	SL	RL	FW	DW	VI-I	VI-II
Genotypes	151	1053.091**	0.472**	62.749**	3.811**	2492.506**	387.296**	1973752.116**	1056898.311**
Error	152	61.68	0.049	3.174	0.480	209.868	281.250	95601.118	101196.241

**P= 0.01

AGP-Anaerobic germination potential (%); DG-Days to Germination; SL-Shoot length (cm); RL-Root length (cm); FW-Seedling Fresh Weight (mg);

DW-Seedling Dry Weight (mg); VI-I-Vigour Index I, VI-II-Vigour index II

Table 2. Estimates of genetic variability parameters of the traits associated with anaerobic germination and early seedling growth of indigenous rice genotypes germinating under submergence

Traits	Range	Mean	CV (%)	PCV (%)	GCV (%)	h^2	GA (%)
AGP	3.57-100.00	53.00	14.79	44.43	41.90	0.89	81.39
DG	3.50-5.00	4.40	5.03	11.61	10.46	0.81	19.41
SL	8.47-41.08	25.96	6.41	21.79	20.83	0.91	41.00
RL	4.31-11.58	7.68	9.02	19.07	16.80	0.78	30.50
FW	21.00-172.00	88.85	15.76	40.94	37.78	0.85	71.84
DW	3.00-171.00	27.14	61.41	66.85	26.41	0.16	21.49
VI-I	67.16-4153.45	1870.93	16.43	54.06	51.50	0.91	101.07
VI-II	23.21-2933.93	1420.77	22.29	53.32	48.44	0.83	90.65

AGP-Anaerobic germination potential (%); DG-Days to Germination; SL-Shoot length (cm), RL-Root length (cm); FW-Seedling Fresh Weight (mg); DW-Seedling Dry Weight (mg); VI-I-Vigour Index I; VI-II-Vigour index II; h^2-heritability; GA-Genetic Advance

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The phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, and genetic advance (GA) were assessed across 152 rice genotypes under study. PCV values ranged from 11.61% (DG) to 66.85% (DW), while GCV values varied from 10.46% (DG) to 51.50% (AVI-I). In all studied traits, PCV values were higher than GCV values, indicating a significant influence of environmental interactions on the expression of these traits (Shanmugam *et al*., 2023). Broad-sense heritability showed a wide range, from 0.16 (DW) to 0.91 (Shoot length and AVI-I). Traits linked to AG potential, such as AGP, seedling Shoot Length (SL) and AVI, exhibited high heritability, indicating strong genetic control. Genetic advance as a per cent of mean (GAM) ranged from 19.41% (DG) to 101.07% (AVI-I). Notably, traits such as AGP, AVI-I, and AVI-II demonstrated both high heritability and high GAM, supporting their utility as a selection index for anaerobic germination tolerance in rice.

Genetic divergence analysis and clustering: Mahalanobis D^2 analysis showed significant genetic divergence among genotypes under study. The genotypes are grouped into eleven clusters based on traits associated with anaerobic germination potential (**Table 3.**). Cluster I comprised of the highest number of genotypes (60), indicating genetic closeness among the genotypes included within cluster, followed by cluster III with 22 genotypes, cluster II with 21 genotypes, cluster IV with 15 genotypes, cluster V with 10 genotypes, cluster VIII with six genotypes, cluster VII and X with five genotypes, cluster VI and IX with three genotypes and cluster XI with least number of genotypes of two, comprising unique genotypes with high divergence.

The means of traits associated with anaerobic germination and early seedling growth within each cluster are given in **Table 4**. Cluster mean analysis revealed a wide range of variation for all the traits studied. For the trait AGP, the maximum value was observed for cluster VI (85.72), while the minimum value was observed in cluster X (12.50). The clusters III, VI and VIII registered a mean AGP greater than 75 per cent. Most of the genotypes that exhibited a mean AGP greater than 75 are included in cluster III. The values for the trait shoot length, ranged from 35.07 (cluster VIII) to 15.12 (cluster X). The highest value for seedling root length was observed in cluster IX (9.94 cm), whereas the lowest value was observed in cluster VII (4.67 cm). For the trait fresh weight, the maximum value was observed for cluster IX (0.16g) while the minimum value was observed for clusters II and VII (0.05g). Vigour index-I was the highest in cluster VIII (3441.26) and the least in cluster X (304.09). The maximum value for Vigour index-II, was observed in cluster III (2445.35) while the minimum value was observed in cluster IV (516.51). The genotypes with high AGP and seedling vigour indices were found to be included in Cluster III and cluster VI. The clusters III, V, VIII and XI showed an average shoot length greater than 30 cm. The values for the trait days to germination, ranged from 4.15 (cluster V) to 5.0 (cluster XI). Tolerant rice genotypes will germinate and develop

Table 3. Grouping of indigenous rice genotypes into clusters based on D^2 values

Cluster	Number of genotypes	Genotypes included in cluster
I	60	L 10, L 20, N 1152 , N 1165, N 1173, N 1194, N 1198, N 1200, N 1201, N 1312, N 1328, N 153, N 173, N 73, TGC 11, TGC 114, TGC 12, TGC 131, TGC 136, TGC 15, TGC 155, TGC 158, TGC 167, TGC 193, TGC 21, TGC 231, TGC 232, TGC 24, TGC 250, TGC 251, TGC 265, TGC 269, TGC 298, TGC 301, TGC 32, TGC 330, TGC 365, TGC 370, TGC 413, TGC 417, TGC 419, TGC 424, TGC 45, TGC 61, TGC 70, TGC 96, IR 42, UN 06, UN 08, UN 103, UN 104, UN 106, UN 11, UN 124, UN 128, UN 13, UN 130, UN 29-22K, UN 33, UN 55
II	21	L 23, L 31, L 34, N 148, N 61, N 88, TGC 121, TGC 13, TGC 153, TGC 297, TGC 306, TGC 324, TGC 328, TGC 339, TGC 350, TGC 355, TGC 374, TGC 387, TGC 395, TGC 442, TGC 78
III	22	N 1144, N 1161, N 127, N 147, N 151, TGC 132, TGC 18, TGC 191, TGC 20, TGC 233, TGC 249, TGC 318, TGC 362, TGC 364, TGC 409, TGC 414, TGC 444, TGC 75, TGC 79, TGC 93, UN 25, UN 27
IV	15	TGC 07, TGC 135, TGC 152, TGC 194, TGC 234, TGC 293, TGC 300, TGC 304, TGC 367, TGC 392, UN 105, UN 107, UN 129, UN 94, UN 99
V	10	L 28, N 1158, N 174, N 176, TGC 140, TGC 416, UN 108, UN 110, UN 123, UN 15
VI	3	N 85, TGC410, UN 07
VII	5	CR 1009 *sub*1, TGC 14, TGC 361, TGC 91, UN 98
VIII	6	N 1172, TGC 16, TGC 87, TGC 88, TGC 94, UN 56
IX	3	N 110, N 1183, UN 14
X	5	TGC 247, UN 109, UN 122, UN 53, UN 96
XI	2	TGC 95, TGC 243

Table 4. Cluster means of traits associated with anaerobic germination and early seedling growth in indigenous rice genotypes

Cluster	AGP	DG	SL	RL	FW	VI-I	VI-II
I	49.73	4.2	26.64	7.59	101.02	1726.56	1271.12
II	61.99	4.88	22.46	8.05	52.12	1903.84	1552.89
III	80.52	4.25	31.57	8.86	121.08	3274.88	2445.35
IV	21.91	4.63	18.03	6.32	59.98	544.41	516.51
V	28.75	4.15	30.46	7.34	121.0	1108.22	869.98
VI	85.72	4.33	25.55	9.79	88.91	2929.61	2430.24
VII	58.79	4.80	19.78	4.67	50.05	1463.07	770.39
VIII	79.76	4.50	35.07	6.96	91.02	3441.26	2020.09
IX	60.12	4.33	28.00	9.94	160.41	2289.50	2289.64
X	12.50	4.50	15.12	7.35	79.86	304.09	741.43
X1	66.97	5.00	34.42	9.25	130.02	2995.64	1963.22

AGP-Anaerobic germination potential (%); DG-Days to Germination; SL-Shoot length (cm), RL-Root length (cm); FW-Seedling Fresh Weight (mg); VI-I-Vigour Index I; VI-II-Vigour index II

Table 5. Average intra-cluster and inter-cluster distances among traditional rice varieties based on the traits associated with anaerobic germination

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	**25.93**	50.64	55.31	63.83	48.14	63.11	62.07	85.55	58.23	116.76	81.64
II		**21.75**	70.21	64.09	109.57	44.03	55.59	107.06	85.29	118.83	103.15
III			**28.49**	137.12	94.59	47.15	110.67	55.25	56.48	195.62	51.95
IV				**24.51**	103.52	118.05	54.88	175.76	122.99	56.53	175.44
V					**35.83**	127.08	109.97	115.09	82.47	161.41	101.94
VI						**34.94**	105.15	106.14	57.25	161.49	88.16
VII							**30.95**	116.73	137.11	123.21	143.03
VIII								**40.04**	127.08	268.94	64.74
IX									**38.65**	147.36	79.38
X										**72.05**	244.41
XI											**89.64**

faster under hypoxia or anoxia stress, with greater seedling length and vigour index (Ismail, 2012). Choosing genotypes based on higher germination per cent, early seedling vigour, and increase in shoot length under anaerobic situations can improve tolerance for anaerobic germination and submergence conditions (Gilbert *et al.*, 2024). Thus, clusters III and VI represents genotypes with superior performance for multiple traits related to anaerobic germination and early seedling vigour, which can be used for further breeding programmes aimed at enhancing anaerobic germination tolerance in rice. While Cluster X recorded the lowest values for AGP, shoot length and VI-I, identifying this as a group of susceptible or poorly performing genotypes under anaerobic stress.

The analysis of genetic divergence among the rice genotypes revealed significant variability in both inter and intra-cluster distances, indicating diverse genetic backgrounds (**Table 5.**) Maximum inter-cluster distance was observed between Cluster VIII (N 1172, TGC 16, TGC 87, TGC 88, TGC 94, UN 56) and Cluster X (TGC 247, UN 109, UN 122, UN 53, UN 96) followed by cluster X and XI, cluster III and X, cluster IV and cluster VIII, and cluster IV and XI, suggesting that hybridization between genotypes from these clusters could yield highly heterotic progenies and broad spectrum of variability in subsequent segregating generations and pave way for further selection and improvement. The minimum inter-cluster distance was observed between cluster II and cluster VI, followed by cluster III and cluster VI, cluster I and cluster V, and cluster I and cluster II. Hence, it is assumed that genotypes included in this clusters are closely related. The maximum intra-cluster distance was exhibited by cluster XI (TGC 95, TGC 243) followed by

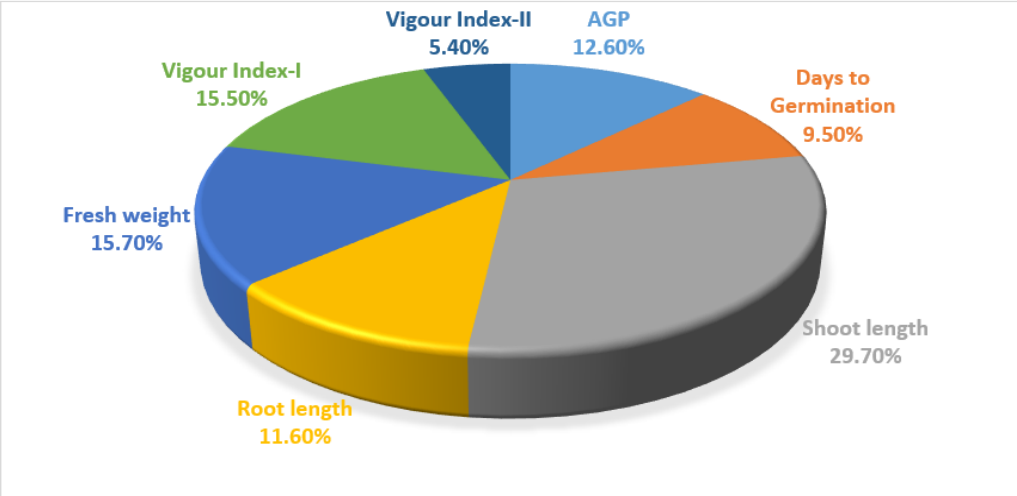

Fig.1. Per cent contribution of component traits associated with anaerobic germination and seedling growth, to the total genetic divergence in indigenous rice genotypes

clusters X (TGC 247, UN 109, UN 122, UN 53, UN 96), VIII (N 1172, TGC 16, TGC 87, TGC 88, TGC 94, UN 56) and IX (N 110, N 1183, UN 14). Cluster II showed minimum intra-cluster distance indicating homogeneity within the group followed by cluster IV.

The per cent contribution of each trait associated with anaerobic germination and seedling growth to total genetic divergence in the indigenous rice genotypes is given in **Fig 1**. Shoot length (29.7%) was the most significant contributing trait to total divergence indicating that it is the most variable and discriminating trait among the studied genotypes, germinating under anaerobic condition. This indicate that shoot elongation shows high genetic variability and plays a role in differentiating genotypes in terms of anaerobic germination stress. Fresh weight (15.7%) and VI-I (15.5%) also showed substantial genetic variability, which represents biomass accumulation and seedling vigour, followed by germination per cent (12.6%) and root length (11.6%).

The study revealed significant genetic divergence within the indigenous rice germplasm of Kerala for traits associated with AG tolerance and early seedling growth under submergence. High heritability and genetic advances recorded for germination per cent, shoot length, and vigour indices under submergence highlight their potential use as a reliable selection criterion in breeding programmes aiming to develop varieties suitable for direct-seeded rice systems as well as flood-prone ecosystems. Cluster analysis further identified genetically diverse genotypes that could serve as parents in such breeding programs. Hybridization between genotypes from genetically distant clusters can be exploited to produce a high level of hybrid vigour and favourable recombination.

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