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PHENOTYPIC SCREENING AND TRAIT CHARACTERIZATION FOR ANAEROBIC GERMINATION TOLERANCE IN RICE (*ORYZA SATIVA* L.) TO IDENTIFY NOVEL DONORS FOR DIRECT SEEDED CULTIVATION IN RAINFED LOWLANDS

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ABSTRACT

The global transition from transplanted rice to Direct Seeded Rice (DSR) is necessitating the development of varieties capable of germinating under anaerobic conditions caused by early flooding. This study evaluates the genetic potential of diverse rice accessions for Anaerobic Germination (AG) tolerance. Screening was conducted under stringent submerged condition (20 cm water depth) for 21 days. Among the survivors, profound phenotypic variability was observed. Germination percentages ranged from 20% to 100%,. The results revealed that only 36% of accessions exhibiting survival seedling Vigour Index (SVI) varied from 40 to 1320. The genotype RL986 was identified as a superior recording 100% germination, maximum coleoptile elongation, and the highest vigour index. Statistical analysis demonstrated a positive correlation between germination percentage and seedling vigour index, confirming that survival is the primary determinant of vigour under hypoxia. These findings highlight RL986, RL988, and RL790 as critical genetic resources for breeding climate-resilient rice varieties suitable for the unpredictable climate of rainfed ecosystems.

***Keywords* :** Direct seeded rice, Anaerobic germination, Hypoxia, Lowlands, Climate resilient.

Introduction

The Imperative for Direct Seeded Rice (DSR)

Rice (*Oryza sativa* L.) production is currently facing a dual challenge: the need to increase yields for a growing population and the urgent requirement to reduce water and labor inputs. Traditional puddled transplantation is resource-intensive, consuming approximately 2,500–5,000 liters of water per kilogram of grain produced (Kumar and Ladha, 2011). Consequently, Direct Seeded Rice (DSR) has emerged as a sustainable alternative, offering water savings of 12–35% and significant reductions in methane emissions (Pathak *et al.,* 2011). However, the widespread adoption of DSR in rainfed lowlands is severely hampered by the sensitivity of modern cultivars to flooding immediately after sowing a condition known as "anaerobic germination" (AG) stress (Miro and Ismail, 2013).

Physiological Barriers under Anaerobic conditions

When rice seeds are submerged, oxygen diffusion to the embryo is restricted (hypoxia) or completely blocked (anoxia). In susceptible varieties, this leads to an energy crisis; the mitochondria cannot perform oxidative phosphorylation, and the seed fails to mobilize starchy endosperm reserves (Ismail *et al.*, 2009). Tolerant landraces, however, employ an "escape strategy." They rapidly degrade starch via amylases into fermentable sugars, fueling anaerobic respiration (glycolysis and ethanol fermentation) to generate ATP. This energy drives the rapid elongation of the coleoptile (the protective sheath), which acts as a "snorkel" to breach the water surface and transport atmospheric oxygen to the submerged root and shoot (Kretzschmar *et al.*, 2015; Magneschi and Perata, 2009).

Genetic Basis and Study Objectives

A major Quantitative Trait Locus (QTL), *qAG-9-2,* derived from the Myanmar landrace Khao Hlan On, has been identified as a key regulator of this tolerance, encoding the *OsTPP7* gene (Angaji *et al.*, 2010; Kretzschmar *et al.,* 2015). While AG1 a potent source of tolerance, diversifying the genetic base is crucial for durable resistance. The objectives of this study were, to evaluate 100 diverse rice accessions under controlled anaerobic conditions to identify novel donors with tolerance comparable to or exceeding known checks. Trait Characterization to analyze the phenotypic variability in coleoptile length, biomass, and survival. Correlation Analysis to determine the statistical relationships between seedling traits to refine selection criteria for breeding programs.

Materials and Methods

The study utilized a panel of 100 rice accessions from ICAR-Indian Institute of Rice Research, Hyderabad, Telangana designated as the 'RL' series representing diverse genetic backgrounds. Genotypes failing to germinate or showing minimal growth served as internal susceptible checks. Phenotypic performance was benchmarked against characteristics typical of known tolerant donors like Khao Hlan On.

Screening Methodology for Anaerobic Germination

The phenotypic evaluation was conducted at ICAR-Indian Institute of Rice Research, using a standardized submerged protocol designed to simulate severe flooding stress in the field (Shanmugam *et al.,* 2022). Dry seeds were sown in plastic cups containing soil. Immediately after sowing, the trays were submerged in a tank to maintain a constant water depth of 20 cm above the soil surface. This depth is sufficient to impose significant anoxic stress and suppress the growth of non-tolerant lines. The submergence stress was maintained for 21 days. The setup ensured that the only oxygen available to the seed was dissolved oxygen in the water, forcing the reliance on coleoptile elongation. Data were recorded on the 21st day after sowing (DAS) for Germination Percentage (%), Seedling Length (cm), Seedling Biomass (g), Number of Leaves, Seedling Vigour Index (SVI).

Plate 1: Showing the protocol for screening of rice genotypes for anaerobic germination tolerance. a) seeding of rice genotypes in cups b) Cups submerged in water c) tolerant genotypes reached water surface d) susceptible and tolerant genotypes on 21st day.

Statistical Analysis

To understand the interrelationships between traits, Phenotypic Correlation Coefficients were calculated using the formula proposed by Webber and Moorthy (1952). The significance of these coefficients was tested against table values for (n-2) degrees of freedom as outlined by Snedecor (1961).

Results

Survival and Genetic Variability

The imposition of 20 cm water depth created a severe selection pressure. Out of the 100 accessions screened, only 36 accessions (36%) exhibited the capacity to germinate and survive by the 21st day. The remaining 64% succumbed to the hypoxic stress, highlighting the rarity of the AG tolerance trait in general germplasm. Among the genotypes which survived, high genetic variability was observed. Germination % ranged from 20% to 100%. Seedling Length varied from 2.0 cm to 13.2 cm. Vigour Index ranged widely from 40 to 1320. Table1 and Table 2 details the performance of the top-performing and lowest-performing genotypes identified. The Phenotypic distribution of genotypes is given in figure 1 and figure 2.

RL986 demonstrated superior tolerance, achieving the maximum values for germination (100%) and seedling length (13.2 cm). This indicates a highly efficient metabolic capability to mobilize starch and elongate the coleoptile to access air. RL988 & RL790 also achieved 100% germination, their shorter seedling lengths resulted in lower vigour indices compared to RL986. They represent tolerant genotypes but perhaps slower in growth. Despite of low germination rate (40%), this genotype RL877 recorded the highest biomass (0.0778 g). This suggests that the few seeds that successfully germinated were extremely vigorous, accumulating significant dry matter. RL856 with only 20% germination and 2 cm growth, failed to establish, effectively acting as a negative check.

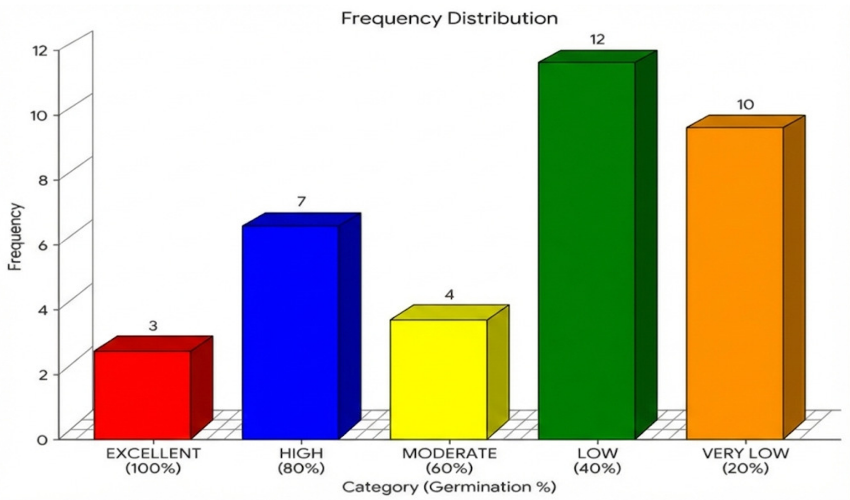

Fig. 1: Frequency distribution of genotypes for germination percentage under anaerobic conditions.

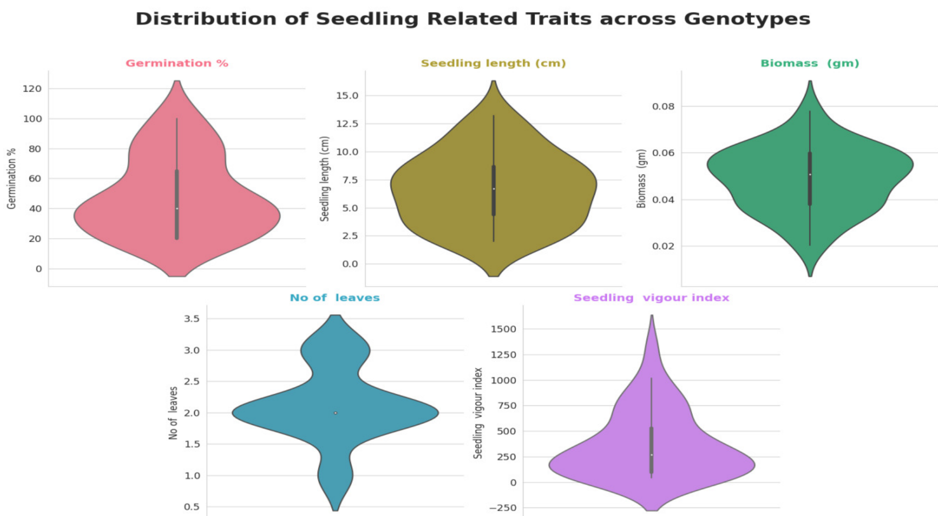

Fig. 2: Violin Plots of genotypes for Seedling traits under Anaerobic Germination

Table 1: Tolerant, moderately tolerant and susceptible genotypes identified for germination percentage under anaerobic condition.

Category	Germ. %	Count	Genotypes Identified
EXCELLENT	100%	3	RL988, RL790, RL986
HIGH	80%	7	RL987, RL920, RL967, RL810, RL873, RL822, RL873
MODERATE	60%	4	RL863, RL902, RL968, RL974
LOW	40%	12	RL989, RL805, RL898, RL873, RL877, RL937, RL954, RL804, RL978, RL961, RL872, RL858, RL821
VERY LOW	20%	10	RL930, RL918, RL892, RL809, RL915, RL912, RL981, RL856, RL816, RL914

Table 2: Maximum and minimum mean values observed among the rice germplasm entries for various anaerobic germination tolerance traits

Trait	Avg.	Max.	Min.
Germination %	48.33	100	20
Seedling length (cm)	6.67	13.2	2
Biomass (gm)	0.05	0.08	0.02
No. of leaves	2.11	3	1
Seedling vigour index	369.39	1320	40

Correlation Studies

The phenotypic correlation coefficients (Table 3 and Fig. 2) reveal the biological associations driving AG tolerance.

Strong Positive Correlations with Germination

Seedling Vigor Index exhibited the strongest positive correlation with both Germination (0.900) and Germination % (0.900). This highly significant association underscored that accessions with better germination rates under anaerobic conditions also tended to produce more vigorous seedlings. This suggested that seedling vigor was an excellent indicator of successful anaerobic germination. Seedling Length also showed a strong positive correlation with Germination (0.582) and Germination % (0.5828). This indicated that higher germination rates were generally associated with greater seedling elongation. Biomass (grams) was positively correlated with Germination (0.499) and Germination % (0.499), suggesting that better germination led to higher seedling dry weight.

Interrelationships among the Traits

The correlation analysis between five key seedling variables Germination %, Seedling length (cm), Biomass (gm), Number of leaves, and Seedling vigour index provides important insights into their interrelationships and the dynamics of seedling growth.

Germination %

Germination % showed a very strong positive correlation with Seedling vigour index (r=0.909), indicating that the success rate of seeds germinating has a direct and substantial effect on overall seedling vigor. This means that lines or treatments with higher germination not only yield more plants but those plants tend to be more vigorous. It also exhibited a strong significant correlation with Seedling length (r=0.64) and Biomass (r=0.575). This suggests that seeds that germinate well tend to produce seedlings that grow longer and accumulate more biomass, both of which are desirable traits for healthy early seedling establishment. The correlation with Number of leaves (r=0.304) was weaker and not statistically significant, implying that high germination does not necessarily result in more leaves per seedling at this early stage.

Seedling Length

Seedling length is extremely well correlated with Biomass (r=0.814), indicating that longer seedlings also tend to accumulate greater mass. This makes sense biologically, as vigorous elongation is often associated with bigger and heavier seedlings. Seedling length also had a moderate to strong association with Number of leaves (r=0.625) and very high correlation with Seedling vigour index (r=0.853). This underlines that increased elongation often coincides with the development of more leaves and a stronger seedling overall. Kim *et al.* (2019) similarly observed that robust seedlings maintain high biomass through efficient anaerobic respiration.

Biomass

Biomass (gm) further shows significant correlations with Number of leaves (r=0.477) and Seedling vigour index (r=0.699), confirming that more robust seedlings not only grow longer but also heavier and have more leaves which is desirable characteristics for early plant vigor and competitive ability in the field. This high positive correlation indicates that coleoptile elongation is driven by substrate mobilization (biomass accumulation), not just cell stretching.

Number of Leaves

Number of leaves, while significantly correlated with most traits, showed generally lower coefficients than other pairs. Its correlation with Seedling vigour index (r=0.438) indicates that while more leaves contribute to overall vigor, the effect is less pronounced compared to traits like elongation and biomass. The lack of significant correlation is physiologically consistent with Baltazar *et al.* (2019), who noted that under anoxia, rice suppresses leaf development to prioritize coleoptile elongation.

Seedling Vigour Index

Seedling vigour index shows significant and strong associations with all other measured variables, most notably with Germination % (r=0.909), Seedling length (r=0.853), and Biomass (r=0.699). This highlights that seedling vigor is a composite measure reflecting multiple underlying growth traits, reinforcing its utility as a practical, holistic selection criterion in breeding and agronomic evaluation. The strongest correlation observed. These results are in agreement with the findings of Yang *et al.* (2022) and Saimohan *et al.* (2025), who reported that survival rate is the primary component of seedling vigour under stress.

Table 3: Correlation Studies for Seedling traits recorded during screening for Anaerobic Germination Tolerance

Variables	Germination %	Seedling length (cm)	Biomass (gm)	No of leaves	Seedling vigour index
Germination %	1.0 **				
Seedling length (cm)	0.64 **	1.0 **			
Biomass (gm)	0.575 **	0.814 **	1.0 **		
No of leaves	0.304	0.625 **	0.477 **	1.0 **	
Seedling vigour index	0.909 **	0.853 **	0.699 **	0.438 **	1.0 **

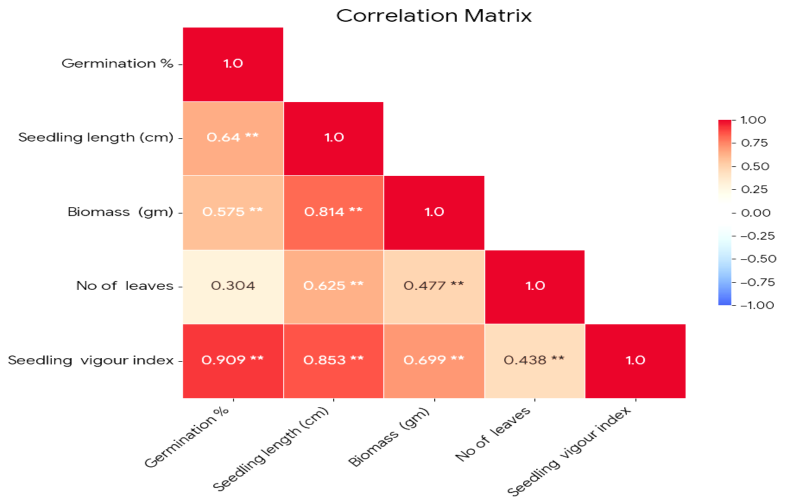

Fig. 2: Correlation plot of anaerobic germination tolerant traits

Discussion

The identification of RL986 is the most significant outcome of this experiment. Its performance (SVI: 1320) rivals that of established donors like Khao Hlan On. The ability of RL986 to maintain 100% germination under 20 cm of water suggests it possesses a robust allele of *OsTPP7* or potentially a novel mechanism for anaerobic starch degradation. This genotype should be prioritized for genotyping and hybridization programs.

The correlation analysis validates the "Escape Strategy" hypothesis. The strong link between seedling length and biomass confirms that successful escape requires metabolic activity. As Kretzschmar *et al.* (2015) elucidated, this energy is derived from the trehalose-6-phosphate phosphatase pathway, which prevents sugar starvation signaling. Tolerant plants do not just survive; they actively grow towards the surface. The Seedling Vigour Index (SVI) emerged as the most reliable selection metric, serving as a composite indicator of both survival and escape potential, a trend also reported by Saimohan *et al.* (2025) in their recent screenings.

For practical breeding, these findings suggest a selection index emphasizing Coleoptile Length and Germination %. Genotypes like RL986 can be used as donors in Marker-Assisted Backcrossing (MABC) programs to improve elite varieties which possess high yield but lack flooding tolerance. Incorporating these traits will secure crop establishment in Direct Seeded Rice systems, directly addressing the water scarcity and labor issues plaguing modern agriculture.

Conclusion

This study successfully screened 100 rice accessions and identified RL986 as a potent donor for anaerobic germination tolerance. The distinct phenotypic correlations established that vigorous coleoptile elongation, supported by biomass accumulation, is the critical mechanism for survival. Integrating RL986 into breeding pipelines will accelerate the development of flood tolerant rice varieties, facilitating the large scale adoption of sustainable Direct Seeded Rice cultivation.

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