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# Phenotypic evaluation of recombinant inbred lines along with parents for anaerobic germination tolerance in rice (*Oryza sativa* L.)

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**Abstract**
The present study was conducted by using an Augmented Block Design to screen 201 recombinant inbred lines (RILs) developed from the cross BPT5204 × Arvatellu, along with their parents and standard check varieties like BPT5204, Arvatellu, IR-4630-11-175, KHO, CST7-1 and CSR-52 for anaerobic germination tolerance during season kharif (2024-2025) at the Indian Institute of Rice Research (IIRR), Hyderabad. The RILs and parents were phenotypically screened under controlled anaerobic conditions by maintaining a 10 cm water level for duration of 21 days from the date of sowing. Key traits like Germination Percentage (G%), Root Length (RL), Shoot Length (SL), Seedling Height (SH), Culm Diameter (CD), Root Dry Weight (RDW) and Shoot Dry Weight (SDW) were recorded after 21 days from the date of sowing. Significant variation was observed among the RILs. Arvatellu and IR-4630-11-175 consistently showed high tolerance to anaerobic stress conditions. While, CSR-52 and BPT5204 exhibited poor performance under anaerobic conditions. A wide variation was observed in the RIL population (Germination percentage ranged from 7-93%; Root Length ranged from: 3-14.0 cm; Shoot Length ranged from: 14.3-44.2 cm; Seedling Height ranged from 17.0-58.2 cm; Culm diameter ranged from 0.2-1.03 mm; Root Dry Weight ranged from 0.010-0.024 g; Shoot Dry Weight ranged from 0.010-0.026 g). Three RILs namely RIL-6**,** RIL-104, andRIL-139 were emerged as most promising exhibited superior performance across multiple traits often surpassing the tolerant parents. These RILs hold potential for breeding programs aimed at enhancing anaerobic tolerance in rice. These findings highlight the potential of RILs as a source of Anaerobic Germination tolerance and provide a foundation for future QTL mapping and molecular breeding efforts aimed at improving DSR adaptation.

**Keywords:** Anaerobic germination, recombinant inbred lines, rice, submergence stress, seedling vigour, Arvatellu, BPT5204

## Introduction
Rice cultivation is often hindered by waterlogging, which leads to low oxygen levels and creates anaerobic conditions (Yang *et al.*, 2019) [12]. In response, rice seeds have adapted by developing the ability to germinate under flooded conditions, known as anaerobic germination. Nevertheless, excessive waterlogging can negatively affect seedling establishment, result in yield losses, and decrease grain quality (Angaj *et al.*, 2010) [1]. Therefore, it is crucial to breed rice varieties that can endure anaerobic conditions during germination and the early growth stages (Septiningsih *et al.*, 2013) [13].

Screening rice for tolerance to anaerobic germination consists of assessing different rice genotypes in waterlogged conditions to find varieties that can effectively germinate and grow in flooded soils. This process simulates anaerobic environments either in controlled laboratory settings or in the field by submerging rice seeds in water and tracking their germination and growth progress. Evaluation criteria for this screening include the germination percentage, the vigor of the seedlings, and their survival rates when exposed to waterlogging stress. Screening rice for tolerance to anaerobic germination consists of assessing different rice genotypes in waterlogged conditions to find varieties that can effectively germinate and grow in flooded soils.

This process simulates anaerobic environments either in controlled laboratory settings or in the field by submerging rice seeds in water and tracking their germination and growth progress. Evaluation criteria for this screening include the germination percentage, the vigor of the seedlings, and their survival rates when exposed to waterlogging stress. The benefits of conducting such screenings are substantial. Firstly, it aids in identifying rice varieties that can endure water logging stress, which enhances seedling establishment and may lead to higher yield potential. Secondly, it supports the creation of climate-resilient rice varieties capable of adapting to changing environmental conditions, particularly in flood-prone regions.

In 2024, Reddy *et al.* [14] screened a diverse set of 103 rice accessions at ICAR-NRRI to identify genotypes with strong anaerobic germination tolerance. The evaluation was carried out for 21 days under anaerobic conditions, measuring germination percentage, first internode length (cm), shoot length (cm), number of leaves, root length (cm), shoot and root biomass (g), and seedling vigour index. The accessions displayed a wide range of tolerance, with germination percentages varying from 0% to 94%. Five genotypes Tulasiphula, Panirohi, BJ1, Chinamali, and ARC14855—were identified as highly tolerant, combining superior anaerobic germination ability with vigorous seedling growth, and recorded significantly higher germination rates than the remaining accessions.

In summary, screening rice for tolerance to anaerobic germination is a crucial method for developing rice varieties capable of withstanding waterlogging stress, thus improving rice yields in flood-prone areas. (Miro and Ismail, 2013) [13]. This screening involves evaluating different rice genotypes under flooded conditions to identify those that can germinate and grow effectively in waterlogged soils.The advantages of this screening process include the potential for enhanced yields, the development of climate-resilient rice varieties, and a decreased dependence on chemicals and fertilizers in rice farming. By fostering varieties that can thrive under challenging conditions, this approach not only boosts agricultural productivity but also supports more sustainable farming practices amid changing climate patterns.

## Materials and Methods

The screening experiment were conducted at Indian Institute of Rice Research (IIRR), Hyderabad during season Kharif (2024-2025). The experimental plant material consisting of 201 RILs mapping population was developed by crossing of BPT5204 and Arvatellu. The 201 RILs along with their corresponding contrast parents screened phenotypically to identify the tolerant RILs of anaerobic germination.

### Plant Materials

The experimental plant material consisting of total of 201 RILs derived from the cross BPT5204 × Arvatellu were evaluated along with the 6 parents *ie*., 6 checks; Arvatellu and IR-4630-11-175 (Tolerant), KHO (Moderately tolerant), BPT5204 and CST7-1 (Sensitive) CSR-52 (Highly sensitive) for AG tolerance at Indian Institute of Rice Research (IIRR), Hyderabad during the Season Kharif (2024-2025).

### Phenotyping for tolerance to anaerobic stress

The anaerobic experiments were conducted by using an Augmented Block Design in 3 Blocks each block with 67 genotypes as per Septiningsih *et al.* (2013) [13]. The anaerobic germination test was conducted for the all the RILs in trays to test the germination ability of RILs under anaerobic conditions. The experiment was done by maintaining the RILs under anaerobic conditions by maintaining 10 cm water level from the soil surface to create anaerobic conditions. The seeds of 201 RILs were sown in each pot which is filled with soil and little quantity of vermicompost. After sowing transfer all the pots in the tank and maintain the water level of 10 cm from the surface of the soil to create anaerobic conditions. As the water level decreases less than 10 cm add required volume of water if needed to maintain water depth of 10 cm. where initially anaerobic stress was created by maintaining water depth of 10 cm to stimulate anaerobic conditions. The 201 RIL’s along with their corresponding contrast parents screened phenotypically and observations were recorded at 21 days after sowing. seeds were germinated under controlled anaerobic conditions.

### Data collection

At the start of the study, we recorded the initial number of seeds sown for each genotype and tracked the number of seedlings that survived at 21 days after seeding. The number of surviving seedling referred to in this study as the ‘number of seeds germinated represented those that had emerged above the soil surface. The main criteria for identifying flood-tolerant RILs was the germination (or survival) percentage, calculated using the formula: Percentage Germination = (number of seeds germinated per entry / total seeds sown per entry) × 100. To enhance the identification of vigorous, anaerobic tolerant lines, we also collected data on several additional growth traits. After seedling establishment traits like Germination percentage (G%), Root length (RL), Shoot length (SL), Seedling height (SH), Culm diameter (CD), Root dry weight (RDW) and Shoot dry weight (SDW) were recorded and level of tolerance was estimated from the recorded traits like Germination percentage (G%), Root length (RL), Shoot length (SL), Seedling height (SH), Culm diameter (CD), Root dry weight (RDW) and Shoot dry weight (SDW) under anaerobic conditions. Trait ranges, means, and parental comparison were calculated. These included seedling height (measured in centimeters) at both 21 days after seeding using a standard ruler. At 21 days, we further measured root length (cm) and culm diameter (mm), using a standard ruler and a digital Vernier caliper, respectively. Lastly, selected seedlings were harvested and dried at 70°C for three days to determine shoot and root dry weights (in grams), which were then recorded. under anaerobic conditions. Trait ranges, means, and parental comparison were calculated. RIL’s were ranked trait-wise and identified for multi-trait superiority.

## Results and Discussion

The present study evaluated both parental lines and recombinant inbred lines (RILs) along with parents for their performance under anaerobic conditions focusing primarily on germination percentage and associated seedling vigour traits. Among the parental lines, Arvatellu showed the highest germination percentage (98%), followed closely by

IR-4630-11-175 (93%). In contrast, CSR-52 consistently underperformed across all measured traits. Several RILs notably outperformed even the tolerant parent lines in multiple traits. In particular RIL-139 stood out with the highest germination (93%), indicating potential for early seedling vigour and flood tolerance. RIL-104 recorded a higher germination rate (87%) with significant shoot and seedling growth. RIL-6 exhibited strong overall performance with a germination rate of 73%, the highest shoot length (44.2 cm), seedling height (58.2 cm), and rootlength (14.0 cm). Table 1.

**Table 1:** Performance of parents and RILs across all traits are:

| Trait | Range | Parents | Range | RILs |
|---|---|---|---|---|
| Germination (%) | 7%-98% | Arvatellu (98%), IR-4630-11-175 (93%) | 7%-93% | RIL-6 (73%), RIL-96 (73%), RIL-104 (87%), RIL-139 (93%) and RIL-183(53%) |
| Root length (cm) | 2.7 cm-6.7 cm | IR-4630-11-175(6.7 cm), Arvatellu and KHO (~6 cm) | 3 cm-14.0 cm | RIL-6 (14.0 cm), RIL-145 (11.6 cm), RIL-81(11.6 cm) and RIL-39 (10.5 cm) |
| Shoot length (cm) | 14.3 cm-23.7 cm | Arvatellu (23.7 cm), KHO (23.3 cm) and IR-4630 (18.7 cm) | 14.3 cm-44.2 cm | RIL-6 (44.2 cm), RIL-104 (43.5 cm), RIL-139(43.9 cm) and RIL-183 (41.9 cm) |
| Seedling height (cm) | 17 cm-29.3 cm | IR-4630-11-175 (29.3 cm) Arvatellu (29.0 cm) and KHO (28.7 cm) | 17 cm-58.2 cm | RIL-6 (58.2 cm), RIL-104 (54.8 cm), RIL-139(55.8 cm) and RIL-183 (54.1 cm) |
| Culm diametre (mm) | 0.2 mm-1.20 mm | IR-4630-11-175(1.20 m), Arvatellu (1.17 mm) and KHO (1.10 mm) | 0.2 mm-1.03 mm | RIL-123 (1.03 mm), RIL-155 (1.00 mm) and RIL-189 (1.00 mm) |
| Root dry weight (g) | 0.010 g-0.018 g | Arvatellu and IR-4630 (0.018 g, KHO (0.016 g) | 0.010 g-0.024 g | RIL-60 (0.024 g), RIL-73 (0.024 g), RIL-155 (0.024 g), RIL-183 (0.024 g) and RIL-134 (0.024 g) |
| Shoot dry weight (g) | 0.010 g-0.015 g | KHO (0.015 g), IR-4630 and Arvatellu (0.014 g) | 0.010 g-0.026 g | RIL-134 (0.026 g), RIL-155 (0.025 g) and RIL-78 (0.025 g) |

Best performing parents are ARVATELLU (most consistent and vigorous) followed by IR-4630-11-175. Poor performer is CSR-52 in all traits. KHO is good in early vigour traits but not consistent across all traits. BPT5204 and CST7-are moderate to low across traits.

**Table 2:** Anova Table

| Source | Df | Mean sum of squares | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | G | | RL | | SL | | SH | | CD | | RDW | | SDW | |
| Treatment (ignoring Blocks) | 167 | 614.30 | ** | 5.86 | ** | 62.76 | ** | 95.81 | ** | 3.40 | ** | 0.001 | ** | 0.001 | ns |
| Treatment: Check | 5 | 3902.09 | ** | 6.99 | ** | 51.14 | ** | 100.10 | ** | 0.81 | ** | 0.001 | ** | 0.001 | ns |
| Treatment: Test | 161 | 440.87 | ** | 3.83 | ** | 36.65 | ** | 54.55 | ** | 3.51 | ** | 0.001 | ** | 0.001 | ns |
| Treatment: Test vs. Check | 1 | 12097.19 | ** | 327.42 | ** | 4325.07 | ** | 6716.52 | ** | 0.24 | ** | 0.001 | ** | 0.001 | * |
| Block (eliminating Treatments) | 2 | 12.06 | * | 0.09 | ns | 2.09 | ns | 2.17 | ns | 0.001 | ns | 0.001 | ** | 0.001 | ns |
| Residuals | 10 | 2.26 | | 0.05 | | 0.87 | | 0.83 | | 0.001 | | 0.001 | | 0.001 | |

The analysis of variance (ANOVA) revealed highly significant differences among treatments for all traits (Table 2). The treatment effect (ignoring blocks) was significant ($p$<0.01) for germination percentage (G), root length (RL), shoot length (SL), shoot height (SH), collar diameter (CD), and root dry weight (RDW), indicating substantial genetic variability among the evaluated entries. Partitioning the treatment sum of squares showed that checks exhibited significant differences ($p$<0.01) for all traits. Similarly, test entries showed significant variation ($p$<0.01) for most traits except SDW. The Test vs. Check comparison was highly significant ($p$<0.01) for G, RL, SL, SH, and CD. The block effect was non-significant for most traits, except for G ($p$<0.05) and RDW ($p$<0.01), indicating that environmental variation among blocks was minimal. The low residual mean squares for all traits further confirm the reliability of the observed differences. Overall, the results demonstrate the presence of considerable variation among the evaluated genotypes, with the differences between test entries and checks suggesting that selection would be effective in improving these traits.

## Conclusion

The study successfully identified several RILs with superior anaerobic germination traits compared to both susceptible and tolerant parents. RIL-6, RIL-139, and RIL-104 emerged as the most promising lines, demonstrating high germination percentage, shoot and seedling height, and biomass accumulation. These RILs are ideal candidates for QTL mapping and subsequent use in AG tolerance breeding programs. The RIL population exhibited wide genetic variability for anaerobic germination traits. RILs such as RIL-6, RIL-104, and RIL-139surpassed tolerant parents in multiple traits, indicating successful recombination and potential for breeding programs. High germination rates with robust seedling growth are essential for anaerobic tolerance. Our phenotypic evaluation reveals that several RILs outperform tolerant parents under anaerobic conditions, especially RIL-6, RIL-104, and RIL-139. These are promising materials for breeding anaerobic-tolerant rice varieties aimed at improving direct-seeded rice in flood-prone systems. These RILs—RIL-6**,** RIL-104**,** andRIL-139 surpassed their parents in key traits making them strong candidates for QTL mapping and incorporation into anaerobic germination (AG) tolerance breeding programs.

## Disclaimer (Artificial Intelligence)

Author(s) hereby declares that no generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing manuscripts.

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**Competing Interests**
Authors have declared that no competing interests exist.

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