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Genetic variability and seedling growth responses of rice parental genotypes and F_2 population under aerobic and anaerobic conditions: Implications for crop biotechnology

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Abstract
Early seedling establishment is a major determinant of crop performance in direct-seeded rice, particularly where seeds encounter waterlogged or oxygen-limited conditions. The present study describes a comparative evaluation of six parental rice genotypes (ADT-36, ADT-43, IR-20, TRV-9, TRV-23 and TRV-26) and four F_2 populations (TRV-9/ADT-43, ADT-36/TRV-23, TRV-26/ADT-36 and TRV-26/ADT-43) using root and shoot length observations recorded under aerobic and anaerobic conditions. The dataset provided one recorded value for each entry and trait. Under aerobic conditions, parental root length ranged from 6.4 cm (IR-20) to 12.0 cm (TRV-26), while parental shoot length ranged from 13.9 cm (IR-20) to 23.9 cm (TRV-23). Among F_2 population, TRV-26/ADT-43 recorded the greatest aerobic root length (13.5 cm) and shoot length (22.3 cm). Under anaerobic conditions, parental root length ranged from 1.0 cm (TRV-26) to 8.5 cm (TRV-9), and shoot length ranged from 5.3 cm (TRV-26) to 15.2 cm (ADT-36). Among F_2 crosses, ADT-36/TRV-23 had the greatest recorded anaerobic root length (6.4 cm), whereas TRV-9/ADT-43 had the greatest recorded anaerobic shoot length (10.4 cm). The pattern indicates that the relative ranking of entries varied with the condition and trait assessed. The findings are descriptive because replication, sample size, variance estimates, treatment duration, and detailed oxygen/water regime were not included in the supplied source record. Therefore, the statistical significance or definitive anaerobic-germination tolerance can be claimed. The observations identify material for further replicated screening using standardized anaerobic-germination protocols and additional establishment traits.

Keywords: *Oryza sativa* L., anaerobic germination, aerobic growth, oxygen deficiency, seedling establishment, root length, shoot length, F_2 population, rice breeding

Introduction

Rice *(Oryza sativa* L.) is adapted to a wide range of aquatic and semi-aquatic production environments, yet successful crop establishment remains vulnerable to flooding during germination and early seedling growth. In direct-seeded rice, seeds are placed directly into the field rather than being raised in a nursery and transplanted. This system can reduce labour and production costs, but rainfall or field-level irregularities may result in standing water soon after sowing. Floodwater restricts the diffusion of oxygen into the soil and creates hypoxic or, under more severe conditions, anoxic conditions around the germinating seed. Such oxygen limitation can impair normal respiratory metabolism and delay or prevent seedling establishment (Yu *et al.,* 2021; Mwakyusa *et al.,* 2023) [23].

The response of rice to oxygen deficiency is physiologically distinctive. Unlike most cereal crops, rice can initiate germination under submerged conditions and can elongate the coleoptile using energy generated through glycolysis and fermentative metabolism. Starch stored in the endosperm is mobilized to soluble sugars, which supply the embryo and growing coleoptile. The coleoptile may elongate toward the water surface, acting as a conduit through which oxygen can subsequently enter submerged tissues. This adaptive response is one reason rice is considered comparatively capable of germinating under low-oxygen conditions (Magneschi and Perata, 2009; Pucciariello, 2020) [19, 25].

However, the capacity to germinate under oxygen deficiency is not uniform among rice genotypes. Genetic variation has been documented for coleoptile elongation, germination percentage, seedling emergence and survival under flooded conditions. Kuya *et al.* (2019) [18] developed an evaluation approach using deoxygenated water and demonstrated that coleoptile elongation was associated with survival in flooded soil. More recent screening studies have combined germination or emergence percentage with seedling height, root length and biomass to identify promising donor genotypes (Mwakyusa *et al.*, 2023) [23].

An important distinction must be made between anaerobic germination and later seedling growth under waterlogging or submergence. During the earliest stages of germination, rice may preferentially support coleoptile growth while root and true-leaf development are restricted. Once the shoot reaches the water surface and oxygen becomes available, further development of roots and leaves can proceed. Consequently, root and shoot measurements taken at a later stage should be interpreted in relation to the developmental stage and the actual flooding protocol. A measurement labelled as shoot length is not necessarily equivalent to coleoptile length unless the measurement method specifically identifies the coleoptile.

In breeding programmes, the comparison of parental genotypes and their crosses can help identify useful variation for subsequent selection. The present source dataset includes six parental entries and four F_2 population assessed under two growth conditions. Although the available observations do not provide replication-level data, they allow a preliminary descriptive comparison of root and shoot length. The specific objectives were: (i) to describe root and shoot length among the parental genotypes under

aerobic and anaerobic conditions; (ii) to compare the recorded performance of four F_2 population under the same conditions; and (iii) to identify entries that warrant further replicated evaluation for early seedling establishment under oxygen-limited conditions.

Materials and Methods

1. Experimental plant materials

The experimental material consisted of ten rice entries. Six were designated as parental genotypes: ADT-36, ADT-43, IR-20, TRV-9, TRV-23 and TRV-26. Four entries were designated as F_2 population: TRV-9/ADT-43, ADT-36/TRV-23, TRV-26/ADT-36 and TRV-26/ADT-43. The entry names and recorded measurements were transcribed from the experiment. The source record specify the pedigree details, source institution, accession numbers, generation history or seed multiplication history; these should be supplied.

Material category	Entries included
Parents	ADT-36; ADT-43; IR-20; TRV-9; TRV-23; TRV-26
F_2 population	TRV-9/ADT-43; ADT-36/TRV-23; TRV-26/ADT-36; TRV-26/ADT-43

2. Aerobic and anaerobic growth conditions

The source record identifies two experimental treatments as “Aerobic Condition” and “Anaerobic Condition”. Photographs show labelled containers arranged under each treatment and a comparative display of uprooted seedlings. The supplied record does state the exact method used to create the anaerobic treatment. It is therefore possible to confirm from the available evidence whether the treatment represented complete submergence, waterlogging, stagnant water, deoxygenated water or another oxygen-restricted system. The final Methods section must describe the actual treatment used in the experiment rather than adopting.

For the aerobic treatment, the manuscript should specify the substrate or medium, irrigation regime, container size, sowing depth, and whether the substrate was maintained at field capacity or another moisture level. For the anaerobic treatment, the authors should state the water depth above the substrate, whether water was stagnant or renewed, whether the containers were sealed or open, and the number of days for which the treatment was maintained. Temperature, light intensity or photoperiod, and the experimental location should also be reported. These details are essential because oxygen availability and seedling development are influenced by water depth, temperature, seed condition and duration of exposure.

3. Seedling growth measurements

Two quantitative traits were included in the root length (cm) and shoot length (cm). Root length represents the recorded length of the root system, while shoot length represents the recorded above-ground seedling axis. The exact endpoints used for measurement, the number of seedlings sampled per entry, and the sampling date were supplied. The final manuscript should state whether root length was measured from the root–shoot junction to the tip of the longest root and whether shoot length was measured from the root–shoot junction to the tip of the longest leaf or coleoptile. These definitions should match the actual laboratory procedure.

4. Experimental design and statistical treatment

The one value per genotype or cross for each trait and condition. It does not identify the number of biological replications, the number of seedlings per replicate, or whether the values are individual measurements, means of several seedlings, or treatment means. In the absence of this information, the present manuscript reports the observations descriptively. Standard deviation, standard error, coefficient of variation, analysis of variance, heritability estimate, correlation coefficient or significance test has been calculated.

If replicate-level data are available, a factorial analysis can be considered with genotype or cross, growth condition and their interaction as fixed effects, and replication or block included according to the actual design. The distribution of residuals and homogeneity of variance should be checked before selecting the final model. Where multiple seedlings are measured within each replicate, the experimental unit must be defined correctly to avoid treating subsamples as independent replications. Mean separation should be conducted only when supported by the design and a statistically significant treatment effect.

4.1 Descriptive statistical formulas and interpretation

The following formulas were used to summarize variation among the recorded entry values. In these calculations, n represents the number of entries in the group (n = 6 for parental genotypes and n = 4 for F_2 population), x_i represents the recorded value for the ith entry, and $\bar{x}$ represents the arithmetic mean of the values.

4.1.1 Arithmetic mean ($\bar{x}$): $\bar{x} = \Sigma x_i / n$

The arithmetic mean is the sum of all recorded values divided by the number of entries. It represents the average recorded root or shoot length for the parental group or F_2-population group. It does not represent a replicated treatment mean.

4.1.2 Sample standard deviation (SD): $SD = \sqrt{[\Sigma (x_i - \bar{x})^2 / (n - 1)]}$

The sample standard deviation describes how far the individual entry values are dispersed around their group mean. A larger SD indicates greater absolute dispersion among the listed entries. Because the observations are entry-level values, this SD describes differences among entries and is not an estimate of within-entry experimental error.

4.1.3 Standard error of the across-entry mean (SE): $SE = SD / \sqrt{n}$

The standard error expresses the dispersion-based precision of the calculated mean across the entries included in that group. Here it is calculated from the across-entry SD and the number of entries. It must not be interpreted as the standard error from biological replicates or as an error bar for individual genotypes.

4.1.4 Coefficient of variation (CV): CV (%) = (SD / $\bar{x}$) × 100

The coefficient of variation expresses the SD relative to the mean as a percentage, allowing comparison of relative dispersion among traits with different means. A higher CV indicates greater relative variability among the recorded entries for that trait and group. In this manuscript, CV is an across-entry descriptive statistic.

4.1.5 Pearson correlation coefficient (r): $r = \Sigma [(x_i - \bar{x})(y_i - \bar{y})] / \sqrt{\{\Sigma (x_i - \bar{x})^2 \times \Sigma (y_i - \bar{y})^2\}}$

Pearson's r measures the direction and strength of the linear association between two variables. In this study, x represents root length and y represents shoot length measured for the same entries within a material group and condition. A positive r indicates that larger root values tend to occur with larger shoot values; a negative r indicates an inverse pattern. Values near zero indicate a weak linear association. These coefficients are exploratory because they are based on only six parental entries or four F_2 population; no statistical significance is inferred.

4.1.6 Range: Range = maximum recorded value − minimum recorded value

The range reports the difference between the largest and smallest recorded entry values. It describes the spread of the observed values but is sensitive to the two extreme observations.

5. Data presentation

The recorded values were organized into separate tables for parental genotypes and F_2 population. Comparative bar charts were prepared from the original values to visualize the difference between the two named conditions for root and shoot length. The charts are descriptive visualizations and do not contain error bars because replicate-level variation was available.

For the present dataset, descriptive means, sample standard deviations, standard errors and coefficients of variation were calculated across the six parental entries and, separately, across the four F_2 population for each trait and condition. Pearson correlation coefficients were calculated between root length and shoot length across entries within each material group and condition. These are across-entry descriptive statistics, not estimates based on biological replication. Because replicate-level observations and the experimental-unit structure were not supplied, inferential ANOVA, experimental error estimates and heritability components were not calculated.

Results

1. Root and shoot length of parental genotypes under aerobic conditions

The six parental genotypes showed a broad range of recorded seedling growth under the aerobic treatment. Root length varied from 6.4 to 12.0 cm. TRV-26 recorded the maximum root length (12.0 cm), followed by ADT-43 (11.1 cm) and TRV-9 (10.6 cm). ADT-36 recorded 8.5 cm, TRV-23 recorded 7.0 cm, and IR-20 had the minimum value of 6.4 cm. The range between the maximum and minimum recorded values was 5.6 cm.

Parental shoot length under aerobic conditions ranged from 13.9 to 23.9 cm. TRV-23 recorded the highest value (23.9 cm), closely followed by TRV-26 (23.5 cm). ADT-43 recorded 19.9 cm, TRV-9 recorded 17.3 cm and ADT-36 recorded 15.5 cm. IR-20 showed the lowest shoot length (13.9 cm). These observations indicate that the genotype with the greatest root length was not exactly the same as the genotype with the greatest shoot length.

Table 1: Recorded root and shoot length of parental rice genotypes under aerobic and anaerobic conditions.

Parental genotype	Aerobic root (cm)	Aerobic shoot (cm)	Anaerobic root (cm)	Anaerobic shoot (cm)
ADT-36	8.5	15.5	5.3	15.2
ADT-43	11.1	19.9	5.5	9.3
IR-20	6.4	13.9	6.4	13.7
TRV-9	10.6	17.3	8.5	9.4
TRV-23	7.0	23.9	6.5	13.5
TRV-26	12.0	23.5	1.0	5.3

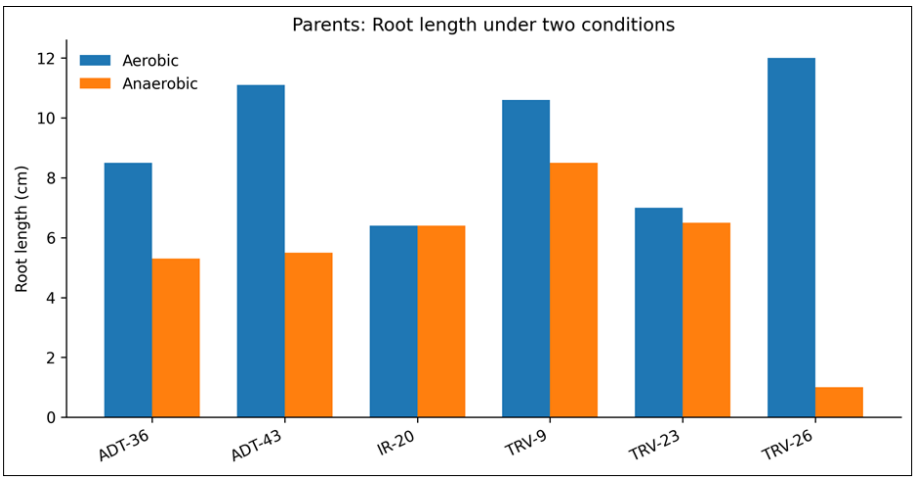


Fig 1: Comparison of root length in parental rice genotypes under aerobic and anaerobic conditions.

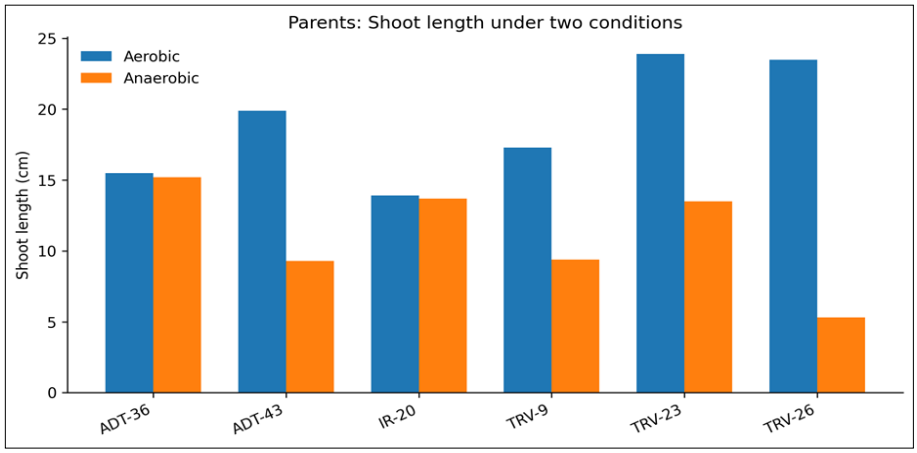


Fig 2: Comparison of shoot length in parental rice genotypes under aerobic and anaerobic conditions.

2. Root and shoot length of parental genotypes under anaerobic conditions

Under the treatment labelled anaerobic, parental root length varied from 1.0 to 8.5 cm. TRV-9 showed the highest recorded root length (8.5 cm), followed by TRV-23 (6.5 cm) and IR-20 (6.4 cm). ADT-43 and ADT-36 recorded 5.5 and 5.3 cm, respectively. TRV-26 had the lowest value (1.0 cm). This pattern contrasts with the aerobic observations, in which TRV-26 recorded the greatest parental root length.

Anaerobic shoot length ranged from 5.3 to 15.2 cm. ADT-36 recorded the maximum value (15.2 cm), followed by TRV-23 (13.5 cm) and IR-20 (13.7 cm). ADT-43 and TRV-9 recorded 9.3 and 9.4 cm, respectively, whereas TRV-26 recorded the minimum shoot length (5.3 cm). Thus, ADT-36 had the highest recorded shoot length under the anaerobic treatment, despite not having the highest value under aerobic conditions.

The contrast between conditions suggests that the relative performance of the parental entries was trait-dependent. TRV-9 showed the greatest root length under the anaerobic treatment, while ADT-36 showed the greatest shoot length. TRV-26, despite its strong aerobic root and shoot growth, had the lowest recorded root and shoot lengths under the anaerobic treatment. These are descriptive observations and should not be interpreted as proof of tolerance or susceptibility without replicated survival and germination measurements.

3. Performance of F_2 population under aerobic conditions

The four F_2 population also differed in their recorded growth under aerobic conditions. Root length ranged from 6.9 to 13.5 cm. TRV-26/ADT-43 recorded the highest root length (13.5 cm), followed by ADT-36/TRV-23 (13.0 cm). TRV-26/ADT-36 recorded 10.0 cm, while TRV-9/ADT-43 had the lowest value (6.9 cm). The two crosses involving TRV-26 as a parent did not show identical root-length values, indicating that the observed phenotype differed between the cross combinations.

Aerobic shoot length ranged from 19.3 to 22.3 cm. TRV-26/ADT-43 recorded the maximum value (22.3 cm), followed by ADT-36/TRV-23 (20.5 cm), TRV-26/ADT-36 (19.5 cm) and TRV-9/ADT-43 (19.3 cm). TRV-26/ADT-43 therefore had the highest recorded value for both root and shoot length among the four crosses under the aerobic treatment.

Table 2: Recorded root and shoot length of F_2 population under aerobic and anaerobic conditions.

F_2 population	Aerobic root (cm)	Aerobic shoot (cm)	Anaerobic root (cm)	Anaerobic shoot (cm)
TRV-9/ADT-43	6.9	19.3	3.4	10.4
ADT-36/TRV-23	13.0	20.5	6.4	5.0
TRV-26/ADT-36	10.0	19.5	1.5	8.2
TRV-26/ADT-43	13.5	22.3	2.2	4.8

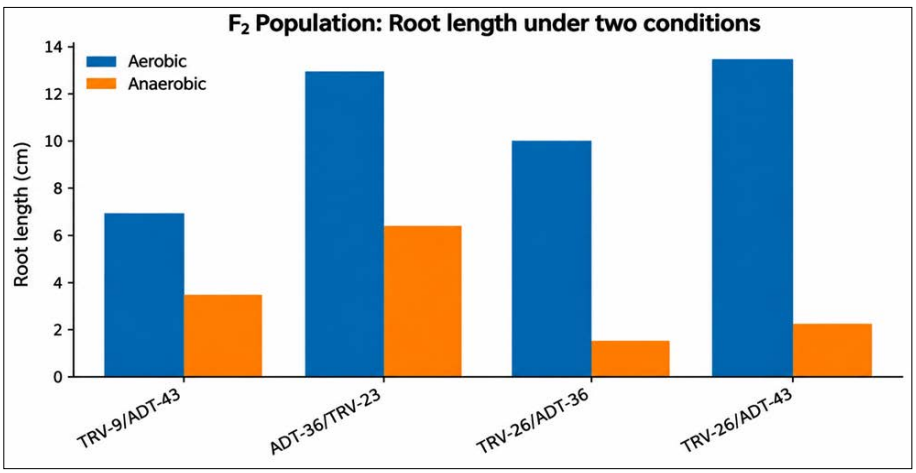


Fig 3: Comparison of root length in four F_2 population under aerobic and anaerobic conditions.

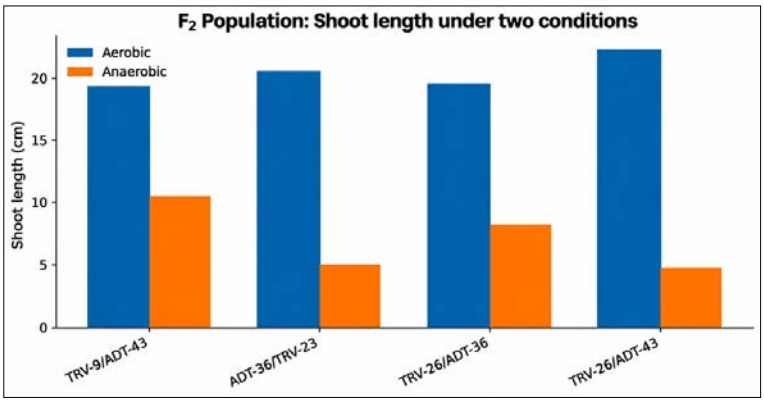


Fig 4: Comparison of shoot length in four F_2 population under aerobic and anaerobic conditions.

4. Performance of F_2 population under anaerobic conditions

Under the anaerobic treatment, root length among the F_2 *population* ranged from 1.5 to 6.4 cm. ADT-36/TRV-23 recorded the highest value (6.4 cm), followed by TRV-9/ADT-43 (3.4 cm), TRV-26/ADT-43 (2.2 cm) and TRV-26/ADT-36 (1.5 cm). The difference between the highest and lowest recorded values was 4.9 cm.

Anaerobic shoot length ranged from 4.8 to 10.4 cm. TRV-9/ADT-43 recorded the highest shoot length (10.4 cm), followed by TRV-26/ADT-36 (8.2 cm). ADT-36/TRV-23 and TRV-26/ADT-43 recorded 5.0 and 4.8 cm, respectively. Therefore, the cross with the highest root length was different from the cross with the highest shoot length under this treatment.

The results show that TRV-26/ADT-43, which recorded the highest aerobic root and shoot lengths, did not retain that ranking under the anaerobic treatment. ADT-36/TRV-23 showed the highest recorded anaerobic root length, whereas TRV-9/ADT-43 showed the highest recorded anaerobic shoot length. These cross-specific differences provide a basis for further testing, but the current dataset cannot establish whether the observed differences are reproducible or statistically significant.

5. Descriptive variability of seedling growth traits

Descriptive statistics were calculated across the entries within each material group to summarize the distribution of root and shoot length under the two growth conditions (Table 4). Among the six parental genotypes, anaerobic root length showed the highest across-entry coefficient of variation (45.07%), followed by anaerobic shoot length (33.59%). Among the four F_2 population, anaerobic root length also showed the highest coefficient of variation (64.12%), whereas aerobic shoot length showed the lowest value (6.72%). These patterns indicate that the listed entries were more dispersed for anaerobic root length than for the other measured traits. The statistics describe variation among entries and should not be interpreted as within-genotype experimental variability.

Table 3: Descriptive statistics for root and shoot length across parental genotypes and F_2 *population*.

Material	Trait / condition	N	Mean (cm)	SD*	SE*	CV (%)*	Range (cm)
Parents	Aerobic root	6	9.27	2.30	0.94	24.87	6.4–12.0
Parents	Aerobic shoot	6	19.00	4.15	1.70	21.86	13.9–23.9
Parents	Anaerobic root	6	5.53	2.49	1.02	45.07	1.0–8.5
Parents	Anaerobic shoot	6	11.07	3.72	1.52	33.59	5.3–15.2
F_2 population	Aerobic root	4	10.85	3.05	1.53	28.14	6.9–13.5
F_2 population	Aerobic shoot	4	20.40	1.37	0.69	6.72	19.3–22.3
F_2 population	Anaerobic root	4	3.38	2.16	1.08	64.12	1.5–6.4
F_2 population	Anaerobic shoot	4	7.10	2.70	1.35	37.97	4.8–10.4

*SD, SE and CV were calculated across genotype/cross entry values within each material group; they are not replicate-based experimental error estimates.

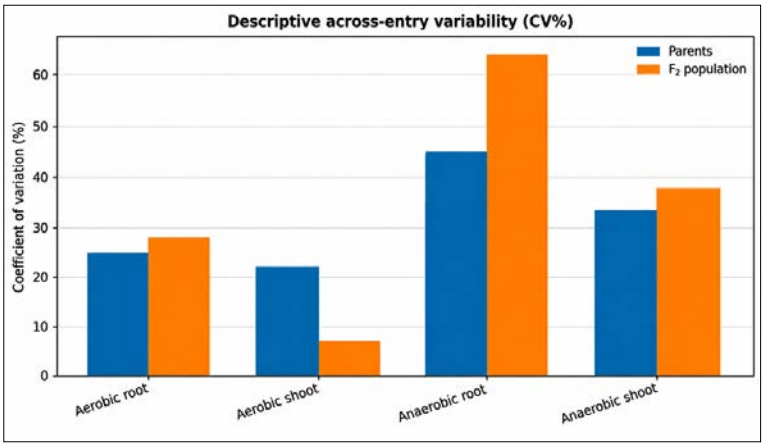


Fig 5: Comparison of across-entry coefficient of variation (CV %) for root and shoot length under aerobic and anaerobic conditions in parental genotypes and F_2 *population*.

Explanation of the descriptive statistical calculations

For example, parental anaerobic root length was recorded for six entries: 5.3, 5.5, 6.4, 8.5, 6.5 and 1.0 cm. The arithmetic mean is $\Sigma x_i/n$ = 33.2/6 = 5.53 cm. The sample SD, calculated using the deviations of the six values from this mean and dividing the sum of squared deviations by n − 1, is 2.49 cm. The SE is 2.49/√6 = 1.02 cm, and the CV is (2.49/5.53) × 100 = 45.07%. These values agree with the parental anaerobic-root row in Table 3.

The same procedure was applied separately to aerobic root length, aerobic shoot length, anaerobic shoot length and the corresponding four traits in the F_2-population group. The calculations were performed across entries within each group; therefore, they describe between-entry dispersion and should not be treated as replicate-based estimates of experimental variability.

6. Correlation between root length and shoot length

Pearson correlation coefficients were calculated descriptively between root length and shoot length across entries within each material group and condition (**Table 5**). In the parental group, root and shoot length were positively correlated under aerobic (r = 0.374) and anaerobic conditions (r = 0.538). In the F_2- *population* group, the association was positive under aerobic conditions (r = 0.825) and negative under anaerobic conditions (r = -0.322). These coefficients are exploratory because they are based on six parental entries and four F_2 *population*, respectively. They do not establish causation and no significance tests are reported.

Table 4: Descriptive Pearson correlation between root length and shoot length within each material group and growth condition.

Material group	Condition	n entries	Pearson r
Parents	Aerobic	6	0.374
Parents	Anaerobic	6	0.538
F_2 population	Aerobic	4	0.825
F_2 population	Anaerobic	4	-0.322

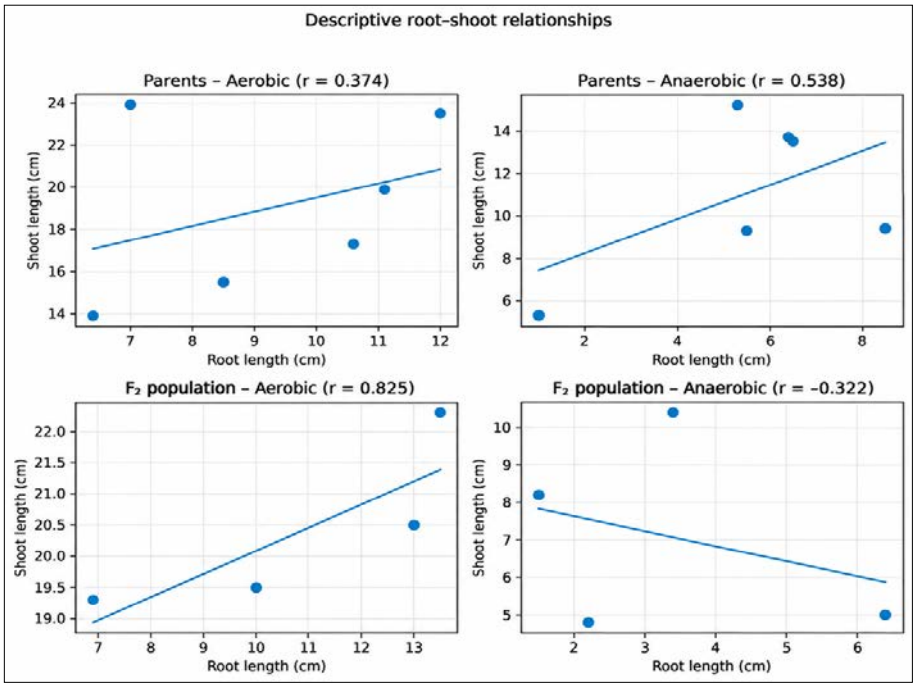


Fig 6: Scatter plots showing the descriptive relationship between root length and shoot length across parental genotypes and F_2 population under aerobic and anaerobic conditions.

Interpretation of the correlation coefficients

The Pearson correlation coefficient was calculated separately for each material group and growth condition using paired root- and shoot-length values from the same entries. For example, the parental aerobic coefficient (r = 0.374) indicates a weak-to-moderate positive linear association in this small set of six genotypes. The F_2-population aerobic coefficient (r = 0.825) indicates a stronger positive descriptive association among the four crosses, whereas the F_2- population anaerobic coefficient (r = −0.322) indicates a negative descriptive association. Because the sample sizes are small and replicate-level data are unavailable, these coefficients should not be interpreted as statistically significant relationships or causal effects.

7. Summary of the highest recorded values

Table 5: Entries with the maximum recorded root and shoot length for each treatment and material group.

Trait and condition	Highest recorded parent	Value (cm)	Highest recorded F_2 population	Value (cm)
Aerobic root length	TRV-26	12.0	TRV-26/ADT-43	13.5
Aerobic shoot length	TRV-23	23.9	TRV-26/ADT-43	22.3
Anaerobic root length	TRV-9	8.5	ADT-36/TRV-23	6.4
Anaerobic shoot length	ADT-36	15.2	TRV-9/ADT-43	10.4

8. Photographic observations

The photographs provide visual documentation of the experimental setup and the apparent differences in seedling growth. The comparative image displays containers labelled for aerobic and anaerobic treatments, while separate images show the entries under each condition. A further photograph presents uprooted seedlings with their root systems arranged for comparison. These images are included as original experimental documentation; they should not be treated as quantitative measurements. The photographs are most useful for illustrating the experimental arrangement and the visible variation among entries.



Fig 7: Comparative photograph showing the labelled aerobic and anaerobic experimental arrangements.

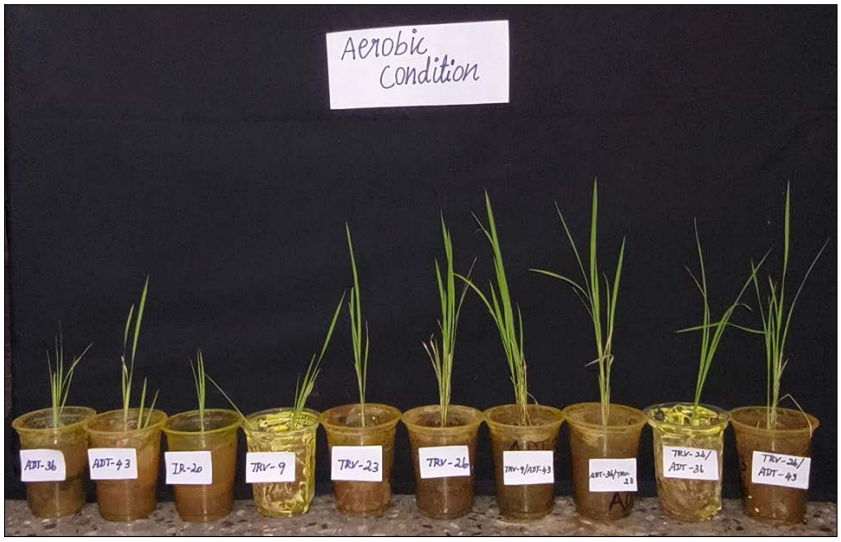


Fig 8: Photograph of rice entries maintained under the aerobic treatment.



Fig 9: Photograph of rice entries maintained under the anaerobic treatment.

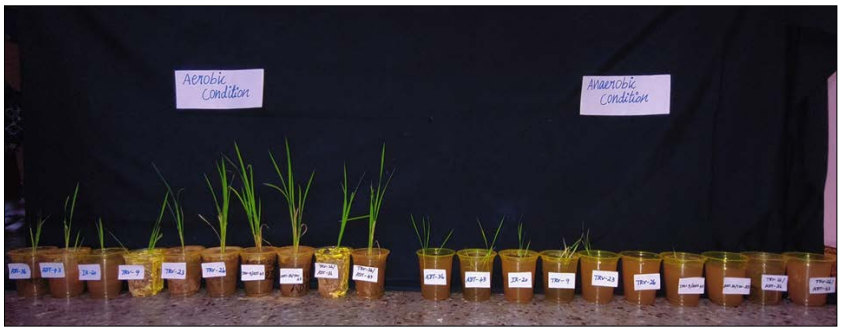


Fig 10: Comparative display of uprooted seedlings and root systems from the two treatments. Labels identify the entries as presented in the source photograph.

Discussion

1. Genotypic variation in seedling growth

The observations revealed variation in both root and shoot length among the six parental genotypes and four F_2 population. The range of values indicates that the tested entries did not respond uniformly across the two conditions. Such variation is relevant to rice improvement because early seedling growth under oxygen limitation is influenced by genetic background as well as environmental conditions. Previous studies have shown substantial genetic variation for anaerobic germination and seedling establishment in rice, and have used this variation to identify donor accessions for breeding (Kuya *et al.*, 2019; Mwakyusa *et al.*, 2023) [18, 23].

The present data suggest that the ranking of the genotypes was not stable between aerobic and anaerobic treatments. TRV-26 had the highest parental root length under aerobic conditions but the lowest root length under the anaerobic treatment. In contrast, TRV-9 recorded the greatest parental root length under the anaerobic treatment. This change in relative ranking illustrates why screening under favourable conditions alone is insufficient for selecting material intended for environments where early flooding or oxygen limitation may occur.

2. Interpretation of root growth under oxygen limitation

Root development is an important component of seedling establishment because roots contribute to water and nutrient acquisition and anchorage. Nevertheless, root growth can be restricted during the earliest stages of rice germination under severe oxygen deficiency. Pucciariello (2020) [25] reviewed evidence that rice germination under submergence can initially favour coleoptile elongation, while radicle and true-leaf development may be delayed. Therefore, a lower root length under an oxygen-limited treatment does not automatically indicate failure of anaerobic germination. The interpretation depends on when the measurement was taken and whether seedlings had emerged or recovered after the stress period.

In the present dataset, TRV-9 recorded the highest parental root length under the anaerobic treatment, and ADT-36/TRV-23 recorded the highest root length among the F_2 population. These entries may be prioritized for additional root-development observations. However, it is important to establish whether the measurements were taken during continuous submergence or after drainage/recovery. Root length after recovery can represent a different biological response from root initiation during complete submergence.

3. Interpretation of shoot growth and coleoptile response

Shoot elongation is often considered an important component of rice adaptation to submerged germination. Rice coleoptiles can elongate under low oxygen by using fermentable sugars derived from endosperm starch. This process helps the emerging shoot approach the water surface, where oxygen availability is greater. The biochemical basis involves starch mobilization, glycolysis and ethanolic fermentation, which help maintain ATP production when mitochondrial aerobic respiration is restricted (Magneschi and Perata, 2009; Pucciariello, 2020) [19, 25].

In this study, ADT-36 showed the highest parental shoot length under the anaerobic treatment, while TRV-9/ADT-43 had the highest shoot length among the F_2 population. These observations may be useful for identifying entries for further screening. However, the recorded trait is described in the source file as shoot length. The measurement specifically targeted the coleoptile, it should be called coleoptile length. Future experiments should measure coleoptile length separately from total shoot or seedling height, particularly during early submerged germination.

4. Relevance of parental and F_2 population comparisons

Comparing parental genotypes and their crosses can provide preliminary information about the phenotypic range represented in a breeding population. The highest recorded aerobic root and shoot lengths among the F_2 population were both observed in TRV-26/ADT-43. Under the anaerobic treatment, however, the highest root and shoot lengths were observed in two different crosses: ADT-36/TRV-23 for root length and TRV-9/ADT-43 for shoot length. This suggests that the cross combinations differ in the traits recorded under the two environments.

It would be premature to conclude that any one cross has superior combining ability, heterosis or transgressed segregation from the table. Such conclusions require appropriate parental and progeny comparisons, replicated data, generation information and a defined statistical framework. In addition, the four entries are identified as F_2 population in the source record; the result confirm whether these are individual F_2 plants, F_2 family means, or bulked populations. The distinction is important because segregation within an F_2 population can produce substantial plant-to-plant variation.

5. Comparison with published screening approaches

Kuya *et al.*, (2019) [18] evaluated anaerobic germination using deoxygenated water and coleoptile length, and compared the results with seedling survival in flooded soil. Their study reported a strong positive association between coleoptile elongation in anoxic water and survival under flooded soil. This provides support for using controlled low-oxygen assays as an initial screening tool, while also showing the value of validating laboratory measurements against survival under a soil-flooding system.

Mwakyusa *et al*., (2023) [23] used a broader phenotyping framework that included germination or emergence at defined days after sowing, seedling height, root length, culm diameter and dry matter. Their approach illustrates that anaerobic-germination tolerance is best characterized using multiple traits rather than relying on root and shoot length alone. The current study contains only root and shoot length values, and its experimental protocol is not sufficiently detailed to make a direct quantitative comparison with those published experiments.

Yu *et al.*, (2021) [35] emphasized that rice responses to oxygen deficiency involve interacting morphological, physiological and molecular mechanisms. The present observations contribute a preliminary phenotypic comparison of a small set of parental genotypes and F_2 population. Further work should combine morphological observations with germination percentage, emergence above water, survival, seedling biomass and, where possible, molecular markers associated with anaerobic germination.

6. Breeding implications and future validation

Based solely on the maximum recorded values in the supplied dataset, TRV-9 and ADT-36 are noteworthy parental entries for anaerobic root and shoot length, respectively. Among the F_2 population, ADT-36/TRV-23 and TRV-9/ADT-43 are noteworthy for anaerobic root and shoot length, respectively. These should be regarded as candidate entries for validation rather than confirmed donors of anaerobic-germination tolerance.

A robust follow-up study should include: (i) uniform, viable seeds with documented seed age and storage conditions; (ii) a standardized aerobic control and an explicitly defined anaerobic or submerged treatment; (iii) adequate biological replication and randomized experimental units; (iv) observations at predetermined days after sowing; (v) germination and emergence percentages; (vi) survival after a defined stress period; (vii) coleoptile length, total shoot length and root length recorded separately; and (viii) fresh and dry biomass where feasible. A subsequent field or screenhouse validation under controlled flooding would help establish whether the laboratory response predicts seedling establishment.

7. Interpretation of descriptive variability and correlation

The across-entry statistics complement the genotype-wise comparisons by indicating how widely the measured traits were distributed within the parental and F_2- population groups. The relatively high CV for anaerobic root length, particularly among the F_2 population, reflects marked differences among the four recorded cross values. The positive parental root–shoot correlations indicate that entries with longer roots tended also to have longer shoots in the observed dataset, while the negative F_2- population. correlation under anaerobic conditions suggests that the ranking of crosses for root length did not parallel their ranking for shoot length. Because these calculations use a small number of entry-level observations and lack replicate-level data, they should be treated as descriptive patterns rather than statistically established genetic relationships.

The requested analysis of variance and heritability estimates could not be obtained from the available manuscript dataset. ANOVA requires a valid estimate of experimental error from independent replications and a clearly specified design. Broad-sense heritability requires suitable estimates of genotypic and environmental variance components, while narrow-sense heritability requires information on additive genetic variance. These parameters should be estimated in a follow-up replicated experiment rather than inferred from the current single-value-per-entry table.

Statistical interpretation and limitations

The descriptive statistical formulas provide a consistent way to summarize the distribution of the available entry values. However, the calculated SD, SE and CV are based on differences among genotypes or crosses, not on repeated measurements within each genotype or cross. Consequently, they cannot be used as estimates of experimental error for hypothesis testing. An analysis of variance would require the original replicate-level observations and a documented randomized design. Broad-sense heritability would require estimates of genotypic and environmental variance components from an appropriate replicated experiment, while narrow-sense heritability would additionally require information about additive genetic variance. These parameters have therefore not been calculated or imputed.

Conclusion

The observations demonstrate variation in root and shoot length among six parental rice genotypes and four F_2 population grown under conditions identified as aerobic and anaerobic. Under aerobic conditions, TRV-26 recorded the highest parental root length (12.0 cm), while TRV-23 recorded the highest parental shoot length (23.9 cm). Among the F_2 population, TRV-26/ADT-43 recorded the highest aerobic root and shoot lengths (13.5 and 22.3 cm, respectively). Under the anaerobic treatment, TRV-9 recorded the highest parental root length (8.5 cm), while ADT-36 recorded the highest parental shoot length (15.2 cm). Among the F_2 population, ADT-36/TRV-23 recorded the highest root length (6.4 cm), and TRV-9/ADT-43 recorded the highest shoot length (10.4 cm). These entries merit further replicated evaluation. Because the available dataset lacks replication information, treatment details and survival measurements, the findings should be interpreted as preliminary descriptive evidence rather than statistically validated anaerobic-germination tolerance.

References

1. Alpi A, Beevers H. Effects of O_2 concentration on rice seedlings. Plant Physiology,1983:71:30–34.
2. Angaji SA, Septiningsih EM, Mackill DJ, Ismail AM. QTLs associated with tolerance of flooding during germination in rice (*Oryza sativa* L.). Euphytica,2010:172:159–168.
3. Anandan A, Mahender A, Sah RP, Bose LK, Subudhi H, Meher J, et al. Non-destructive phenotyping for early seedling vigor in direct-seeded rice. Plant

Methods,2020:16:127. https://doi.org/10.1186/s13007-020-00666-6
4. Illangakoon TK, Marambe B, Keerthisena RSK, Kumar V. Importance of anaerobic seed germination and seedling development in direct-seeded rice with special reference to Sri Lanka. Sri Lanka Journal of Food and Agriculture,2019:5(2):47–57. https://doi.org/10.4038/sljfa.v5i2.76
5. Bailey-Serres J, Fukao T, Ronald P, Ismail A, Heuer S, Mackill D. Submergence tolerant rice: SUB1's journey from landrace to modern cultivar. Rice,2010:3:138–147. https://doi.org/10.1007/s12284-010-9048-5
6. Baltazar MD, Ignacio JCI, Thomson MJ, Ismail AM, Mendioro MS, Septiningsih EM. QTL mapping for tolerance to anaerobic germination in rice from IR64 and the aus landrace Kharsu 80A. Breeding Science,2019:69(2):227–233. https://doi.org/10.1270/jsbbs.18159
7. Colmer TD, Pedersen O. Oxygen dynamics in submerged rice (*Oryza sativa*). New Phytologist,2008:178:326–334.
8. Dey D, Sah RP, Beena R, Dunna V, Sahu AK, Pandit M, et al. Early seedling vigor for direct-seeded rice: traits, mechanisms, and improvement strategies—a review. Plant Growth Regulation,2026:106:19. https://doi.org/10.1007/s10725-026-01429-4
9. Farooq M, Siddique KHM, Rehman H, Aziz T, Lee DJ, Wahid A. Rice direct seeding: experiences, challenges and opportunities. Soil and Tillage Research,2011:111:87–98. https://doi.org/10.1016/j.still.2010.10.008
10. Ghosal S, Casal C Jr, Quilloy FA, Septiningsih EM, Mendioro MS, Dixit S. Deciphering genetics underlying stable anaerobic germination in rice: phenotyping, QTL identification, and interaction analysis. Rice,2019:12:50. https://doi.org/10.1186/s12284-019-0305-y
11. Ghosal S, Quilloy FA, Casal C Jr, Septiningsih EM, Mendioro MS, Dixit S. Trait-based mapping to identify the genetic factors underlying anaerobic germination of rice: phenotyping, G×E, and QTL mapping. BMC Genetics,2020:21:6. https://doi.org/10.1186/s12863-020-0808-y
12. Hsu SK, Tung CW. Genetic mapping of anaerobic germination-associated QTLs controlling coleoptile elongation in rice. Rice,2015:8:38. https://doi.org/10.1186/s12284-015-0072-3
13. Vinitha A, Vijayalakshmi D, Raveendran M, Ravichandran V, Parthipan T. Identifying donors for anaerobic germination tolerance and direct seeded rice cultivation by exploring seed and seedling traits. Agricultural Science Digest,2024:44(2):333–339. https://doi.org/10.18805/ag.D-5848
14. Ismail AM, Singh US, Singh S, Dar MH, Mackill DJ. The contribution of submergence-tolerant (Sub1) rice varieties to food security in flood-prone rainfed lowland areas in Asia. Field Crops Research,2013:152:83–93. https://doi.org/10.1016/j.fcr.2013.01.007
15. Jackson MB, Ram PC. Physiological and molecular basis of susceptibility and tolerance of rice plants to complete submergence. Annals of Botany,2003:91:227–241.
16. Kretzschmar T, Pelayo MAF, Trijatmiko KR, et al. A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. Nature Plants,2015:1:15124. https://doi.org/10.1038/nplants.2015.124
17. Kumar V, Ladha JK. Direct seeding of rice: recent developments and future research needs. Advances in Agronomy,2011:111:297–413. https://doi.org/10.1016/B978-0-12-387689-8.00001-1
18. Kuya N, Sun J, Iijima K, Venuprasad R, Yamamoto T. Novel method for evaluation of anaerobic germination in rice and its application to diverse genetic collections. Breeding Science,2019:69(4):633–639. https://doi.org/10.1270/jsbbs.19003
19. Magneschi L, Perata P. Rice germination and seedling growth in the absence of oxygen. Annals of Botany,2009:103(2):181–196. https://doi.org/10.1093/aob/mcn121
20. Mahender A, Anandan A, Pradhan SK. Early seedling vigour, an imperative trait for direct-seeded rice: an overview on physio-morphological parameters and molecular markers. Planta,2015:241:1027–1050. https://doi.org/10.1007/s00425-015-2273-9
21. Menard G, Sandhu N, Anderson D, Catolos M, Hassall KL, Eastmond PJ, et al. Laboratory phenomics predicts field performance and identifies superior indica haplotypes for early seedling vigour in dry direct-seeded rice. Genomics,2021:113(6):4227–4236. https://doi.org/10.1016/j.ygeno.2021.11.006
22. Miro B, Ismail AM. Tolerance of anaerobic conditions caused by flooding during germination and early growth in rice (*Oryza sativa* L.). Frontiers in Plant Science,2013:4:269. https://doi.org/10.3389/fpls.2013.00269
23. Mwakyusa LM, Heredia MC, Kilasi NL, Madege RR, Herzog M, Dixit S. Screening of potential donors for anaerobic stress tolerance during germination in rice. Frontiers in Plant Science,2023:14:1261101. https://doi.org/10.3389/fpls.2023.1261101
24. Perata P, Pozueta-Romero J, Akazawa T, Yamaguchi J. Effect of anoxia on starch breakdown in rice and wheat seeds. Planta,1992:188:611–618. https://doi.org/10.1007/BF00197056
25. Pucciariello C. Molecular mechanisms supporting rice germination and coleoptile elongation under low oxygen. Plants,2020:9(8):1037. https://doi.org/10.3390/plants9081037
26. Rathod R, Dinesh A, Sreedhar M, Charan SM, Basavaraj PS, Vanisri S. Genetic assessment of germplasms for anaerobic germination in rice. Asian Journal of Soil Science and Plant Nutrition,2024:10(1):272–285. https://doi.org/10.9734/ajsspn/2024/v10i1233
27. Sandhu N, Torres RO, Cruz MTS, Maturan PC, Jain R, Kumar A, et al. Traits and QTLs for development of dry direct-seeded rainfed rice varieties. Journal of Experimental Botany,2015:66(1):225–244. https://doi.org/10.1093/jxb/eru413
28. Sandhu N, Subedi SR, Singh VK, Sinha P, Kumar S, Singh SP, et al. Deciphering the genetic basis of root morphology, nutrient uptake, yield, and yield-related traits in rice under dry direct-seeded cultivation systems. Scientific Reports,2019:9:137. https://doi.org/10.1038/s41598-019-45770-3
29. Sandhu N, Yadav S, Catolos M, Cruz MTS, Kumar A. Developing climate-resilient, direct-seeded, adapted

multiple-stress-tolerant rice applying genomics-assisted breeding. Frontiers in Plant Science,2021:12:637488. https://doi.org/10.3389/fpls.2021.637488
30. Lee KW, Chen JJW, et al. Auxin plays a role in the adaptation of rice to anaerobic germination and seedling establishment. Plant, Cell & Environment,2023:46(4):1157–1175. https://doi.org/10.1111/pce.14434
31. Saraswathi R, Suresh R, Renganayaki R, Sritharan PNBSK. Characterization of rice (*Oryza sativa* L.) germplasm for early seedling vigour-related traits under direct seeded condition. International Journal of Plant & Soil Science,2023:35(19):2106–2114. https://doi.org/10.9734/ijpss/2023/v35i193762
32. Septiningsih EM, Ignacio JCI, Sendon PMD, Sanchez DL, Ismail AM, Mackill DJ. QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan Red. Theoretical and Applied Genetics,2013:126:1357–1366. https://doi.org/10.1007/s00122-013-2057-1
33. Sudeepthi K, Srinivas T, Ravi Kumar BN VSR, Jyothula DPB, Umar SKN. Genetic variability, character association and path analysis for anaerobic germination traits in rice (*Oryza sativa* L.). Journal of Pharmacognosy and Phytochemistry,2020:9(1):553–556. https://doi.org/10.22271/phyto.2020.v9.i1i.10489
34. Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, et al. Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature,2006:442:705–708. https://doi.org/10.1038/nature04920
35. Yu SM, Lee HT, Lo SF, Ho TD. How does rice cope with too little oxygen during its early life? New Phytologist,2021:229(1):36–41. https://doi.org/10.1111/nph.16395