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Screening and Evaluation of Indica Rice (*Oryza sativa L.*) Landraces for Anaerobic Germination and Early Seedling Growth under Hypoxic Conditions

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Abstract

Rice (*Oryza sativa L.*) is the primary food crop for more than half of the global population and is cultivated extensively under diverse agro ecological conditions. In eastern India, particularly in Odisha, frequent and unpredictable flooding events during the early stages of crop establishment severely constrain direct seeded rice (DSR) cultivation. Germination under low oxygen (hypoxic) or fully submerged (anoxic) conditions is a critical adaptive trait for ensuring crop survival and yield stability in such ecosystems. The present study evaluated 10 rice genotypes, including traditional landraces, released varieties, and basmati types, for their ability to germinate and establish seedlings under hypoxic conditions. Screening was performed using a 10 cm water column in test tubes and earthen pots. Among all genotypes tested, only ten exhibited more than 50% germination under submerged conditions. Notably, the landraces Parbatjeera and Kalikati recorded over 70% germination and vigorous coleoptile elongation, indicating strong anaerobic germination (AG) tolerance. These genotypes demonstrated the ability to extend coleoptile growth beyond 1 cm, allowing rapid emergence through the water surface and early establishment. The findings reveal considerable genetic variation in AG tolerance among traditional landraces from the Jeypore tract of Odisha, a region known as a secondary center of rice diversity. The identified tolerant genotypes represent valuable genetic resources for breeding rice cultivars resilient to flooding during germination and early seedling stages, thereby supporting climate resilient and sustainable DSR production systems.

Keywords: Anaerobic Germination Tolerance; Rice Landraces Under Submergence; Direct Seeded Rice (DSR); Flood Tolerant Rice Genotypes; Hypoxia Stress in Rice

Introduction

Seed germination marks the transition from dormancy to the onset of active metabolism and growth a critical phase determining plant establishment and productivity. In rice (*Oryza sativa* L.), this process often occurs in environments where oxygen availability fluctuates dramatically, such as in flooded soils or waterlogged fields. Under normal aerobic

conditions, oxygen supports efficient energy production through oxidative phosphorylation. However, when rice seeds are submerged, oxygen diffusion is limited, forcing the embryo to switch to alternative metabolic pathways to sustain growth and survival.

Aerobic Germination: Energy Efficiency and Oxidative Metabolism

In the presence of oxygen, rice seeds undergo a tightly coordinated metabolic sequence starting with water imbibition, enzyme activation, and mobilization of starch reserves stored in the endosperm. Oxygen acts as the final electron acceptor in the mitochondrial electron transport chain, enabling aerobic respiration to generate adenosine triphosphate (ATP) efficiently via the tricarboxylic acid (TCA) cycle and oxidative phosphorylation [1]. During this phase, enzymes such as α amylase hydrolyze starch into maltose and glucose, which feed into glycolysis, producing pyruvate and NADH. Pyruvate is then oxidized in the TCA cycle to yield CO_2, NADH, and $FADH_2$, whose electrons drive ATP production in mitochondria. This high energy yield approximately 36 ATP per glucose molecule fuels rapid cell division, root protrusion, and shoot development [2]. Aerobic germination also involves the activation of reactive oxygen species (ROS) as signaling molecules that regulate hormonal balance, particularly gibberellins (GA) and abscisic acid (ABA), which together control seed dormancy and early growth. Under well aerated conditions, rice seeds maintain low internal ethylene concentrations, allowing coordinated root and coleoptile growth [3].

Anaerobic and Hypoxic Conditions: The Energy Crisis

When rice fields experience flooding immediately after seeding as in direct seeded rice (DSR) systems — oxygen levels around the seeds drop sharply. The diffusion of oxygen in water is 10,000 times slower than in air, leading to hypoxia (low oxygen) or anoxia (complete absence) in the soil microenvironment. This oxygen shortage disrupts oxidative respiration, halting the mitochondrial electron transport chain and drastically reducing ATP generation. Without sufficient oxygen, pyruvate from glycolysis cannot enter the TCA cycle and is instead redirected into fermentative metabolism, yielding ethanol and lactate through the coordinated action of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) enzymes. Although anaerobic fermentation provides only 2 ATP per glucose molecule far less than aerobic respiration this minimal energy output sustains essential cellular processes long enough for survival. The rice seed's ability to survive and grow under such conditions is an evolutionary adaptation rarely found in other cereals. Most crop seeds, such as maize or wheat, fail to germinate under anoxia due to rapid energy depletion and toxic metabolite accumulation [2]. In contrast, rice maintains metabolic flexibility through efficient carbohydrate mobilization and the activation of anaerobic responsive genes that modulate energy production and cell wall expansion [4].

Molecular Mechanisms Under Oxygen Deficiency

The shift from aerobic to anaerobic metabolism involves an extensive reprogramming of gene expression and enzyme activity. Transcriptome analyses have revealed upregulation of genes encoding glycolytic enzymes, PDC, ADH, and sucrose synthase, which together maintain glycolytic flux and ensure ATP production under anoxia [1,4]. Rice also triggers the expansin (EXPA7 and EXPB12) gene families, which loosen the cell wall, enabling coleoptile elongation a vital escape mechanism that allows seedlings to reach the water surface and access oxygen. This rapid elongation is fueled by stored carbohydrates and supported by hormonal signaling involving ethylene and gibberellin, which stimulate cell expansion even under submergence [5]. Metabolically, tolerant rice genotypes accumulate higher levels of soluble sugars, disaccharides, and fatty acid derivatives, which stabilize membranes and support cell viability during hypoxia. In contrast, sensitive genotypes accumulate lactate and organic acids, causing cytosolic acidification and reduced growth [6].

Coleoptile Elongation: The Key to Survival

The coleoptile, a protective sheath covering the emerging shoot, plays a central role in rice seedling survival under water. In the absence of oxygen, rice coleoptiles elongate faster than roots, functioning as a snorkel like structure that transports oxygen from the water surface to submerged tissues once it emerges [7]. This elongation depends on efficient starch degradation, glycolytic energy production, and cell wall loosening mediated by expansins. High α amylase activity releases sugars from starch reserves, while sucrose synthase converts sucrose into glucose and fructose, providing substrates for fermentation [8]. These biochemical reactions enable coleoptile cells to maintain turgor and elongate even when oxidative respiration is inhibited. Recent genomic analyses have identified several quantitative trait loci (QTLs) and genes regulating this process. For example, OsVP1, OsGA2ox8, and OsDi19 1 influence gibberellin and abscisic acid balance under oxygen deficiency, promoting elongation while preventing premature energy exhaustion. Moreover, the OsDREB6 gene acts as a transcriptional regulator of sugar and starch metabolism during anaerobic germination [9]. These findings highlight the coordinated control between hormonal signaling and metabolic adaptation in rice's anaerobic germination tolerance.

Seed Energy Balance and Recovery

Even under extreme oxygen shortage, some rice varieties demonstrate remarkable resilience by maintaining metabolic quiescence slowing down respiration and conserving carbohydrates until oxygen becomes available. This "pause and recover" strategy ensures high survival and seedling vigor after reoxygenation [10].

Upon re aeration, seeds rapidly restore mitochondrial activity, resume the TCA cycle, and metabolize accumulated ethanol and lactate. The reactivation of oxidative enzymes allows continued seedling development and establishment. The genetic basis for this recovery capacity lies in efficient redox regulation and activation of antioxidant systems, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which prevent oxidative damage during the oxygen transition phase [3].

Physiological and Genetic Implications

Understanding rice seed metabolism under oxygen stress is critical for breeding flood tolerant and direct seeded rice cultivars. With climate change causing erratic rainfall and frequent flooding, the demand for rice varieties capable of anaerobic germination (AG) has surged. Recent genome wide association studies (GWAS) and QTL mapping have pinpointed multiple loci associated with AG tolerance, particularly those influencing coleoptile length, sugar metabolism, and cell wall dynamics.

The combination of *SUB1A* (submergence tolerance at vegetative stage) and AG related QTLs enables varieties to withstand both early stage flooding and complete submergence later in growth, offering multi stage flood resilience [11]. In essence, the germination behavior of rice under contrasting oxygen environments illustrates its unique physiological plasticity. While aerobic germination relies on efficient oxidative phosphorylation for energy supply, anaerobic germination depends on fermentation driven ATP generation, sugar metabolism, and coleoptile elongation to sustain life without oxygen.

These adaptive mechanisms governed by intricate molecular networks and hormonal controls underpin rice's capacity to survive flooding during germination.Understanding these mechanisms provides vital insight for developing climate resilient, direct seeded rice systems, ensuring food security in flood prone regions.

The *indica* sub-species is considered to be less responsive and recalcitrant to tissue culture compared to *japonica* lines [12]. Genotypes within the *indica* sub-species show different response to *in vitro* culture [13].

Reduced growth and necrosis in some rice genotypes during callus culture is mainly due to the accumulation of ethylene and to some extent carbon dioxide in the culture medium. This causes depletion of oxygen in the nutrient medium as ethylene is found to restrict callus growth [14]. The genotypes which show high callus induction frequency when inoculated in the culture medium are able to tolerate high concentrations of ethylene. The response towards tissue culture is genotype dependent, same in the case of genotypes which possess certain QTLs associated with tolerance to low oxygen stress and can germinate under high concentrations of ethylene [15,16].

The landraces belonging to the *aus* group of *indica* sub-species which has evolved in the hilly terrains can tolerate many biotic and abiotic stresses. Experiments were conducted to identify the capacity of promising landraces to germinate and grow under low oxygen conditions. Our study was focused to relate the capacity of a genotype to germinate under anaerobic (hypoxic) conditions and subsequent seedling development with its callus induction frequency (CIF) and shoot regeneration efficiency (SRE) respectively.

Materials and Methods

Plant Materials and Genotype Selection

The research work was conducted in the tissue culture laboratory, ICAR-National Rice Research Institute, Cuttack and Regional Research and Technology Transfer Station (RRTTS), Bhawanipatna (OUAT).

Ten rice genotypes including nine local landraces and one released variety of Odisha obtained from RRTTS, Bhawanipatna, were inoculated in culture media, using mature seed embryos as explants. This study was conducted to evaluate the anaerobic germination (AG) tolerance and tissue culture response of selected *Oryza sativa L.* landraces and varieties from Odisha, India. A total of 10 rice genotypes, released varieties, and traditional landraces, were obtained from the Regional Research and Technology Transfer Station (RRTTS), Bhawanipatna, Kalahandi, Odisha.

Initial screening for submergence tolerance identified ten promising genotypes (Tables 1-5). These were subsequently used for detailed analysis of hypoxic germination, seedling development, and in vitro tissue culture response through callus induction and shoot regeneration.

Sl. No	Genotype	Type	Origin	Duration (Days)	Average yield/plant (g)
1	Basumati	Landrace	Kandhamal	138	10.21
2	Kalikati	Landrace	Kalahandi	134	12.88
3	Parijat	Landrace	Kalahandi	141	9.27
4	Kanakachampa	Landrace	Bolangir	140	9.83
5	Jeerakasal	Landrace	Wayanad	137	10.02
6	Karpurajeera	Landrace	Koraput	134	9.15
7	Parbatjeera	Landrace	Kalahandi	138	8.89
8	Gangabali	Landrace	Kandhamal	146	12.48
9	Dhanaprasad	Landrace	Koraput	133	14.09
10	CR Dhan 907	Released variety	Cuttack	145	25.16

Table 1: Rice genotypes used in the experiment.

All seeds were cleaned, surface sterilized, and stored at ambient conditions (28 ± 2 °C, 12% seed moisture) before experimentation.

Experimental Design

The entire study consisted of **three distinct experimental modules**, each addressing specific physiological and developmental responses under controlled conditions:
Hypoxic germination and seedling development
Callus induction under in vitro conditions
Shoot regeneration and plantlet formation
All experiments were conducted in **Completely Randomized Block Design (CRBD)** with four replications per genotype to ensure statistical reliability.

Hypoxic Germination and Seedling Development

Experimental Setup

To simulate anaerobic germination conditions, ten seeds per genotype were placed in 12 cm long test tubes, each filled with 15 mL of dechlorinated tap water (representing a 10 cm standing water column). The tubes were maintained in a vertical position for 10 days at 30 ± 2°C under a 12 hour photoperiod.

Water levels were monitored daily to maintain consistent hypoxia. The oxygen concentration in the water column ranged between 0.8–1.2 mg L^{-1}, representing typical hypoxic conditions in flooded fields [18].

Data Collection

After 10 days of incubation:
Germination (%) was calculated as:

A seed was considered germinated when coleoptile emergence exceeded 2 mm.
Following 21 days, water was drained, and the partially germinated seeds were transferred to Petri dishes lined with moist filter paper to evaluate subsequent seedling growth.

Seedling Growth and Survival

Seedling performance was assessed on the 3rd and 7th days after transfer, with observations including:
Root length (cm)
Shoot length (cm)
Seedling survival (%)

The **seedling development (%)** was computed as:
These parameters quantified the genotypes' ability to sustain post anoxic growth once oxygen was reintroduced, a key indicator of physiological resilience.

Pot Experiment under Submergence

To validate laboratory findings under semi natural conditions, 25 dry seeds of each genotype were directly sown in earthen pots (30 cm height × 25 cm diameter) lined with plastic grow bags. Each pot contained 15 cm of finely ground field soil (pH 6.8, organic carbon 0.65%) saturated with water 24 hours before sowing.

After sowing, pots were flooded to maintain a 10 cm standing water layer for three weeks, mimicking field level flooding.

Observations recorded:
Seedling emergence rate (%)
Coleoptile length (cm)
Seedling survival (%) at 21 DAS.
Seedling emergence was calculated as:

Genotypes were classified as:
Highly tolerant: >70% emergence and coleoptile length >1.5 cm
Moderately tolerant: 50–70% emergence
Susceptible: <50% emergence

This two stage (lab + pot) method reliably identified Parbatjeera and Kalikati as high performing genotypes under flooding stress.

Callus Induction Studies

Objective

To assess in vitro regenerative potential, callus induction was studied for all ten selected genotypes. This experiment aimed to determine their tissue culture responsiveness, essential for future molecular breeding and transformation programs.

Basal Media Preparation

Callus induction medium was prepared using Murashige and Skoog (MS) basal salts supplemented with the following hormones:
2,4 Dichlorophenoxyacetic acid (2,4 D): 2.0 mg L^{-1}
Naphthaleneacetic acid (NAA): 0.5 mg L^{-1}
6 Benzylaminopurine (BAP): 0.25 mg L^{-1}
Kinetin (Kn): 0.1 mg L^{-1}
Sucrose: 3% (w/v)
Agar: 0.8% (w/v)
Media pH was adjusted to 5.8 ± 0.1, followed by autoclaving at 121 °C, 15 psi for 20 minutes [19].

Inoculation and Culture Conditions

Approximately 12–15 surface sterilized seeds were inoculated per sterile Petri plate (50 mL medium per plate).

A total of 100 seeds per genotype were tested, with each treatment replicated three times.

Plates were incubated at 25 ± 2°C in complete darkness to promote callus formation.

Callus Induction Frequency (CIF%)

After 4 weeks, callus formation was quantified as:
Calli were classified based on color and texture:
Friable yellow calli: indicative of embryogenic potential
Compact white calli: non embryogenic
Genotypes exhibiting high CIF% and friable calli morphology were considered tissue culture responsive, suitable for regeneration and transformation studies.

Shoot Regeneration and Plantlet Formation

Media Composition

For shoot regeneration, MS medium was modified as follows:
BAP (6 Benzylaminopurine): 2.0 mg L^{-1}
Kinetin: 0.25 mg L^{-1}
NAA: 0.1 mg L^{-1}
Sucrose: 3%
Agar: 0.8%
This hormonal combination promotes cytokinin auxin balance, favoring shoot initiation from embryogenic calli [19].
Prepared medium (25 mL) was poured into autoclaved culture tubes, plugged with cotton, and solidified at a 45° angle to increase the culture surface.

Inoculation Procedure

Calli (2–4 mm diameter) obtained from the previous step were placed (2–4 per tube) on the regeneration medium.
Each genotype was represented by 50 calli, with three replicates per treatment.
Cultures were maintained at 25 ± 2°C, under a 12 hour photoperiod (3000 lux light intensity).

Shoot Regeneration Efficiency (SRE%)

After **3–4 weeks**, regenerated shoots were counted, and SRE was computed as:

Shoot morphology (number, height, color) was recorded. Regenerated shoots were later transferred to **half strength MS medium without hormones** for root induction.

Statistical Analysis

All experiments followed a completely randomized design (CRD) with three or four replications. Data for CIF%, SRE%, and germination traits were arcsine transformed prior to analysis. Analysis of variance (ANOVA) was performed using R (v4.2.2) and SPSS (v25). The Least Significant Difference (LSD) test at $p \leq 0.05$ was used to determine statistically significant differences among genotypes [20]. Results were expressed as mean ± standard error (SE). Graphical data visualization was done in Microsoft Excel 2023.

Environmental Monitoring and Quality Control

Throughout experiments:
Temperature: 25–30°C (monitored daily)
Dissolved Oxygen: 0.8–1.2 mg L^{-1} during hypoxic tests
pH: Maintained between 6.8–7.2
Light intensity: 3000 lux during regeneration
All media and instruments were sterilized to maintain aseptic conditions. Random plates were periodically checked for contamination.

Validation of Results

Positive and negative control genotypes were included to validate experimental conditions:
Tolerant check: *Khao Hlan On* (Myanmar), known AG tolerant genotype.
Susceptible check: *IR64*, standard lowland rice cultivar with poor AG tolerance.
Control responses confirmed the validity of stress conditions and experimental reproducibility.

Ethical and Biosafety Considerations

All experiments adhered to institutional biosafety and ethical guidelines. The study utilized **non transgenic rice genotypes**, ensuring compliance with the **Biosafety Guidelines for Plant Research (ICAR, 2020)**. All waste culture materials were autoclaved before disposal.

Experimental Phase	Objective	Medium/Condition	Measured Parameter	Duration
Hypoxic germination	Assess tolerance to oxygen deficiency	10 cm standing water in test tubes	Germination %, coleoptile length	10 days
Pot submergence	Validate field level emergence	10 cm flooded soil (3 weeks)	Seedling emergence %, survival	21 days
Callus induction	Evaluate embryogenic response	MS + 2.0 mg L^{-1} 2,4 D + 0.5 mg L^{-1} NAA + hormones	CIF%	4 weeks
Shoot regeneration	Assess regeneration ability	MS + 2.0 mg L^{-1} BAP + 0.25 mg L^{-1} Kinetin + 0.1 mg L^{-1} NAA	SRE%	3–4 weeks

Table 2: Summary of Methodological Workflow.

Results and Discussion

Callus Induction Frequency (CIF) among the Ten Genotypes

Callus induction frequency (CIF) reflects the ability of rice embryos to dedifferentiate and form totipotent callus tissues under in vitro stress conditions. This response often varies across genotypes due to differences in hormonal sensitivity, carbohydrate metabolism, and stress response genes [21]. The ten selected genotypes showed significant variation in CIF (Figure 1). The highest CIF was observed in **Kalikati (72.3%)**, followed by **Basumati (66.6%)**, **Dhanaprasad (61.0%)**, and **Parbatjeera (55.6%)**. Moderate CIF was recorded in **Jeerakasal (53.0%)** and **Kanakachampa (44.3%)**, while **Parijat (36.0%)**, **Karpurajeera (33.3%)**, and **Gangabali (19.6%)** exhibited comparatively low CIF. The lowest response (26.0%) was found in **CR Dhan-907**, the released variety used as a check.

Genotype	CIF (%)	Remarks
Basumati	66.6	High callus formation
Kalikati	72.3	Highest embryogenic potential
Parijat	36	Moderate response
Kanakachampa	44.3	Intermediate response
Jeerakasal	53	Moderately high
Karpurajeera	33.3	Low CIF
Parbatjeera	55.6	Good response
Gangabali	19.6	Poor callus formation
Dhanaprasad	61	High embryogenic potential
CR Dhan-907	26	Low CIF

Table 3: Callus Induction Frequency (CIF%) among Ten Rice Genotypes.

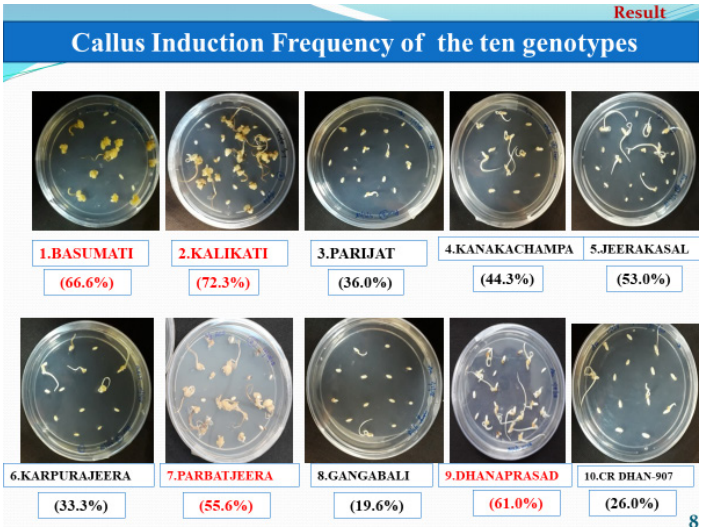

Figure 1: Callus induction frequency (CIF) of ten rice genotypes under standard MS medium supplemented with 2,4-D and NAA.

Interpretation

The wide range of CIF values suggests **genotype-specific response** to auxin and cytokinin balance in the callus induction medium. The superior callusing ability of *Kalikati* and *Basumati* might be linked to their higher endogenous auxin levels and enhanced carbohydrate mobilization capacity, promoting embryogenic tissue proliferation [21]. Conversely, *Gangabali* and *CR Dhan-907* exhibited lower callus formation, likely due to reduced sensitivity to 2,4-D and stress-induced ethylene accumulation, which inhibits callus proliferation [16]. High CIF values often correlate with tolerance to low-oxygen stress during germination, as oxygen deficiency and hormonal imbalance (especially ethylene buildup) affect both callus induction and anaerobic seedling development. Thus, genotypes with **high CIF under in vitro stress** may inherently possess **stronger hypoxia tolerance mechanisms**, reflected in their field-level germination behavior.

Shoot Regeneration Efficiency (SRE)

Shoot regeneration efficiency (SRE) indicates the potential of embryogenic calli to redifferentiate into organized shoots, signifying regeneration competence and genetic stability. The ten rice genotypes displayed wide variation in SRE values ranging from **24.3% to 79.3%** (Figure 2) (Table 4).

Genotype	SRE (%)	Remarks
Basumati	54	High regeneration
Kalikati	68	Vigorous shoot regeneration
Parijat	35.3	Moderate regeneration
Kanakachampa	44.6	Intermediate
Jeerakasal	25	Low regeneration
Karpurajeera	62	High
Parbatjeera	32	Moderate
Gangabali	79.3	Exceptional regeneration
Dhanaprasad	24.3	Poor regeneration
CR Dhan-907	43	Intermediate

Table 4: Shoot Regeneration Efficiency (SRE%) of Ten Rice Genotypes.

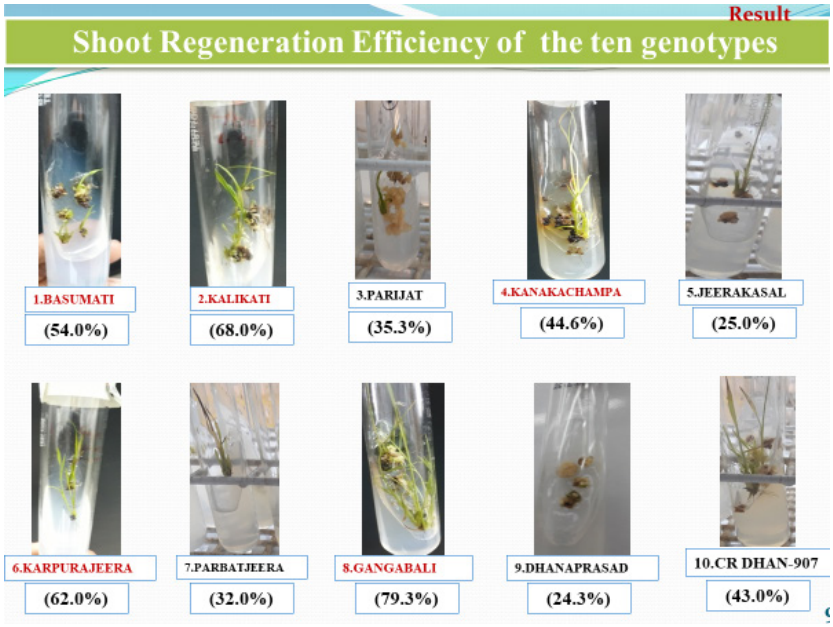

Figure 2: Shoot regeneration efficiency (SRE) of ten rice genotypes on MS medium supplemented with BAP, kinetin, and NAA.

Interpretation

The highest SRE (79.3%) was recorded in *Gangabali*, followed by *Kalikati (68.0%)* and *Karpurajeera (62.0%)*, while *Dhanaprasad* and *Jeerakasal* showed the lowest regeneration rates. Interestingly, while *Gangabali* performed poorly in callus induction, it exhibited remarkable shoot regeneration, suggesting that its calli, though fewer, possessed high organogenic potential [22]. In contrast, *Kalikati* and *Basumati* maintained high values for both CIF and SRE, indicating robust in vitro adaptability and a balanced hormonal response that supports both dedifferentiation and redifferentiation processes. The positive correlation between CIF and SRE observed in *Kalikati* and *Basumati* aligns with earlier findings that tissue culture response is controlled by genotype-specific quantitative trait loci (QTLs) that regulate cell division, auxin transport, and ethylene signaling [23,24].

Germination and Seedling Development under Hypoxic Conditions

To validate in vitro findings under real anaerobic stress, genotypes were subjected to **10 cm water submergence** for three weeks. The results revealed pronounced differences in germination percentage and seedling development among genotypes (Figure 3) (Table 5).

Genotype	Germination (%)	Seedling Development (%)	Coleoptile Length (cm)	Remarks
Basumati	65	60	1.2	Moderately tolerant
Kalikati	75	70	1.5	Highly tolerant
Parijat	45	40	0.9	Moderate
Kanakachampa	52	48	1	Moderate
Jeerakasal	56	51	1	Moderate
Karpurajeera	58	53	1.1	Moderate
Parbatjeera	72	68	1.4	Highly tolerant
Gangabali	20	15	0.6	Susceptible
Dhanaprasad	64	59	1.2	Moderately tolerant
CR Dhan-907	42	37	0.8	Sensitive

Table 5: Germination and Seedling Development under Hypoxia.

Figure 3: Germination and seedling development of rice genotypes under hypoxic condition (10 cm standing water for 21 days).

Interpretation

Among the tested genotypes, *Kalikati* and *Parbatjeera* exhibited superior anaerobic germination (AG) performance, with >70% germination and robust seedling emergence. These genotypes also showed rapid coleoptile elongation (>1.4 cm), facilitating oxygen transport and early emergence through water. In contrast, *Gangabali* and *CR Dhan-907* displayed poor germination (<40%) and weak seedlings, indicating sensitivity to hypoxic stress.

Such variation in AG tolerance is primarily linked to metabolic flexibility and hormonal balance. Tolerant genotypes maintain higher activities of α-amylase, pyruvate decarboxylase (PDC), and alcohol dehydrogenase (ADH), ensuring sustained glycolytic flux and ATP generation during oxygen deprivation [1,17]. Additionally, their ability to elongate coleoptiles rapidly allows them to "escape" hypoxia by reaching oxygenated layers at the surface, an adaptive trait associated with ethylene and gibberellin-mediated growth signaling.

Relationship between Hypoxic Germination, CIF, and SRE

A significant finding of this study was the positive correlation between anaerobic germination (AG) ability, CIF, and SRE across genotypes. Genotypes exhibiting high CIF also tended to perform well in hypoxic germination assays, suggesting a shared physiological basis for stress tolerance in both systems [16]. For instance, *Kalikati* (CIF 72.3%; SRE 68.0%) and *Parbatjeera* (CIF 55.6%; SRE 32.0%) demonstrated strong anaerobic germination (>70%), while *Gangabali* exhibited both low CIF and poor AG performance. This pattern implies that both in vitro regeneration and in vivo hypoxia tolerance may involve similar stress-response genes and QTLs, especially those regulating ethylene metabolism, carbohydrate utilization, and energy conservation.In rice, ethylene accumulation under submergence inhibits root elongation but promotes coleoptile growth. However, excessive ethylene buildup during callus culture can cause necrosis and reduced callusing, paralleling hypoxic stress *in vivo* [25]. Therefore, genotypes capable of modulating ethylene signaling efficiently exhibit better CIF and AG tolerance. This association is further supported by QTL mapping studies identifying shared loci for callus induction and AG tolerance, notably on chromosomes 7 and 9 [22]. These loci control carbohydrate metabolism, auxin sensitivity, and cell wall expansion all essential for both embryogenic callus formation and coleoptile elongation.

Correlation between SRE and Seedling Development

Interestingly, genotypes with high shoot regeneration efficiency (SRE) in vitro also displayed strong seedling development under oxygen stress. For example, *Kalikati* and *Karpurajeera* exhibited both high SRE (>60%) and good seedling establishment under submergence. This relationship indicates that tissue regeneration ability in vitro mirrors resilience mechanisms in vivo, particularly the capacity for cellular redifferentiation after stress-induced dedifferentiation. Similar metabolic pathways including reactive oxygen species (ROS) detoxification, mitochondrial recovery, and hormonal rebalancing operate during both processes. Therefore, genotypes that can regenerate shoots efficiently after callus stress are also better equipped to recover from anaerobic stress at the seedling level. This suggests the presence of co-regulated QTLs controlling cellular recovery mechanisms.

Integrative Analysis and Modern Relevance

The integration of in vitro morphogenic response (CIF and SRE) with in vivo hypoxic tolerance (AG and seedling development) represents a novel approach in identifying stress-resilient genotypes. This multidimensional analysis helps bridge cellular-level plasticity with whole-plant adaptability. Recent genomic insights show that genes like OsTPP7 (trehalose-6-phosphate phosphatase) and OsDREB6 regulate energy metabolism and sugar mobilization under oxygen limitation [26]. Such genes may also influence tissue culture responses by maintaining carbohydrate homeostasis and stress recovery in vitro. Furthermore, advances in transcriptomics reveal that both anaerobic germination and regeneration processes share activation of sucrose synthase, expansin, and cell wall-loosening enzymes, linking cell elongation under hypoxia with cell division during shoot organogenesis [27]. Therefore, the observed correlations in this study not only validate genotype-specific adaptability but also highlight potential markers for marker-assisted selection (MAS) in breeding programs targeting flood-resilient and tissue-culture-amenable rice varieties.

Conclusion

The present study successfully identified genotypic differences in the ability of rice (*Oryza sativa* L.) landraces and varieties to tolerate oxygen deficiency during germination and to perform efficiently under in vitro culture conditions. The combined evaluation of callus induction frequency (CIF), shoot regeneration efficiency (SRE), and anaerobic germination (AG) capacity revealed that these physiological and morphogenic responses are genotype-dependent and interrelated. Among the ten tested genotypes, the landrace Kalikati consistently exhibited superior performance, achieving the highest CIF (72.3%), strong SRE (68.0%), and excellent germination under hypoxic conditions (75%). Other promising performers included Parbatjeera, Basumati, and Dhanaprasad, which also showed high regenerative capacity and moderate AG tolerance. Conversely, Gangabali and CR Dhan-907 were found to be highly sensitive to oxygen stress, with low callus formation and poor germination. The positive correlation observed between CIF, SRE, and AG tolerance suggests that common physiological mechanisms such as efficient carbohydrate mobilization, ethylene regulation, and hormonal homeostasis govern both in vitro and in vivo stress responses. Genotypes capable of regenerating shoots effectively after cellular stress in vitro also demonstrated strong seedling development following hypoxic stress, indicating the involvement of shared genetic loci (QTLs) like

OsTPP7 and *OsDREB6*, which regulate energy balance and recovery under low-oxygen condition.

Both CIF and SRE are genotype dependent. Genes responsible for callus induction are different from shoot regeneration. The positive association of anaerobic (hypoxic) germination capacity with CIF and seedling development with SRE of the genotypes may be due to the presence of QTLs associated with these characters. This relationship can be a key in selecting cultivars for capacity to germinate and establish under standing water conditions during seed germination.

Overall, the study establishes a functional link between anaerobic germination ability and tissue culture responsiveness, emphasizing that genotypes with high regeneration potential are also inherently more stress-adaptive. The landrace Kalikati, combining excellent AG tolerance and regeneration efficiency, represents a valuable genetic resource for breeding climate-resilient, direct-seeded rice varieties suited to flood-prone ecosystems. These insights contribute to developing integrated selection strategies for rice improvement, where in vitro screening can complement physiological evaluations for enhanced precision and speed in breeding flood-tolerant cultivars.

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