Discover Plants

RESEARCH Open Access

Unveiling submergence tolerance in improved restorer lines of rice (*Oryza sativa* L.) seedlings at varied durations: evaluation through chlorophyll fluorescence and morphological responses

Y. Manasa[1], P. Beulah[1], G. Karthika[1], V. Jaldhani[1], V. G. N. Tripura Venkata[1], K. K. Raghuraman[1], M. Arivin[1], D. Sanjeeva Rao[1], S. K. Mangrauthia[1,2], A. S. HariPrasad[1], R. M. Sundaram[1], A. Krishna Satya[2], P. Sudhakar[2], P. Raghuveer Rao[1] and P. Senguttuvel[1*]

*Correspondence:
P. Senguttuvel
senguttuvel@gmail.com
[1]Crop Improvement Section, Indian Council of Agricultural Research (ICAR) - Indian Institute of Rice Research (IIRR), Hyderabad 500030, India
[2]Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh 522510, India

Abstract

Submergence stress caused by flash floods is a major abiotic challenge that hampers plant growth and threatens sustainable crop production in the era of climate change. The rice restorer 'KMR-3R' plays a crucial role in hybrid rice breeding, but its susceptibility to submergence stress poses a significant limitation. To enhance its submergence tolerance, we employed marker-assisted backcross breeding to introgress the *Sub1* gene from Swarna-Sub1 into KMR-3R. This study assessed the morpho-physiological responses of 13 backcross inbred lines (BILs) of KMR-3R (BC_2F_6) at the 14-days-old seedling stage under two submergence durations (7- and 14-days). Prolonged submergence significantly impacted survival rates, chlorophyll fluorescence parameters (F_v/F_m, Y(II), ETR, qN, qP) and total chlorophyll content in both BILs and parental lines compared to the control. Shoot elongation was restricted at 14-days after submergence (DAS). Notably, seven BILs (TCP18, TCP28, MB44, TCP25, TCP15, TCP02 and TCP10) exhibited limited shoot elongation, stable PS II activity, while TCP18 and TCP15 showed highest survival percentage exceeding that of Swarna-Sub1 at 14-DAS highlighting their enhanced tolerance. These promising BILs have the potential to serve as improved restorer lines for the developing submergence-tolerant rice hybrids in flash floods prone ecosystem.

Keywords Hybrid rice, Submergence, Sub1, MABB, Chlorophyll fluorescence

1 Introduction

Rice (*Oryza sativa* L.) is a globally significant staple food crop, playing a crucial role in food security and livelihoods of growing population. Global rice production stands at 527.61 million metric tons (MMT) across 167.55 million hectares, while India contributing 138 MMT from 48.50 million hectares [1]. However, recurrent flooding poses a

major challenge to rice cultivation, adversely affecting production and productivity. The flooding stress is classified as either submergence or water logging stress, depending on the depth of the water table [2]. Among the various abiotic stresses impacting rice production, submergence stress is particularly detrimental [3]. It occurs when the rice plants are completely submerged due to flash floods, especially during the vegetative or seedling stages and can last up to two weeks. The frequent occurrence of flooding discourages the adoption of high-yielding varieties that lack submergence tolerance, leading to reduced farm income (with annual losses estimated at Rs. 432 crores) and jeopardizing food security and livelihoods [4].

While submergence stress known to cause substantial yield losses in inbred rice varieties, its impact on hybrid rice remains less explored. Although India has developed and released 152 rice hybrids covering 3.5 million hectares (http://aicrip-intranet.in/), none exhibit significant submergence tolerance. The rice hybrids cultivars such as 'Zheou-18' and 'Yliangyou-689' have shown significant yield reductions under submergence conditions [3]. Given the rising global demand for rice, breeding programs must prioritize the development of high yielding, submergence tolerant hybrids, particularly for flood-prone regions.

As a semi-aquatic species, rice can withstand short-term flooding for up to one week [5, 6]. However, only genotypes with specific tolerance mechanisms can survive prolonged submergence [6]. Developing such cultivars requires a deep understanding of the physiological and metabolic responses of rice to flooding. Under submergence, plants undergo complex physiological changes, including reduced growth rates [7]. Prolonged submergence negatively affects key physiological processes such as chlorophyll degradation, gas exchange, stomatal conductance, and photosynthetic efficiency, often before visible symptoms of plant stress appear [8] [9].

Screening 14-days old seedling has been effective in identifying genotypes with improved survival rate under submergence stress [10]. The duration of submergence also influences the expression of *Sub1*, a gene linked to submergence tolerance, enabling rice plants to survive complete submergence for up to 14-days [11]. Key indicators of submergence tolerance at 14-days after submergence (DAS) stress include shoot elongation percentage and seedling survival rate, which reflect a plant's ability to withstand flooding conditions [9, 12]. Controlled environment screening, such as artificial tanks with stable water levels (55 cm), allows precise evaluation of plant responses to submergence while minimizing environmental variability [13]. Significant progress has been made in introgressing the *Sub1A* gene into major rice varieties, enhancing their tolerance to submergence and flash floods for up to two weeks [14, 15].

To address these challenges, a marker-assisted backcross breeding (MABB) strategy was employed to improve the submergence tolerance of the widely used restorer line 'KMR-3R' by introgressing the *Sub1* gene derived from Swarna-Sub1, the *Sub1* plays a critical role in regulating ethylene signalling and plant growth under low-oxygen conditions [11], thereby enhancing submergence tolerance. This study hypothesizes that rice plants possessing the *Sub1* gene will exhibit increased photosynthetic activity, greater physiological resilience, restricted shoot elongation, and improved post-stress recovery compared to non-*Sub1* plants under submergence durations of 7- and 14-days at the 14-day-old seedling stage [16]. The study focuses on evaluating the morpho-physiological responses of backcross inbred lines (BILs) carrying the *Sub1* gene under varying

submergence stress durations. The findings provide valuable insights into photosynthetic changes during submergence and contribute to a deeper understanding of plant adaptation to prolonged submergence.

2 Materials and methods

The plant material used in this study comprised 13 advanced backcross *Sub1* introgressed lines (BILs) at the BC_2F_6 generation, developed from the KMR-3R × Swarna-Sub1, along with the parental lines. The BILs included are RP-6342-VTCP02 (TCP02), RP-6342-VTCP10 (TCP10), RP-6342-VTCP11 (TCP11), RP-6342-VTCP12 (TCP12), RP-6342-VTCP14 (TCP14), RP-6342-VTCP15 (TCP15), RP-6342-VTCP18 (TCP18), RP-6342-VTCP23 (TCP23), RP-6342-VTCP25 (TCP25), RP-6342-VTCP26 (TCP26), RP-6342-VTCP28 (TCP28), RP-6342-VTCP32 (TCP32), and RP-6342-MB44 (MB44).

2.1 Screening of BILs using fertility restoration markers

The submergence tolerant conferring QTL- *Sub1* from Swarna-sub1 were introgressed into KMR-3R using MABB method. Two successive backcrosses and pedigree selection was followed for further advancement of generations up to BC_2F_6. KMR-3R being a restorer, the derived BILs were also screened for fertility restoration using gene specific markers RMS-PPR9-1, DRCG-RF4-14 for *Rf4* and RMS-SF-21-5 for *Rf3* genes.

Screening of BILs for fertility restoration using molecular markers was performed following the protocol reported in our earlier study [17]

2.2 Screening for submergence tolerance

The experiment was conducted during *Kharif* 2021 in the artificial screening facility under standard environmental conditions at the Department of Hybrid Rice, Crop Improvement Section, Indian Council of Agricultural Research (ICAR)—Indian Institute of Rice Research (IIRR), Hyderabad, India (17.53°N and 78.27°E). Seedlings of all the BILs along with their parents were grown in rows within four plastic trays (56 cm × 36 cm × 11.5 cm) filled with fertilized soil. Each tray was systematically divided into two halves to facilitate replication following a completely randomized design (CRD). Each row represented a distinct genotype, with one half designated as replication 1 and the other as replication 2. Three plants per genotype were randomly selected as biological replicates within each replication. Among the four trays, two trays were subjected to submergence stress for 7- and 14-days individually, while the other two trays were maintained as control under normal conditions. Submergence stress was induced by immersing the trays with 14-days-old seedlings into individual cement tanks (84.5 cm × 54.5 cm × 55 cm), ensuring a water level of 55 cm from the base of the trays for the specified durations (7- and 14-days). After each stress duration, the trays were removed from the tanks and kept in dark conditions for one hour to facilitate physiological analysis. Following the stress treatment and sample collection seedlings were allowed a recovery period of two weeks (14-days) under normal environmental conditions to examine their survival and recovery. The revival ability of the seedlings was evaluated visually using the SES scale (1, 3, 5, 7, and 9), whereas the scores 1 and 9 indicates 100 (%) and 0–49 (%) survival of plants as described by [18]. Morphological and physiological parameters were recorded to assess submergence tolerance.

2.3 Seedling survival (%)

Seedling survival under submergence stress was calculated using the following formula given by [19]

$$\text{Seedling survival } (\%) = \left(\frac{\text{Number of plants reemerged after the recovery period of stress}}{\text{Total number of plants before stress}}\right) * 100$$

2.4 Plant height and shoot elongation percent

Plant height and shoot elongation were assessed in three randomly selected plants from each replicate under both control and stress conditions across submergence durations. The height was measured from the ground level to the tip of the top leaf using a ruler. The shoot elongation percent was calculated by subtracting the height of the control seedlings from the height of the seedlings subjected to submergence stress. The result was then expressed as a percentage of the plant height under control conditions using the formula given by [19]

$$\text{Shoot Elongation } (\%) = \left(\frac{\text{Plant height after submergence stress - Plant height under control conditions}}{\text{Plant height under control conditions}}\right) * 100$$

2.5 Chlorophyll fluorescence and total chlorophyll content

Chlorophyll fluorescence was measured at the seedling stage using a portable fluorometer (PAM-210, Effeltrich, Germany). The fluorescence parameters comprise of actual photosynthetic efficiency [Y(II)], electron transport rate (ETR), coefficient of photochemical quenching (qP), coefficient of non-photochemical quenching (qN), and maximal quantum yield of PSII (F_v/F_m). Measurements were taken on the leaves of three to four seedlings from each genotype, in two replicates, under dark-adapted conditions (two hours) for specified submergence durations (7- and 14-days) as well as under control conditions. After recording chlorophyll fluorescence parameters, the same leaves were used to estimate the total chlorophyll content under stress and control conditions at 14-days (Total chlorophyll content at 7-days stress could not be analysed due to technical constraints).

For total chlorophyll content estimation, 100 mg of fresh leaves from each genotype were individually weighed and incubated in screw-cap tubes containing 25 ml of 80 (%) acetone (v/v) for 48 h in the dark. Absorbance was measured at 645 nm and 663 nm using a spectrophotometer (GE Ultrospec) as per the method described by [20]. The total chlorophyll content (mg g^{-1} FW) was calculated using the formula:

$$\text{Total chlorophyll content } (\mu g/\text{mL}) = 20.2\text{A}645 + 8.02A663$$

where A_{645}—Absorbance at 645 nm; A_{663}—Absorbance at 663 nm.

2.6 Statistical analysis

The data were analysed using two-way ANOVA to evaluate the effects of genotype and treatment (control and stress conditions) and their interactions. Tukey's post-hoc test was performed to compare means and identify significant differences between treatments and genotypes. All statistical analyses were conducted using GenStat version 15

(VSN International Pvt. Ltd). ANOVA of Seedling survival (%) was done in the BILs excluding KMR-3R and TCP32 to maintain the uniformity of the data, since KMR-3R died due to the induced submergence stress and also devoid of *Sub1* gene.

3 Results

3.1 Phenotypic screening of BILs for submergence tolerance under 7- and 14-durations

The current study revealed the submergence tolerance of BILs over varying durations (7- and 14-days). The scoring of BILs screened at 7- and 14-days stress was presented in the Supplementary Table 1. Among the studied thirteen BILs, seven BILs at 7-DAS and six BILs at 14-DAS showed varying levels of tolerance respectively (Fig. 1). Notably, Swarna-Sub1 exhibited tolerance mechanisms, whereas KMR-3R was highly sensitive.

3.2 ANOVA results

The ANOVA results revealed significant effects of duration (D), treatment (T), and their interaction (D × T) on seedling survival percentage, Actual photosynthetic efficiency [Y(II)] and electron transport rate (ETR). The coefficient of photochemical quenching (qP) was significantly affected by duration (D), and its interaction with treatment (D × T). Total chlorophyll content (TC) was significantly affected by treatment while the plant height (PH) and maximum quantum yield of photosystem II (F_v/F_m) were significantly influenced by duration (D) and treatment (T) (Table 1).

3.3 Morphological parameters

The BILs recovered after 7- and 14-days of submergence were evaluated for their visual performance using the Standard Evaluation System (SES) scale [18]. Their scores were compared against the donor parent, Swarna-Sub1 (Supplementary Table 1).

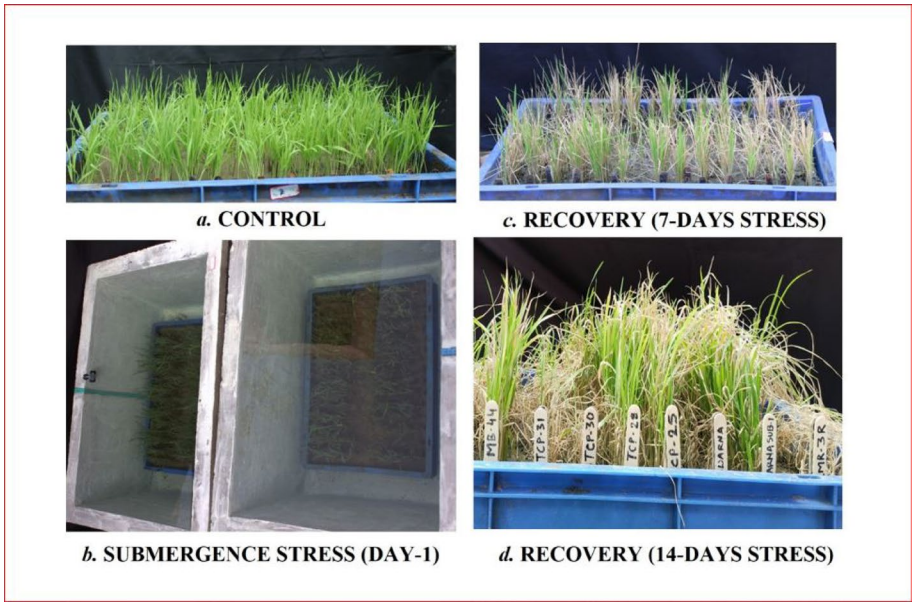

Fig. 1 Showing BILs, parents in control, stress and recovery for tolerance to submergence stress at varied time durations

Table 1 Analysis of variance (ANOVA) of BILs, submergence durations and their interactions for morpho-physiological parameters in rice

Source of variation	d.f	Seedling survival (%)	Plant height (cm)	Y(II)	ETR	qP	qN	F_v/F_m
Duration (D)	1	8127.4***	99.736***	0.043701***	204.624***	0.072104***	0.000411ns	0.013409**
Treatment (T)	1	12,267.2***	394.34***	0.013125ns	51.352**	0.002054ns	0.000161ns	0.068235***
Duration x Treatment (DXT)	1	8127.4***	5.461 ns	0.051709***	270.901***	0.103283***	0.036505***	0.000768ns
Residual	116	262.1	2.082	0.001727	8.775	0.003029	0.001926	0.001989
CV (%)		18.2	9.0	19.0	19.5	17.2	25.5	5.8

Y(II)–Actual photosynthetic efficiency; ETR–Electron transport rate; qP–Coefficient of Photochemical quenching; qN–Coefficient of non- photochemical quenching; F_v/F_m–Maximum efficiency of PSII photochemistry

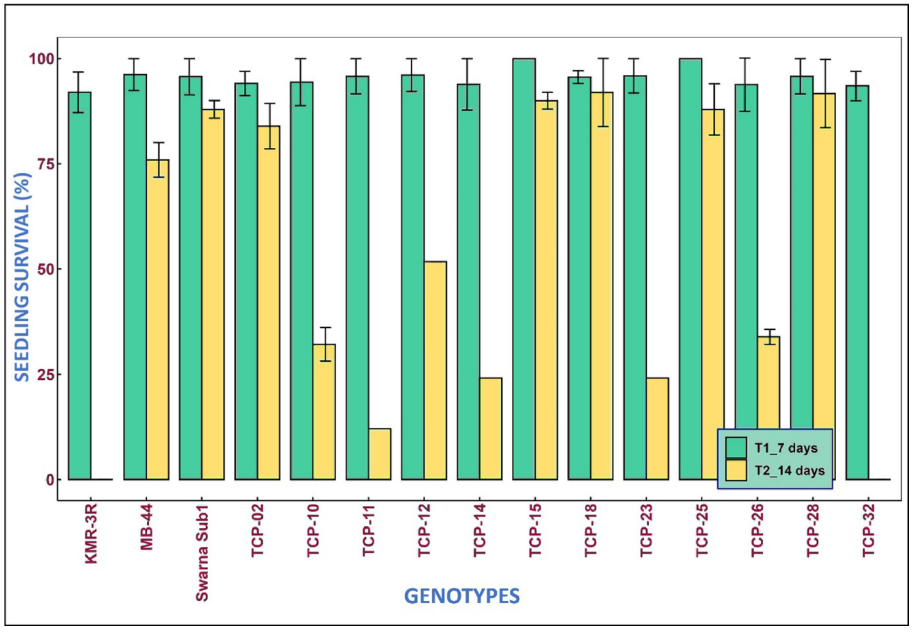

Fig. 2 Seedling survival (%) of BILs after 14-days of recovery of de-submerged plants subjected to stress for 7- and 14- days

3.4 Seedling survival (%)

The mean seedling survival (%) of the genotypes decreased as the duration of submergence increased from 7 to 14-days. Following recovery after 14-days of stress, the mean survival rates were recorded at 95.5 (%) (7-DAS) and 67.7 (%) (14-DAS) (Supplementary Table 2). At 7-DAS, seven BILs exhibited higher survival rates than the parental lines, with TCP15 and TCP25 achieving 100 (%) survival. At 14-DAS, TCP18, TCP28, and TCP15 demonstrated superior survival rates, while TCP25 performed comparably to Swarna-Sub1. In contrast, KMR-3R experienced complete mortality at 14-DAS (Fig. 2). Tukey's test revealed significant reduction of mean at 14-days stress compared to 7-days stress. No significant differences were found among the genotypes at both stress durations. (Note: Standard error was not shown because of the identical replicate values, since the error is zero).

3.5 Plant height

The mean plant height of BILs exhibited an increasing trend with prolonged submergence stress. The BILs after desubmergence were significantly shorter than KMR-3R at both 7- and 14-DAS, while TCP18 maintained a consistently lower plant height compared to Swarna-Sub1 across all stress durations. Significant genotypic variations in plant height were observed at 7- and 14-D under both control and stress conditions (Supplementary Table 3). TCP15, TCP18, TCP25, fand TCP32 showed significantly lesser plant height than KMR-3R.

3.6 Shoot elongation percentage (%)

Shoot elongation percentage (%) increased with the duration of submergence, reaching 23.98 (%) at 7-DAS and 27.18 (%) at 14-DAS (Supplementary Table 4). All BILs exhibited lower shoot elongation than KMR-3R, whereas TCP26 and TCP23 displayed the highest elongation among the BILs and Swarna-Sub1 at both time points. At 14-DAS, TCP11, TCP15, TCP18, TCP25, TCP26, TCP28, TCP32, and MB44 exhibited significantly lower shoot elongation than Swarna-Sub1. Notably, TCP18 consistently recorded the lowest shoot elongation percentage across submergence durations (Fig. 3). No significant differences were observed among the genotypes.

3.7 Effect of submergence stress on total chlorophyll content and fluorescence parameters

Submergence stress led to a reduction in total chlorophyll content and chlorophyll fluorescence parameters, including actual photosynthetic efficiency [Y(II)], electron transport rate (ETR), coefficient of photochemical quenching (qP), coefficient of non-photochemical quenching (qN), and maximal quantum yield of PSII (F_v/F_m) over 7- and 14-DAS across the studied genotypes (Table 2). However, certain BILs exhibited higher values for these parameters at specific time points.

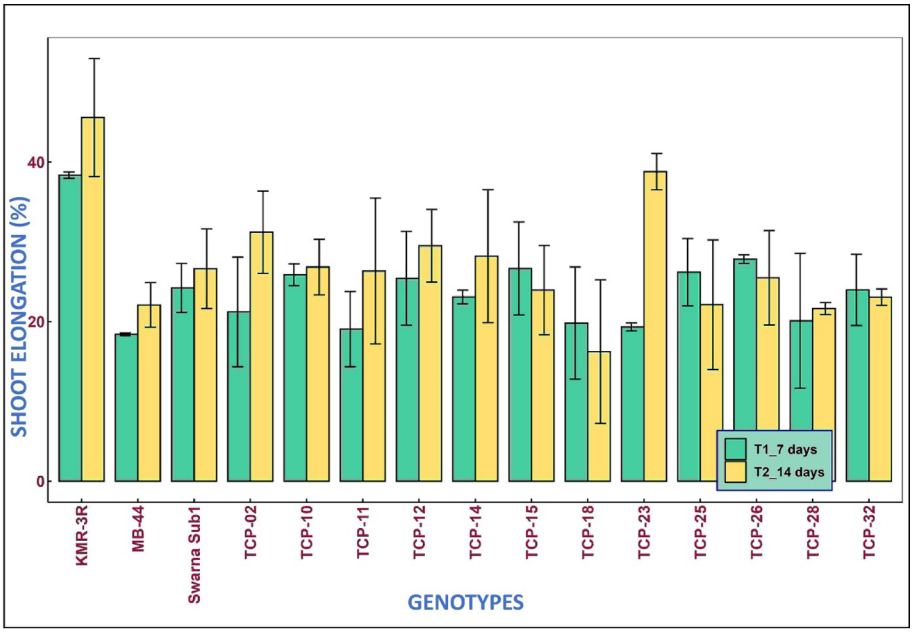

Fig. 3 Shoot elongation (%) of BILs at 7- and 14- days of stress (after de-submergence)

Table 2 Physiological performance and variations of BILs after 7- and 14-days of submergence stress

Genotypes	Total chlorophyll content (mg/ml)	Actual photosynthetic efficiency [Y(II)]		Electron transport rate (ETR)		Coefficient of photochemical quenching (qP)		Coefficient of non-photochemical quenching (qN)		Maximum efficiency of PSII photochemistry (F_v/F_m)	
	14-DAS	7-DAS	14-DAS	7-DAS	14-DAS	7-DAS	14-DAS	7-DAS	14-DAS	7-DAS	14-DAS
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
KMR-3R	1.83 ± 0.20	0.23 ± 0.04	0.19 ± 0.04	15.7 ± 2.7	13.6 ± 2.73	0.32 ± 0.05	0.28 ± 0.04	0.13 ± 0.01	0.14 ± 0.04	0.77 ± 0.01	0.75 ± 0.01
Swarna-Sub 1	2.12 ± 0.29	0.23 ± 0.01	0.22 ± 0.03	16.38 ± 0.93	15.6 ± 2.4	0.34 ± 0.03	0.32 ± 0.04	0.16 ± 0.02	0.17 ± 0.04	0.79 ± 0.01	0.77 ± 0.02
TCP02	2.26 ± 0.44	0.26 ± 0.01	0.22 ± 0.02	18.38 ± 0.58	15.05 ± 1.35	0.38 ± 0.01	0.33 ± 0	0.18 ± 0.04	0.16 ± 0.03	0.8 ± 0.01	0.78 ± 0.01
TCP10	2.16 ± 0.16	0.29 ± 0.01	0.18 ± 0.04	20.43 ± 0.47	12.53 ± 2.73	0.41 ± 0.01	0.27 ± 0.05	0.16 ± 0.02	0.17 ± 0.02	0.79 ± 0	0.78 ± 0.03
TCP11	1.46 ± 0.29	0.21 ± 0.01	0.2 ± 0.02	14.6 ± 0.75	13.96 ± 1.76	0.3 ± 0.02	0.3 ± 0.03	0.13 ± 0.02	0.18 ± 0.03	0.78 ± 0	0.74 ± 0.03
TCP12	1.48 ± 0.06	0.26 ± 0.03	0.2 ± 0.02	18.02 ± 1.92	14.06 ± 1.71	0.37 ± 0.03	0.3 ± 0.04	0.18 ± 0.03	0.16 ± 0.05	0.77 ± 0.01	0.76 ± 0.01
TCP14	2.12 ± 0.15	0.23 ± 0.03	0.16 ± 0.04	16.02 ± 1.63	11.34 ± 2.66	0.33 ± 0.03	0.25 ± 0.04	0.16 ± 0.03	0.18 ± 0.02	0.78 ± 0.02	0.75 ± 0.05
TCP15	1.91 ± 0.04	0.24 ± 0.03	0.17 ± 0.01	16.6 ± 1.75	11.82 ± 0.93	0.34 ± 0.04	0.25 ± 0.02	0.2 ± 0.02	0.17 ± 0.02	0.78 ± 0.01	0.72 ± 0.01
TCP18	1.68 ± 0.02	0.25 ± 0.01	0.2 ± 0.01	17.55 ± 0.75	14.18 ± 0.98	0.36 ± 0.03	0.29 ± 0.02	0.2 ± 0.02	0.17 ± 0.02	0.79 ± 0.02	0.75 ± 0.02
TCP23	1.90 ± 0.67	0.21 ± 0.03	0.21 ± 0.01	14.18 ± 1.73	14.66 ± 0.54	0.3 ± 0.03	0.31 ± 0.02	0.17 ± 0.02	0.19 ± 0.01	0.8 ± 0.01	0.78 ± 0.02
TCP25	2.00 ± 0.29	0.24 ± 0.03	0.2 ± 0.02	16.93 ± 2.18	13.88 ± 1.38	0.35 ± 0.05	0.3 ± 0.02	0.22 ± 0.02	0.18 ± 0.05	0.77 ± 0.01	0.75 ± 0.01
TCP26	3.09 ± 0.63	0.21 ± 0.03	0.23 ± 0.02	14.4 ± 1.95	15.97 ± 1.25	0.29 ± 0.04	0.33 ± 0.02	0.15 ± 0.01	0.17 ± 0.01	0.79 ± 0.02	0.75 ± 0.01
TCP28	2.13 ± 0.24	0.25 ± 0.02	0.21 ± 0.01	17.57 ± 1.58	14.74 ± 0.56	0.36 ± 0.03	0.31 ± 0.02	0.16 ± 0.03	0.17 ± 0.02	0.79 ± 0.01	0.76 ± 0.01
TCP32	2.57 ± 0.27	0.24 ± 0.01	0.19 ± 0.01	16.68 ± 0.38	13.22 ± 0.53	0.35 ± 0.01	0.29 ± 0.02	0.2 ± 0.01	0.18 ± 0	0.79 ± 0.01	0.76 ± 0.02
MB44	2.15 ± 0.48	0.21 ± 0.06	0.2 ± 0.02	14.35 ± 4.3	13.99 ± 1.01	0.35 ± 0.04	0.29 ± 0.03	0.21 ± 0.05	0.18 ± 0.03	0.79 ± 0.1	0.74 ± 0.02
Mean	2.06	0.24	0.20	16.52	13.91	0.34	0.30	0.17	0.17	0.78	0.76
F value	< 0.001	0.088	< 0.001	0.051	< 0.001	0.003	< 0.001	0.03	0.003	0.003	< 0.001
LSD	0.42	0.032	0.025	2.264	1.781	0.043	0.032	0.032	0.028	0.036	0.024
CV (%)	29.7	19.4	18.3	19.9	18.6	18.0	15.9	27.1	23.7	6.8	4.6

DAS–days after submergence; SE–Standard error

Total chlorophyll content was highest in TCP26, TCP32, TCP02, TCP10, MB44 and TCP28 at 14-DAS, while TCP26 and MB44 maintained higher chlorophyll content post-submergence (Fig. 4). At 7-DAS, TCP10 demonstrated higher Y(II), while TCP26 exhibited the highest Y(II) at 14-DAS (Table 2). A significant reduction of mean Y(II) was observed under 14-DAS. TCP10 at 7-DAS and TCP26 at 14-DAS recorded a higher ETR than the parental lines. Significant reduction of mean at 14-DAS was observed for ETR and no variation among the genotypes was observed. For qP, TCP10 and TCP02 exhibited superior values at 7-DAS, whereas TCP02 and TCP26 outperformed the parental lines at 14-DAS. Significant reduction in mean qP was observed at 14-DAS. No genotypic differences were found among the genotypes.

The mean qN remained stable at 0.17 across both time periods; however, TCP25, TCP23, and TCP18 recorded the highest qN values compared to the parental lines at 7- and 14-days of stress durations, respectively. The mean F_v/F_m ratio showed a slight decline from 0.78 at 7-DAS to 0.76 at 14-DAS. Nevertheless, BILs such as TCP02 and TCP23 consistently exhibited higher F_v/F_m values than the parental lines at both time points, along with TCP10 (Fig. 5). No significant differences among the genotypes were observed for qN and F_v/F_m under 7- and 14-DAS. Reduction in mean values were observed for total chlorophyll content, Y(II), ETR, qP, and F_v/F_m ratio after 14-DAS (Table 2).

4 Discussion

4.1 Screening of BILs for fertility restoration using markers

The BILs used in the present study were identified as promising restorers possessing fertility restoration genes along with *Sub1* [17]. Based on the presence of *Sub1* gene and fertility restoration genes, 13 BILs along with their parents were evaluated for physiological and morphological parameters for assessing their tolerance under varied submergence stress. These changes were discussed as follows:

4.2 Impact of submergence stress on rice survival and tolerance mechanisms

Submergence stress limits oxygen and light availability, thereby disrupting key physiological and metabolic processes [21]. Most rice cultivars experience severe damage or mortality after a week or prolonged submergence [11, 22]. The extent of submergence-induced damage and subsequent recovery depends on the duration of stress, with prolonged submergence often resulting in irreversible injury or plant death [6]. Submergence tolerance is a complex trait regulated by genetic, physiological, and biochemical mechanisms that enable plants to withstand and recover from flooding stress [23]. However, the degree of submergence impact varies due to multiple factors, including seedling age, submergence duration, water turbidity, depth, temperature, light intensity, and carbohydrate availability [13, 24]. Screening BILs across different submergence durations facilitates the identification of tolerant genotypes. This study demonstrated that prolonged submergence significantly reduced the survival ability of all genotypes. The variation in survival rates among genotypes was primarily attributed to the presence or absence of *Sub1A*, a major gene conferring submergence tolerance in rice [25].

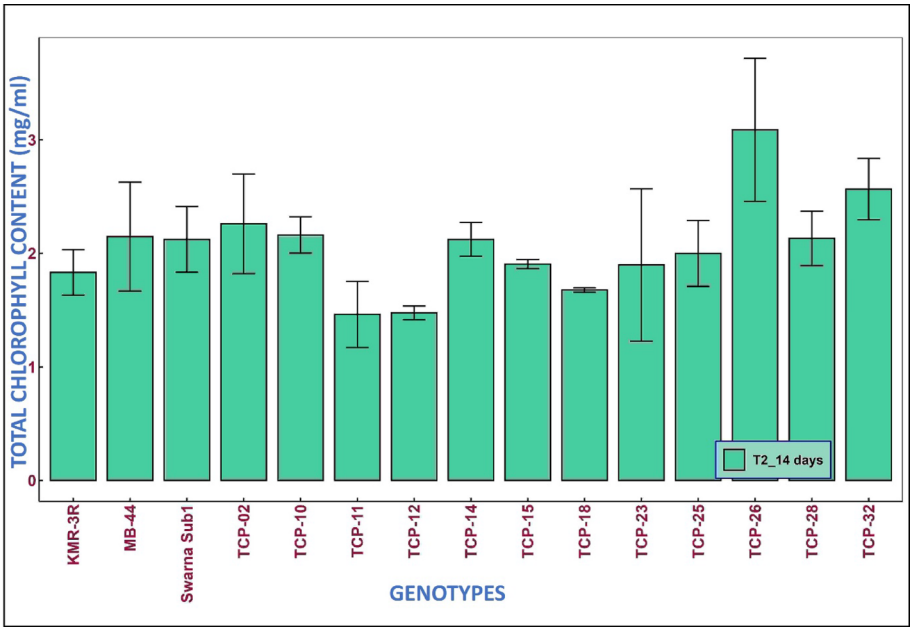

Fig. 4 Total chlorophyll content (mg/ml) of BILs at 14-days of submergence stress (after de-submergence)

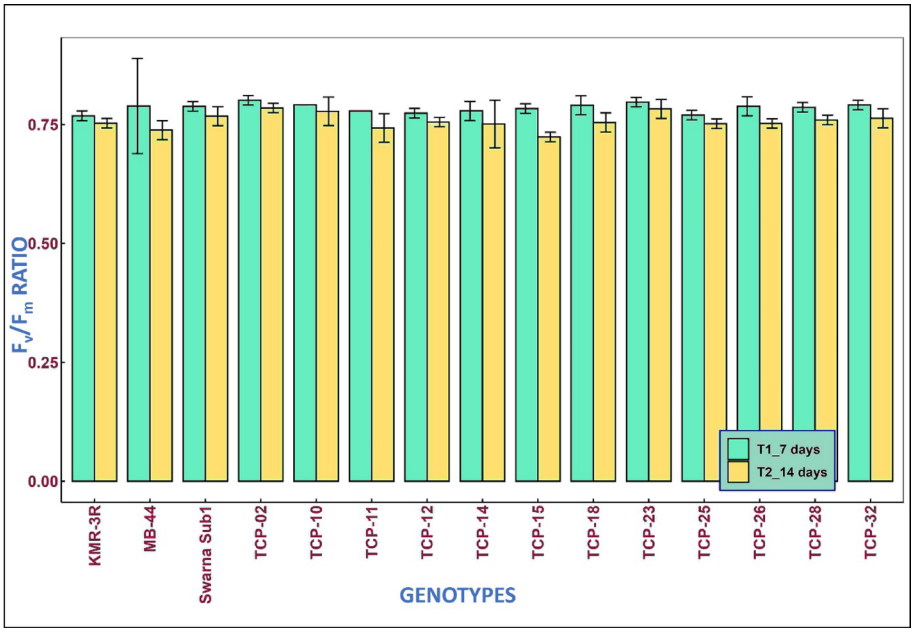

Fig. 5 F_v/F_m ratio of BILs at 7- and14-days of submergence stress

4.3 Responses of BILs to submergence stress

Rice cultivars generally tolerate up to a week of flooding, but prolonged submergence beyond this threshold significantly reduces survival, with only tolerant genotypes capable of recovery [6]. To withstand hypoxia/anoxia, tolerant rice genotypes undergo a series of adaptive modifications that mitigate damage and enhance survival [26, 27]. The *Sub1A* gene plays a critical role in regulating physiological and molecular responses during submergence and de-submergence, conferring enhanced tolerance [28].

Following stress removal, genotypes were allowed a two-week recovery period [29]. TCP15 and TCP25 exhibited 100 (%) survival under 7-DAS, assessed 14-days after recovery (DAR). Similarly, TCP18, TCP15, TCP25, and TCP28 demonstrated the highest survival among the BILs and parental lines under 14-DAS, consistent with earlier reports [28–30]. While *Sub1A* introgression generally enhances submergence tolerance, extended submergence (e.g., 20-days) can still cause severe damage, as reported by Sarkar and Bhattacharjee [19].

4.4 Morphological adaptations and shoot elongation response

All BILs exhibited shorter plant heights than KMR-3R at 7- and 14-DAS, with TCP18 consistently shorter than Swarna-Sub1 across all stress durations. Tolerant cultivars generally maintain reduced elongation to conserve energy for post-submergence recovery, as observed in previous studies [31]. The shoot elongation percentage (SE (%) in all BILs remained lower than KMR-3R at both 7- and 14-DAS. Furthermore, TCP11, TCP15, TCP18, TCP25, TCP26, TCP28, TCP32, and MB44 exhibited lower SE (%) than Swarna-Sub1 at 14-DAS, indicating a quiescence response that minimizes carbohydrate depletion during submergence [32, 33]. In contrast, KMR-3R and TCP23 exhibited greater plant height and SE (%), suggesting an elongation-driven escape strategy. While this mechanism allows access to oxygen and light, it also increases lodging risk and accelerates carbohydrate depletion, ultimately leading to mortality [22, 34]. The failure of recovery in TCP32, despite the presence of *Sub1* gene, may be due to several factors, including variations in *Sub1A* and *Sub1C* gene expression and genetic background interactions of recurrent parent and duration [35], carbohydrate exhaustion [36], and ROS accumulation [37]. These factors highlight that while *Sub1* confers submergence tolerance, its effectiveness depends on genetic regulation, physiological energy reserves, oxidative defense mechanisms, and environmental suitability. Reported studies have demonstrated that submergence tolerance in rice is influenced by haplotypic variation at the *Sub1A* locus (*Sub1A-1* vs. *Sub1A-2*), differential gene expression under stress conditions, and genetic interactions with other loci that collectively contribute to the modulation of submergence tolerance in rice [11, 28, 38]. Although the improved submergence tolerance observed in the BILs correlates with the introgression of *Sub1A*, only the *Sub1A-1* allele with a serine at position 186 confers strong induction and tolerance, whereas the *Sub1A-2* allele (bearing proline at this site) is poorly induced and typically ineffective [39]. Additionally, submergence resistance can also arise from *Sub1A*–independent physiological mechanisms, such as slowed starch hydrolysis, enhanced carbohydrate conservation, and quiescent under water [40, 41].

4.5 Chlorophyll content, chlorophyll fluorescence and PSII stability

Chlorophyll degradation was more pronounced in sensitive cultivars, consistent with previous findings [29, 42–44]. Except for TCP26, TCP32, TCP02, TCP10, MB44 and TCP28, the remaining BILs exhibited elevated chlorophyll degradation after 14-days of stress.

The increase in Y(II) in TCP10 at 7-DAS and TCP26 at 14-DAS suggests superior photochemical efficiency in PSII [26], aligning with earlier reports [26, 45]. Similarly, TCP10 and TCP26 exhibited higher ETR than the parental lines at both 7- and 14-DAS, with TCP15 surpassing Swarna-Sub1 at 14-DAS. These results confirm that submergence

stress inhibits PSII activity and reduces photosynthetic efficiency in sensitive genotypes [45, 46].

The qP values progressively declined with increasing submergence duration, from 0.34 at 7-DAS to 0.30 at 14-DAS, indicating increased PSII damage over time [45]. Notably, TCP10, TCP02, and TCP26 maintained higher qP values than the parents at 7- and 14-DAS, suggesting superior photoprotective mechanisms.

Meanwhile, qN, an indicator of non-photochemical quenching, was significantly higher in TCP25 at 7-DAS and in TCP23, TCP11, TCP25, and MB44 at 14-DAS, highlighting their enhanced photoprotection and reduced energy conversion under stress [26]. These findings align with previous reports [45, 47]

4.6 Submergence tolerance and genetic implications

Several BILs, including TCP02, TCP10, TCP12, TCP14, TCP23, and TCP18, exhibited superior F_v/F_m values compared to the parental lines, indicating greater PSII stability under prolonged submergence. These findings agree with prior studies [26, 29, 42, 45, 47–49]. The increased tolerance of these BILs to 14-days of submergence may be attributed to their enhanced photosynthetic efficiency and photoprotective mechanisms. The observed variations in physiological responses among the genotypes highlight the genetic diversity influencing submergence tolerance. Significant differences were observed in total chlorophyll content, ETR, and F_v/F_m ratio at 14-DAS, reinforcing the impact of prolonged submergence on these physiological traits. Prior studies have also demonstrated that submergence depth and duration critically influence seedling survival [50]. Our findings suggest that the selected BILs hold promise as improved restorer lines and genetic resources for submergence tolerance up to 14-days of flooding.

5 Conclusion

This study is the first to report the enhancement of restorer lines for submergence tolerance in rice in India. The findings revealed significant genotype-specific variations in response to different durations of submergence stress. All genotypes exhibited stability up to 7-DAS. Notably, the BILs- TCP15, TCP18, TCP25, and TCP28 demonstrated superior regeneration ability compared to Swarna-Sub1, highlighting their stable photosystem II (PS-II) activity at 14-DAS. Based on assessments of chlorophyll fluorescence parameters and regeneration potential, the BILs TCP15, TCP18, TCP25, TCP28, MB44, and TCP02 emerged as promising restorer lines for submergence tolerance at 14-DAS. These lines hold significant potential for developing submergence-tolerant rice hybrids, particularly suitable for flood-prone regions.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44372-025-00382-2.

Supplementary Material 1

Acknowledgements

The authors were grateful to Indian council of Agriculture Research (ICAR), Indian Institute of Rice Research (IIRR), Department of Biotechnology (DBT).

Author contributions

Dr P Senguttuvel conceptualized and designed the study. Material preparation and data collection were carried out by Mrs Y Manasa, Ms P Beulah, Mr KK Raghuraman and Mr M Arivin. Data analysis was conducted by Dr G Karthika, Mr Tripura Venkata VGN and Dr V Jaldhani. Dr D Sanjeeva Rao and Dr P Raghuveer Rao provided support for the chlorophyll

fluorescence analysis in the physiology department. Mrs Y Manasa drafted the initial version of the manuscript, with all authors contributing to revisions. The manuscript was edited by Dr SK Mangrauthia, Dr AS HariPrasad, Dr P Sudhakar, Dr A Krishna Satya and Dr RM Sundaram. All authors reviewed and approved the final version.

Funding
This study was supported by the funding received from Department of Biotechnology (DBT) [Sanction order Number and Date: BT/PR7488/AGIII/103/865/2013] and Indian council of Agriculture Research (ICAR)-Indian Institute of Rice Research (IIRR).

Data availability
The datasets generated and/or analysed during the current study are included in this article and its supplementary information files.

Declarations

Ethics approval and consent to participate
All the experiments carried out on plants were carried out in accordance with the guidelines of ICAR – Indian Institute of Rice Research.

Consent for publication
Not applicable.

Plant reproducibility
Rice (*Oryza sativa* L.) varieties used in this study were developed through research experiments conducted at the ICAR–Indian Institute of Rice Research (ICAR–IIRR), Hyderabad, India. The plant material was taxonomically authenticated by Dr. P. Senguttuvel, Principal Scientist (Hybrid Rice), ICAR–IIRR. Representative seed samples are preserved at ICAR–IIRR for future reference. All procedures for plant collection and experimentation adhered to institutional, national, and international guidelines and regulations, in accordance with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

Competing interests
The authors declare no competing interests.

Received: 6 March 2025 / Accepted: 15 October 2025
Published online: 26 October 2025

References

1. FAS/USDA. Approved by the World Agricultural Outlook Board Russia Wheat: Frost and Dryness Damage Crops Across European Russia World Agricultural Production, 1–40. 2024.
2. Fukao T, Barrera-Figueroa BE, Juntawong P, Peña-Castro JM. Submergence and waterlogging stress in plants: a review highlighting research opportunities and understudied aspects. Front Plant Sci. 2019;10:437311. https://doi.org/10.3389/FPLS.2019.00340/PDF.
3. Chen Y, Song J, Yan C, Hong X. Effects of submergence stress at the vegetative growth stage on hybrid rice growth and grain yield in China. Chil J Agric Res. 2021;81(2):191–201. https://doi.org/10.4067/S0718-58392021000200191.
4. Dar MH, Zaidi NW, Waza SA, Verulkar SB, Ahmed T, Singh PK, et al. No yield penalty under favorable conditions paving the way for successful adoption of flood tolerant rice. Sci Rep. 2018;8(1):1–7. https://doi.org/10.1038/s41598-018-27648-y.
5. Voesenek LACJ, Bailey-Serres J. Flood adaptive traits and processes: an overview. New Phytol. 2015;206(1):57–73. https://doi.org/10.1111/nph.13209.
6. Das G, Pradhan B, Bastia DN, Verma R. Effect of submergence stress on agro-morphological and physiological traits in rice (*Oryza Sativa* L.) genotypes. Asian J Microbiol Biotechnol Environ Sci. 2022;24(2):327–34. https://doi.org/10.53550/ajmbes.2022.v24i02.019.
7. Zhang Y, Xu J, Li R, Ge Y, Li Y, Li R. Plants' response to abiotic stress: mechanisms and strategies. Int J Mol Sci. 2023;24(13):1–17. https://doi.org/10.3390/ijms241310915.
8. Debabrata P, Kumar SR. Improvement of photosynthesis by Sub1 QTL in rice under submergence: probed by chlorophyll fluorescence OJIP transients. J Stress Physiol Biochem. 2011.
9. Panda D, Barik J. Flooding tolerance in rice: focus on mechanisms and approaches. Rice Sci. 2021;28:43–57. https://doi.org/10.1016/j.rsci.2020.11.006.
10. Goswami S, Kar RK, Paul A, Dey N. Genetic potentiality of indigenous rice genotypes from Eastern India with reference to submergence tolerance and deepwater traits. Curr Plant Biol. 2017;11–12:23–32. https://doi.org/10.1016/j.cpb.2017.10.002.
11. 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(7103):705–8. https://doi.org/10.1038/NATURE04920.
12. Sarkar RK, Reddy JN, Sharma SG, Ismail AM. Physiological basis of submergence tolerance in rice and implications for crop improvement. Review Articles Current Science. 2006.
13. Das KK, Panda D, Sarkar RK, Reddy JN, Ismail AM. Submergence tolerance in relation to variable floodwater conditions in rice. Environ Exp Bot. 2009;66(3):425–34. https://doi.org/10.1016/j.envexpbot.2009.02.015.
14. Hussain W, Anumalla M, Ismail AM, Walia H, Singh VK, Kohli A, et al. Revisiting FR13A for submergence tolerance: beyond the SUB1A gene. J Exp Bot. 2024. https://doi.org/10.1093/JXB/ERAE299.
15. 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 Res. 2013;152:83–93. https://doi.org/10.1016/J.FCR.2013.01.007.

16. Goswami S, Kar RK, Paul A, Dey N. Differential expression of Sub1A loci in rice under submergence. J Plant Biochem Biotechnol. 2018;27(4):473–7. https://doi.org/10.1007/s13562-018-0456-8.
17. Manasa Y, Beulah P, Nagaraju P, Jaldhani V, Arivin M, Raghuraman KK, et al. Evaluation of submergence tolerant improved restorer lines for fertility restoration and phosphorus use efficiency using gene-specific markers in rice (*Oryza sativa* L.). J Adv BiolBiotechnol. 2024;27(12):391–400. https://doi.org/10.9734/jabb/2024/v27i121787.
18. IRRI (International Rice Research Institute). 2014. Standard Evaluation System for rice (SES) 5th edition. Los Baños (Philippines): International Rice Research Institute. 5 th Edition June 2014. 2014.
19. Sarkar RK, Bhattacharjee B. Rice genotypes with SUB1 QTL differ in submergence tolerance, elongation ability during submergence and re-generation growth at re-emergence. Rice. 2011;5(1):1–11. https://doi.org/10.1007/s12284-011-9065-z.
20. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 1949;24(1):1–15. https://doi.org/10.1104/pp.24.1.1.
21. Fukao T, Bailey-Serres J. Submergence tolerance conferred by Sub1A is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc Natl Acad Sci U S A. 2008. https://doi.org/10.1073/pnas.0807821105.
22. 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(2–3):138–47. https://doi.org/10.1007/s12284-010-9048-5.
23. Alpuerto JB, Hussain RMF, Fukao T. The key regulator of submergence tolerance, SUB1A, promotes photosynthetic and metabolic recovery from submergence damage in rice leaves. Plant Cell Environ. 2016;39(3):672–84. https://doi.org/10.1111/pce.12661.
24. Das KK, Sarkar RK, Ismail AM. Elongation ability and non-structural carbohydrate levels in relation to submergence tolerance in rice. Plant Sci. 2005;168(1):131–6. https://doi.org/10.1016/j.plantsci.2004.07.023.
25. Fukao T, Xu K, Ronald PC, Bailey-Serres J. A variable cluster of ethylene response factor-like genes regulates metabolic and developmental acclimation responses to submergence in rice. Plant Cell. 2006;18(8):2021–34. https://doi.org/10.1105/tpc.106.043000.
26. Pradhan B, Chakraborty K, Prusty N, Deepa, Mukherjee AK, Chattopadhyay K, et al. Distinction and characterisation of rice genotypes tolerant to combined stresses of salinity and partial submergence, proved by a high-resolution chlorophyll fluorescence imaging system. Funct Plant Biol. 2019;46(3):248–61. https://doi.org/10.1071/FP18157.
27. Samanta P, Ganie SA, Chakraborty A, Dey N. Study on regulation of carbohydrate usage in a heterogeneous rice population under submergence. J Plant Biochem Biotechnol. 2020;30(1):138–46. https://doi.org/10.1007/s13562-020-00577-6.
28. Fukao T, Yeung E, Bailey-Serres J. The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. Plant Cell. 2011;23(1):412–27. https://doi.org/10.1105/tpc.110.080325.
29. Vijayalakshmi D, Muthulakshmi S, Arumugaperumal M, Raveendran M. Chlorophyll fluorescence and photosynthetic gas exchange in submergent tolerant CO 43 sub1 lines. Plant Physiol Rep. 2020;25(2):245–54. https://doi.org/10.1007/s40502-020-00520-w.
30. Elanchezhian R, Kumar S, Singh SS, Dwivedi SK, Shivani S, Bhatt BP. Plant survival, growth and yield attributing traits of rice (*Oryza sativa* L.) genotypes under submergence stress in rainfed lowland ecosystem. Indian J Plant Physiol. 2013;18(4):326–32. https://doi.org/10.1007/s40502-013-0050-y.
31. Panda D, Rao DN, Das KK, Sarkar RK. Role of starch hydrolytic enzymes and phosphatases in relation to under water seedling establishment in rice. Indian J Plant Physiol. 2017;22(3):279–86. https://doi.org/10.1007/s40502-017-0305-0.
32. Colmer TD, Voesenek LACJ. Flooding tolerance: suites of plant traits in variable environments. Funct Plant Biol. 2009;36(8):665–81. https://doi.org/10.1071/FP09144.
33. Oladosu Y, Rafii MY, Arolu F, Chukwu SC, Muhammad I, Kareem I, et al. Submergence tolerance in rice: review of mechanism, breeding and future prospects. Sustainability (Switzerland). 2020. https://doi.org/10.3390/su12041632.
34. Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, et al. The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. Nature. 2009;460(7258):1026–30. https://doi.org/10.1038/nature08258.
35. Masuduzzaman ASM, Haque MM, Shamsuddin AKM, Salam MA, Kabir MS, Masouleh AK, et al. Variation in expression of Sub1 gene confers differential response to submergence tolerance in rice (*Oryza sativa* L.). Aust J Crop Sci. 2017;11(6):757–67. https://doi.org/10.21475/ajcs.17.11.06.p446.
36. Fukao T, Yeung E, Bailey-Serres J. The submergence tolerance gene SUB1A delays leaf senescence under prolonged darkness through hormonal regulation in rice. Plant Physiol. 2012;160(4):1795–807. https://doi.org/10.1104/pp.112.207738.
37. Saha I, De AK, Sarkar B, Ghosh A, Dey N, Adak MK. Cellular response of oxidative stress when sub1A QTL of rice receives water deficit stress. Plant Sci Today. 2018;5(3):84–94. https://doi.org/10.14719/pst.2018.5.3.387.
38. Singh N, Dang TTM, Vergara GV, Pandey DM, Sanchez D, Neeraja CN, et al. Molecular marker survey and expression analyses of the rice submergence-tolerance gene SUB1A. Theor Appl Genet. 2010;121(8):1441–53. https://doi.org/10.1007/s00122-010-1400-z.
39. Wang J, Han M, Huang Y, Zhao J, Liu C, Ma Y. Flooding tolerance of rice: regulatory pathways and adaptive mechanisms. Plants. 2024. https://doi.org/10.3390/plants13091178.
40. Banerjee S, Ghosh N, Mandal C, Dey N, Kumar AM. Physiological basis of submergence tolerance in rice genotypes with reference to carbohydrate metabolism. Plant Gene Trait. 2015. https://doi.org/10.5376/pgt.2015.06.0002.
41. Sasidharan R, Voesenek LACJ. Lowland rice: high-end submergence tolerance. New Phytol. 2013. https://doi.org/10.1111/nph.12148.
42. Beulah P, Manasa Y, Karthika G, Nagaraju P, Jaldhani V, Gobinath R, et al. Physiological changes and evaluation of Saltol1 introgressed backcrossed inbred lines (BILs) for early seedling stage salinity tolerance in rice (*Oryza sativa* L.). Russ J Plant Physiol. 2024;71(1):10. https://doi.org/10.1134/S1021443723602057.
43. Panda D, Sharma SG, Sarkar RK. Chlorophyll fluorescence parameters, CO_2 photosynthetic rate and regeneration capacity as a result of complete submergence and subsequent re-emergence in rice (*Oryza sativa* L.). Aquat Bot. 2008;88(2):127–33. https://doi.org/10.1016/j.aquabot.2007.08.012.
44. Panda D, Sarkar RK. Leaf photosynthetic activity and antioxidant defense associated with Sub1 QTL in rice subjected to submergence and subsequent re-aeration. Rice Sci. 2012;19(2):108–16. https://doi.org/10.1016/S1672-6308(12)60029-8.
45. Barik J, Panda D, Mohanty SK, Lenka SK. Leaf photosynthesis and antioxidant response in selected traditional rice landraces of Jeypore tract of Odisha, India to submergence. Physiol Mol Biol Plants. 2019;25(4):847–63. https://doi.org/10.1007/s12298-019-00671-7.

46. Hazrati S, Tahmasebi-Sarvestani Z, Modarres-Sanavy SAM, Mokhtassi-Bidgoli A, Nicola S. Effects of water stress and light intensity on chlorophyll fluorescence parameters and pigments of *Aloe vera* L. Plant Physiol Biochem. 2016;106:141–8. https://doi.org/10.1016/j.plaphy.2016.04.046.
47. Olorunwa OJ, Adhikari B, Shi A, Barickman TC. Screening of cowpea (*Vigna unguiculata* (L.) Walp.) genotypes for waterlogging tolerance using morpho-physiological traits at early growth stage. Plant Sci. 2022;315:111136. https://doi.org/10.1016/j.plantsci.2021.111136.
48. Jaldhani V, Rao DS, Beulah P, Srikanth B, Rao PR, Subrahmanyam D, et al. Assessment of heat-tolerance potential in QTL introgressed lines of hybrid rice restorer, KMR-3R through PS-II efficiency. Int J Environ Clim Change. 2021;11(11):258–66. https://doi.org/10.9734/IJECC/2021/V11I1130540.
49. Bathula S, Jaldhani V, Suman K, Sanjeeva Rao D, Subrahmanyam D, Raghuveer Rao P, et al. Screening rice genotypes for nitrogen efficiency under graded nitrogen application. Plant Science Today, 2025; 12(1). https://doi.org/10.14719/pst.4525
50. Rachmawati D, Maryani MM, Kusumadewi S, Rahayu F. Survival and root structure changes of rice seedlings in different cultivars under submergence condition. Biodiversitas. 2019;20(10):3011–7. https://doi.org/10.13057/biodiv/d201033.

Publisher's note