J. Appl. Res Plant Sci. Vol. 5(2), 237-248, **2024,** https://doi.org/10.38211/joarps.2024.05.277

Research Article

OPEN ACCESS

Available on https://www.joarps.org
Journal of Applied Research in Plant Sciences (JOARPS)
ISSN: 2708-3004 (Online), 2708-2997 (Print)

Phenotypic and Genotypic Screening of Green Super Rice Genotypes for Submergence Tolerance at Seedling Stage

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Article Received 24-02-2023, Article Revised 20-04-2024, Article Accepted 12-05-2024.

Abstract

Climate change threatens rice-based systems, especially in areas where crops are sensitive to temperature fluctuations. Rice production is significantly impacted by extreme weather events, and persistent and heavy rainfalls which cause floods lead to submergence stress. The primary focus of this research was the evaluation of rice genotypes under controlled flooding conditions. The study included nine green super rice lines and one local control variety based on better tolerance against submergence stress. Using genotyping based on SSR markers, the goal was to better understand how rice germplasm responded to submergence stress at the seedling stage. The treatments included: T0, the control without submergence stress; T1, submergence for 10 days with ethylene treatment; and T2, submergence for 10 days without ethylene treatment. Ten genotypes were tested under complete seedling submergence. Submergence-tolerant genotypes were identified using the RM23877 SSR marker for genotyping. The results highlighted significant genotypic variations (alleles and genetic markers associated with submergence tolerance) in response to submergence stress affected by ethylene treatment, with variable effects observed for different genotypes. Most genotypes had zero survival except for GSR-4 and GSR-61, followed by GSR-5, GSR-13, GSR-2, and Chenab basmati. SSR marker-based genotyping further revealed that six out of ten genotypes present the submergence tolerance allele. We observed significant genotypic variations in the alleles associated with submergence tolerance, including differences in the *Sub1A-1*, *Sub1B*, and *Sub1C* genes among the GSR genotypes. These findings lay the groundwork for marker-assisted selection in breeding programs to develop rice varieties with enhanced submergence tolerance.

Keywords: Climate change; extreme weather events; rice; sustainability; improved breeding.

Introduction

Rice is the primary staple crop in many developing countries, especially those located in the humid tropics. Asia and other tropical and subtropical developing countries account for nearly 90% of rice production and consumption (Al-Hashimi, 2023). Rice, scientifically known as Oryza sativa, is one of the most significant crops globally, providing over half of the world's daily calorie intake and feeding almost half of the world's population (Muthayya, Sugimoto, Montgomery, & Maberly, 2014). Climate change has a significant impact on agricultural production, especially for temperature-sensitive crops (Vasilyev, Kuzichkin, Surzhik, & Koskin, 2023). Changes in global temperature, rainfall patterns, and variability of rainfall during the monsoon season put pressure on agricultural practices (Habib-ur-Rahman *et al.*, 2022). Rice crop variability is strongly influenced by the variability of monsoon rainfall from year to year (Bowden, Foster, & Parkes, 2023). Rising temperatures during the summer in Asia have contributed to past and present climate change trends and variability, with the greatest impact of climate change seen through extreme weather events affecting rice production and food security (Malhi, Kaur, & Kaushik, 2021). Its adaptability to diverse environments and high nutritional value makes it an indispensable crop, crucial for ensuring food security in flood-prone areas. However, the escalating impacts of climate change, including the increased frequency and severity of flooding events, pose significant challenges to rice production and food security.

Rainfall frequency and duration are critical factors in rice farming. Heavy rainfall during July and August in South Asian countries can negatively impact rice

production, causing flooding or rising river water levels in rivers and reducing rice yield (Su & Kuo, 2023). Climate extremes, including salinity, drought, flooding, and temperature extremes, continue to challenge rice and other agricultural production systems (Hasanuzzaman *et al.*, 2018; Liaqa, Shakeel, Khalid, Amjad, & Saeed, 2023; Nawaz *et al.*, 2023; Saeed, Hayat, Shafiq, & Tareen, 2023). Submergence is another abiotic factor that negatively affects the growth and yield of rice due to delayed gas exchange rates, intense water shading, mechanical damage, and solute transportation (Mahmood *et al.*, 2019; Michael & Phool, 2001; Sarma *et al.*, 2023). Submergence is a critical abiotic stress factor that adversely affects rice production, particularly in low-lying and flood-prone regions. Prolonged inundation of rice fields leads to oxygen deprivation, hindering plant respiration and nutrient uptake which results in reduced photosynthesis, impaired growth, and ultimately significant yield losses. The vulnerability of rice to submergence stress underscores the urgent need for developing submergence-tolerant varieties to mitigate the negative impacts on food security and agricultural sustainability (Mahmood *et al.*, 2019; Michael & Phool, 2001; Sarma *et al.*, 2023). Pakistan's agriculture sector has suffered from climate change, with excessive variability in floods and droughts reducing rice yields by 6 to 18% in Punjab's arid and semi-arid regions (A. Ali & Erenstein, 2017; S. Ali *et al.*, 2023). The country has also been significantly affected by floods in recent years, with the 2022 floods causing severe damage to crop, livestock, and infrastructure. Grain storage facilities holding millions of tonnes of grain were severely impacted, posing a risk to the nation's food security. The flood inundation resulted in a projected loss of 1.9 million tons of rice, which represents an 80% loss of total rice production (Stallworth, Shrestha, Schumaker, Roma-Burgos, & Tseng, 2021). Incorporating submergence tolerance traits into GSR varieties is paramount for maintaining productivity in flood-prone areas. As climate change intensifies, the frequency and severity of flooding events are expected to increase, posing a significant threat to rice production. GSR varieties with enhanced submergence tolerance offer a sustainable solution by ensuring crop resilience and minimizing yield losses in flood-affected regions. Rice cultivars have varying levels of resilience to submersion, with older plants being more tolerant of complete submergence than younger ones. Semi-dwarf cultivars and inhibited leaf elongation and lower carbohydrate consumption while submerged have been linked to survival and tolerance of rice cultivars (Gao, Chao, & Lin, 2007; Stallworth *et al.*, 2021). The purpose of this study is to evaluate the response of GSR lines against submergence. Climate change poses a significant threat to rice productivity, particularly in South Asia, where the frequency and intensity of extreme weather events such as floods and heavy rainfall are increasing. Submergence stress due to prolonged flooding can cause severe damage to rice crops, leading to substantial yield losses. This region, heavily dependent on rice as a staple food and a major agricultural commodity, faces heightened risks to food security and the livelihoods of millions of farmers. Developing submergence-tolerant rice varieties through advanced breeding techniques is essential to mitigate these impacts and sustain rice production under changing climatic conditions.

Material and Methods

Experimental Site and Material Description: The experiment for this study was carried out at Rice Molecular Breeding Laboratory and field area of Rice Research Program, Crop Sciences Institute, National Agriculture Research Centre, Islamabad between June and July 2023. In this experiment, 08 coarse rice GSR lines obtained from PGRI, and NARC with two check varieties were used against submergence stress (Table 1) at the seedling stage. The genotypes were selected based on their known potential submergence tolerance traits which encompass a mix of GSR genotypes and other promising lines identified through extensive screening for submergence tolerance traits.

Table 1. List of Genotypes employed in the current study.

Sr. No.	Source 2021	Codes 2022
1	GSR-5	S-1
2	GSR-61	S-2
3	GSR-13	S-3
4	GSR-2	S-4
5	GSR-4	S-5
6	GSR-16	S-6
7	GSR-62	S-7
8	GSR-59	S-8
9	IR-6	S-9
10	Chenab basmati	S10

Experimental Design: The experiment was conducted in two replications with three treatments; (T0) control not submerged, (T1) plants treated with ethylene completely submerged, and (T2) plants without ethylene treatment and submerged. A nursery bed was set up, and 10 different cultivars' seeds were planted in

it individually. Seedlings were transplanted into tiny pots 15 days after planting. In water tanks with a 45 cm water depth, seedlings were entirely submerged 15 days after transplanting. Regular water additions to the water tanks kept the water at the desired depth. Plants were retrieved from the water tank after 10 days of total submergence. Using the rate of seedling survival 10 days after de-submergence, submergence-tolerant genotypes were found. Data of seedlings was recorded before submergence stress and 10 days after submergence stress. Plant height, root length, and shoot length data were measured using the scale in centimeters. The Standard Evaluation System (SES) score for submergence tolerance in rice (IRRI, 2002) was used to evaluate the genotypes.

The stress indices were calculated by using the following formulas (Barik, Kumar, Lenka, & Panda, 2020):

$$Survival\ \% = \frac{Total\ No.of\ survived\ seedlings}{Total\ No.of\ seedlings\ before\ submergence} \times\ 100$$

$$Elongation\ \% = \frac{P.height\ before\ submergence\ -\ P.height\ after\ desubmergece}{Plant\ height\ after\ desubmergece} \times\ 100$$

SSR marker-based selection: In the DNA extraction process, DNA was obtained from fresh leaves at the 3-leaf stage through two methods. Firstly, the GeneJET Plant Genomic DNA Purification Mini Kit was utilized according to Thermo Fisher Scientific's standard protocol. Additionally, a Cetyl Trimethyl Ammonium Bromide (CTAB) based method with minor adjustments was employed. This involved grinding 2-3 young rice leaves into powder using liquid nitrogen, followed by incubation in pre-heated 2×CTAB extraction buffer at 65°C for 45 minutes. After centrifugation and ethanol washes, the DNA was dissolved in nuclease-free water, and RNA was digested with RNAse A at 37°C. Subsequently, DNA was precipitated, the pellet was washed, and it was finally dissolved in 100µl of nuclease-free water. The quantification of DNA was carried out using the QubitTM 4 Flourometer.

For Polymerase Chain Reaction (PCR), the Bio-Rad T100TM Thermal Cycler was used to perform PCR on all genotypes. The PCR reaction mixture contained 2× Dream Master Mix, forward and reverse primers, template DNA, and ddH2O. The PCR protocol included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 53-64°C (depending on primer melting temperature), and extension at 72°C for 30 seconds. A final extension was conducted at 72°C for 10 minutes. The resulting amplified products were stored at -20°C for future use.

In the context of Gel Electrophoresis, Documentation, and Allele Scoring, the amplified PCR products were separated by size in a 2% agarose gel at 80V for 1.5 hours using 1×TAE buffer. Visualization of genotype-specific bands was achieved through ethidium bromide staining, which was then digitally documented using a Gel Documentation system. The size of the amplicons was determined by comparison with a 100 bp DNA ladder. Qualitative scoring was conducted to assess the presence (score 1) or absence (score 0) of the marker allele in each genotype. Null alleles were assigned in cases where no amplification product was detected. Regarding the design of the SSR Primer Pair, an SSR primer pair was selected for the evaluation of rice genotypes with submergence tolerance based on a well-established map of rice microsatellites. Specifically, the RM23877-linked SSR marker (F: TGCCACATGTTGAGAGTGATGC; R: TACGCAAGCCATGACAATTCG), located on Chromosome 9, was chosen following prior research conducted by (Sultana, Islam, Hassan, Rahman, & Haque, 2019).

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Results

Morphological Parameters: Average aggregated data for yield components represented that the mean values were evidentiary varying among genotypes as well as between treatments. Table 2 provides a comprehensive dataset illustrating the complex relationships between genotypic variations, treatments, and various physiological parameters. Genotype 2 consistently displays higher mean values, especially in SL (shoot length) and WDW (whole plant dry weight). Genotypic and treatment-based differences were observed, with Genotype 2 exhibiting longer root length shoot length, and plant height. Discussing the plant height (Figure 1) shows the maximum plant height in (control) T0 before stress in genotype 2 (16.16cm) whereas the minimum was recorded in genotype 10. Phenotypic screening involves the evaluation of observable traits in plants, such as survival rate, growth recovery, and vigor after submergence. These traits are crucial indicators of submergence tolerance in rice genotypes, reflecting their ability to withstand and recover from flooding stress. By conducting phenotypic screening, we can identify promising genotypes with enhanced submergence tolerance, which is essential for developing resilient rice varieties capable of maintaining productivity in flood-prone areas

Table 2: Morphological Data of the Rice genotypes before and after submergence.

	Genotype	TRT	PH mv±sd	RL mv±sd	SL mv±sd	WDW mv±sd	wfw mv±sd
Before	1	0	10.131 ± 0.02 g	9.1833±1.5AB	1.3146±0.5D	0.1195±0.02BCD	0.1635±0.02BCD
	1	1	9.9812±0.02 G	9.3500±1.06ABC	3.0646±0.5C	0.0235±0.02BCD	0.0835±0.02BCD
	1	2	9.0896±0.02 I	9.4000±1.4A	2.6062±0.8DEF	0.0214±0.01D	0.0671±0.01D
	2	0	16.165±0.02A	10.983±2.4A	5.4812±1.13A	0.1870±0.002A	0.2310±0.002A
	2	1	16.015±0.02A	10.483±2.4AB	7.2312±1.13A	0.0910±0.002A	0.1510±0.002A
	2	2	12.356±0.02D	8.1167±0.02AB	4.6896±0.25A	0.0607±0.02B	0.1064±0.02B
	3	0	10.331±0.02F	7.9167±0.11BCD	3.6979±0.77B	0.0965±0.003D	0.1405±0.003D
	3	1	10.181± 0.02F	7.4167±0.11CD	5.4479±0.77B	0.0102±0.01D	0.0605±0.003D
	3	2	11.856±0.02E	6.2500±0.2BCD	3.0063±0.75DEF	0.0593±0.0001BC	0.1050±0.0001BC
	4	0	11.531±0.02C	8.7167±2.0AB	3.3979±0.35BC	0.1322±0.010BC	0.1762±0.010BC
	4	1	11.381±0.02C	8.2167±2.0BC	5.1479±0.35B	0.0362±0.01BC	0.0962±0.010BC
	4	2	11.523±0.02F	4.7000±0.56CDE	2.1562±0.87F	0.0305±0.005CD	0.0762±0.005CD
	5	0	12.465±0.02B	5.3333±1.6D	6.0479±0.18A	0.1415±0.01B	0.1855±0.01B
	5	1	12.315±0.02B	4.8333±1.6D	7.7979±0.18A	0.0455±0.01B	0.1055±0.01B
	5	2	12.690±0.02B	5.3000±0.14CDE	3.2396±0.09CDEF	0.0613±0.011B	0.1070±0.011B
	6	0	9.8313±0.02H	5.4333±0.65CD	2.3479±0.42CD	0.1317±0.0009BC	0.1757±0.0009BC
	6	1	9.6812±0.02H	4.9333±0.65D	2.7646±0.51C	0.0357±0.0009BC	0.0957±0.0009BC
	6	2	12.690±0.02B	4.2333±0.32DE	4.2562±0.35ABC	0.0410±0.0001BCD	0.0867±0.0001BCD
	7	0	10.331±0.02F	10.100±0.84AB	3.4313±0.58BC	0.1137±0.007CD	0.1577±0.007CD
	7	1	10.181±0.02F	12.033±0.04A	3.3479±0.35C	0.0237±0.0009BCD	0.0777±0.007CD
	7	2	12.456±0.02C	3.3667±0.32E	3.6896±0.44ABCD	0.0601±0.0009BC	0.1058±0.009BC
	8	0	10.965±0.02E	8.4000±0.70ABC	3.5146±0.04BC	0.1135±0.01CD	0.1575±0.01CD
	8	1	10.815±0.02E	7.9000±0.70BC	2.5979±1.22C	0.0175±0.01CD	0.0775±0.01CD
	8	2	13.523±0.02A	6.6500±2.19BC	4.5562±0.02AB	0.0405±0.01BCD	0.0862±0.01BCD
	9	0	11.165±0.02D	7.1167±1.24BCD	3.8146±0.80B	0.1392±0.008B	0.183±0.0082B
	9	1	11.015±0.02D	6.6167±1.24CD	3.1812±0.21C	0.0432±0.008B	0.1032±0.008B
	9	2	11.156±0.02G	5.7833±1.10CD	3.4896±0.54BCDE	0.0993±0.02A	0.1450±0.02A
	10	0	8.6313±0.02I	7.7500±0.35BCD	2.3479±0.14CD	0.1052±0.01D	0.1492±0.015D
	10	1	8.4812±0.02I	7.2500±0.35CD	1.7312±1.03C	0.0332±0.001BC	0.0692±0.015D
	10	2	9.6563±0.02H	6.8167±1.10BC	2.5063±0.18EF	0.0228±0.004D	0.0685±0.004D
	Genotype	**TRT**	**PH mv±sd**	**RL mv±sd**	**SL mv±sd**	**WDW mv±sd**	**wfw mv±sd**
After	1	0	15.780±0.05G	10.307±0.05B	7.3917±0.05H	0.1420±0.05A	0.2200±0.05A
	1	1	10.265±5.22CD	3.8650±0.51BC	4.7250±4.63BCD	0.0105±0.01BC	0.0602±0.05CD
	1	2	13.500±0.70ABC	8.6667±1.88A	5.8333±1.6A	0.0831±0.007A	0.3009±0.08A
	2	0	21.801±0.05A	9.3067±0.05D	10.392±0.05B	0.1950±0.05A	0.2730±0.05A
	2	1	18.667±0.94AB	5.8333±4.00AB	7.0000±1.41ABC	0.1091±0.02A	0.1733±0.03B

	2	2	15.225±2.5A	9.500021.A	5.8917±2.9A	0.0618±0.01AB	0.3211±0.14A
	3	0	15.968±0.05F	7.8733±0.05F	8.8583±0.05D	0.1000±0.05A	0.1780±0.05A
	3	1	19.167±1.17A	4.1667±0.23BC	10.667±0.47A	0.0372±0.02BC	0.3157±0.12A
	3	2	14.150±3.0AB	9.4167±0.8A	5.9833±5.6A	0.0557±0.01AB	0.2155±0.11A
	4	0	17.16±0.05C	10.173±0.05C	9.3583±0.05C	0.1453±0.05A	0.2233±0.05A
	4	1	13.167±1.17ABC	2.6667±0.94BCD	6.5000±0.70ABC	0.0436±0.03BC	0.1192±0.03BC
	4	2	12.825±0.24ABC	3.8333±0.23B	4.3350±2.3A	0.0688±0.008AB	0.2501±0.10A
	5	0	18.101±0.05B	4.2067±0.05J	11.625±0.05A	0.1400±0.05A	0.2180±0.05A
	5	1	14.150±0.21ABC	1.7333±1.60CD	6.6667±0.94ABC	0.0588±0.02ABC	0.1440±0.03BC
	5	2	14.694±0.43AB	3.9333±0.51B	5.3602±0.9A	0.0678±0.004AB	0.2560±0.09A
	6	0	15.468±0.05H	5.0067±0.05I	7.7583±0.05G	0.1383±0.05A	0.2163±0.05A
	6	1	11.945±2.90BCD	4.7167±1.107BC	5.1333±0.61ABCD	0.0527±0.03ABC	0.1501±0.06BC
	6	2	12.845±0.21ABC	3.1667±0.23B	4.5117±0.7A	0.0560±0.01AB	0.2029±0.05AB
	7	0	15.968±0.05F	10.740±0.05A	8.7250±0.05E	0.1143±0.05A	0.1923±0.05A
	7	1	11.744±2.20BCD	6.2167±0.40AB	9.0250±6.04AB	0.0450±0.03BC	0.0857±0.01BCD
	7	2	9.7450±3.8C	3.9167±0.11B	4.2250±3.4A	0.0580±0.02AB	0.2328±0.10A
	8	0	16.601±0.05E	8.9400±0.05E	9.2583±0.05C	0.1270±0.05A	0.2050±0.05A
	8	1	5.4000±7.63D	0.0000±0D	0.0000±0D	0.0000±0C	0.00000±0D
	8	2	13.750±0.3ABC	2.5000±3.5B	5.0000±7.07A	0.0000±0C	0.0000±0C
	9	0	16.801±0.05D	6.2733±0.05H	8.9583±0.05D	0.1513±0.05A	0.2293±0.05A
	9	1	11.480±0.67BCD	8.4500±2.4A	3.2333±0.32CD	0.0313±0.01BC	0.0604±0.01BCD
	9	2	12.595±0.5ABC	5.1667±0.23B	4.0333±0.04A	0.0373±0.04BC	0.2113±0.04A
	10	0	14.268±0.05I	7.5400±0.05G	7.9583±0.05F	0.1223±0.05A	0.2003±0.05A
	10	1	11.995±1.26ABCD	4.9167±0.02ABC	1.7500±2.19CD	0.0653±0.04AB	0.0497±0.002CD
	10	2	10.750±1.06BC	3.3333±0.47B	3.6667±0.94A	0.0506±0.05AB	9.40E-03±0.0005BC

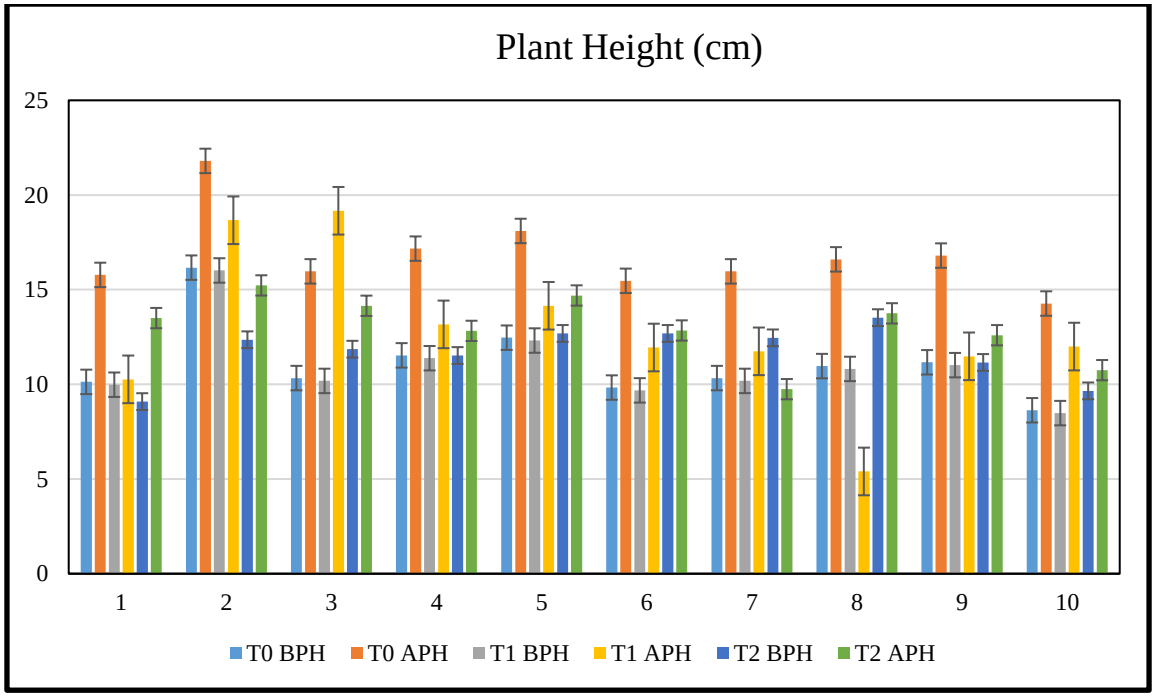

Figure 1. Variation in Plant height (cm) of different genotypes before and after submergence stress.

After submergence stress APH (after stress plant height), plant heights range from approximately 10.27 cm to 21.80 cm. The shift from BPH to APH signifies the response to submergence stress under control conditions. In T1, BPH (before stress plant height) values range from approximately 8.48 cm to 16.01 cm, and after submergence stress (APH), plant heights range from approximately 5.40 cm to 19.17 cm. This indicates how ethylene treatment influences the response to submergence stress. In T2, BPH values range from approximately 9.09 to 13.52, and after submergence stress (APH), plant heights range from approximately 9.75 cm to 15.23 cm, reflecting the response without ethylene treatment. Overall, submergence stress tends to impact plant height, with varied magnitudes across treatments.

The data demonstrates a consistent impact of submergence stress on plant height, with an increase in heights after stress across all treatments. Ethylene treatment in T1 yields a diverse response, including instances of decreased plant height, emphasizing the modulatory effect of ethylene. Similarly, T2, without ethylene treatment, exhibits varying responses. Analysis of the root length data reveals variations among genotypes. Figure 2 illustrates root length measurements (before stress root length) BRL in T0 before stress, ranging from approximately 5.33 cm to 10.98 cm. After submergence stress ARL (after stress root length), root lengths range from approximately 1.73 cm to 10.31 cm (Figure 2), representing the response under control conditions. In T1, BRL (before stress root length) values range from approximately 4.21 to 10.48, and after submergence stress (ARL), root lengths range from approximately 0 to 8.67cm (Figure 2). This indicates how ethylene treatment influences the response to submergence stress.

In T2, BRL values range from approximately 3.17 cm to 9.42 cm, and after submergence stress (ARL), root lengths range from approximately 2.5 cm to 9.5 cm, reflecting the response without ethylene treatment. For instance, genotype 4 shows a substantial increase in ARL under T1, suggesting a potential positive effect of ethylene, while genotype 8 exhibits a marked reduction, indicating a possible negative impact. The root length data underscores the genetic diversity in responses to ethylene treatment and submergence stress. Variations among genotypes, such as the substantial increase in root length in genotype 4 and reduction in genotype 8, emphasize the multifaceted role of ethylene in root development.

Analysis of shoot length responses reveals significant variations among genotypes, indicating the intricate nature of ethylene signaling and its interaction with submergence stress in rice. (Figure 3) demonstrates shoot length measurements BSL (before stress shoot length) in T0 before stress, ranging from approximately 1.31 cm to 6.05 cm. After submergence stress ASL (after stress shoot length), shoot lengths range from approximately 1.50 cm to 11.63 cm (Figure 3), representing the response under control conditions. In T1, BSL values range from approximately 1.73 cm to 7.80 cm, and after submergence stress (ASL), shoot lengths range from approximately 2.43 cm to 9.43 cm (Figure 3). This indicates how ethylene treatment influences the response to submergence stress. In T2, BSL values range from approximately 2.51 cm to 5.98 cm, and after submergence stress (ASL), shoot lengths range from approximately 3.33 cm to 5.89 cm, reflecting the response without ethylene treatment

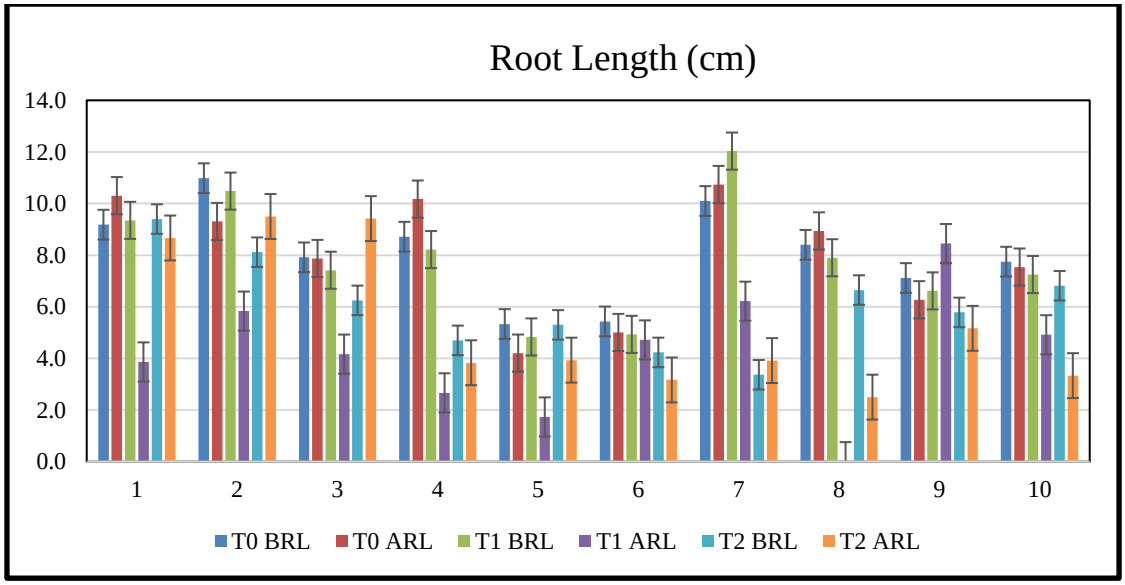

Figure 2. Variation in Root length (cm) of different genotypes before and after submergence stress.

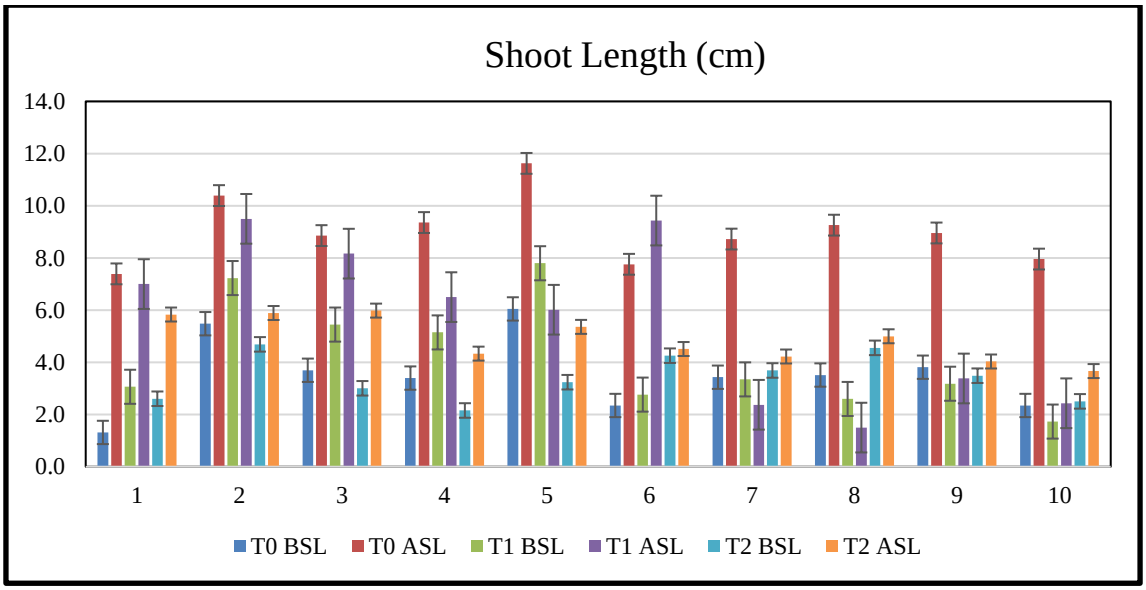

Figure 3. Variation in shoot length (cm) of different genotypes before and after submergence stress.

Analysis of the shoot length data highlights significant variations among genotypes. For instance, genotype 5 shows a considerable increase in ASL under T1, suggesting a potential positive effect of ethylene, while genotype 8 demonstrates a decrease, suggesting a possible negative impact. The contrasting responses are exemplified by the considerable increase in shoot length in genotype 5 and the decrease in genotype 8 under ethylene treatment. Analysis of the whole plant fresh weight data reveals significant variations among genetic lines. (Figure 4) displays whole plant fresh weight measurements BWF (before stress whole plant fresh weight) in T0 before stress, ranging from approximately 0.14 mg to 0.23 mg. After submergence stress AWF (after stress whole plant fresh weight), ranged from approximately 0.01 mg to 0.32 mg (Figure 4), representing the response under control conditions. In T1, BWF values range from approximately 0.06 mg to 0.15 mg, and after submergence stress (AWF), whole plant fresh weights range from approximately 0.00 to 0.27 mg. This indicates how ethylene treatment influences the response to submergence stress. In T2, BWF values range from approximately 0.01mg to 0.15mg (Figure 4), and after submergence stress (AWF), whole plant fresh weights range from approximately 0 to 0.30mg, reflecting the response without ethylene treatment. For instance, genetic line 5 exhibits a substantial increase in AWF under T1, indicating a potential positive effect of ethylene, while genetic line 8 demonstrates a marked decrease, suggesting a possible negative impact. The observed diversity in whole plant fresh weight responses highlights the intricate nature of ethylene signaling and its interaction with submergence stress in rice. The substantial increase in fresh weight in genetic line 5 under ethylene treatment and the marked decrease in genetic line 8 underscore the need for in-depth exploration into the underlying molecular mechanisms influencing whole plant fresh weight

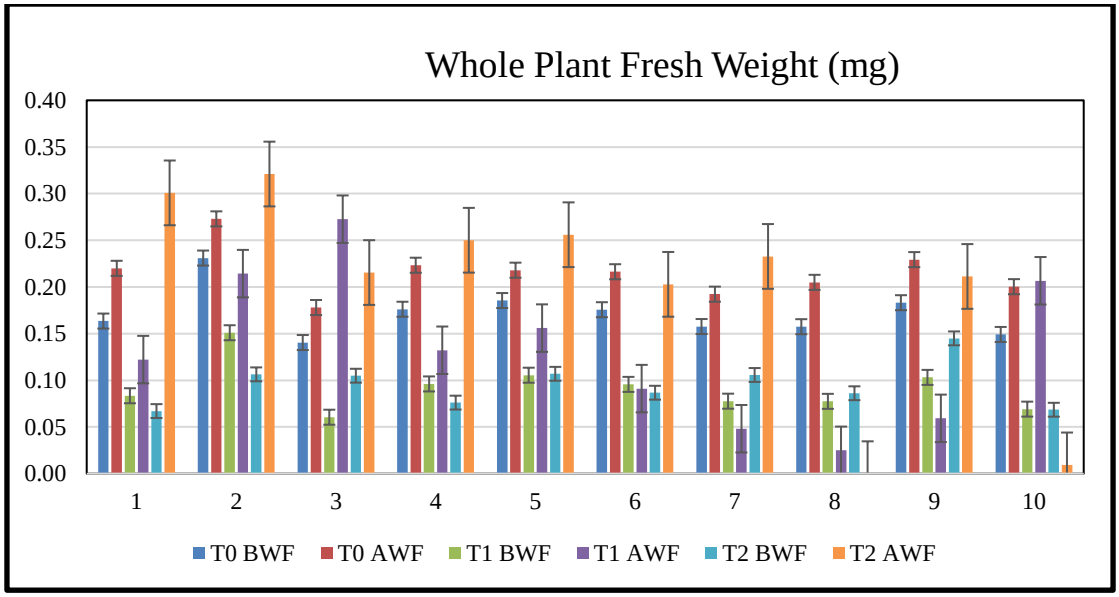

Figure 4. Variation in whole plant fresh weight (mg) of different genotypes before and after submergence stress.

Analysis of the whole plant dry weight data reveals significant variations among genetic lines. (Figure 5) shows whole plant dry weight measurements before stress (BWD), ranging from approximately 0.10 mg to 0.19 mg. After submergence stress whole plant dry weights (AWD) range from 0.00 to 0.32 mg, representing the response under control conditions. In T1, BWD values range from approximately 0.01mg to 0.09mg (Figure 5), and after submergence stress (AWD), whole plant dry weights range from approximately 0.00 to 0.27 mg. This indicates how ethylene treatment influences the response to submergence stress. In T2, BWD values range from approximately 0.00 to 0.10 mg, and after submergence stress (AWD), whole plant dry weights range from approximately 0.00 to 0.25 mg, reflecting the response without ethylene treatment. For instance, genetic line 9 exhibits a substantial increase in AWD under T1, indicating a potential positive effect of ethylene, while genetic line 8 demonstrates a marked decrease, suggesting a possible negative impact. The diversity in whole plant dry weight responses emphasizes the multifaceted roles of ethylene in stress responses, involving both growth promotion and inhibition. A substantial increase in dry weight in genotype 9 under ethylene treatment and a recorded decrease in dry weight in genotype 8.

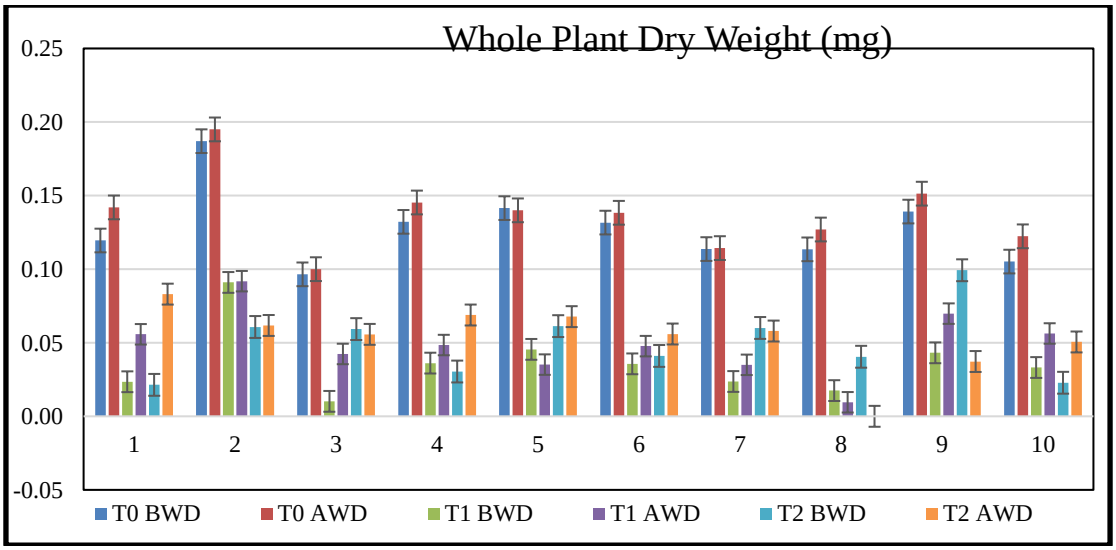

Figure 5. Variation in whole plant dry weight (mg) of different genotypes before and after submergence stress.

Table 3 presents the mean values and standard deviations for survival, mortality, and recovery rates following exposure to submergence stress. The analysis reveals distinctive patterns among the different experimental genotypes. Genotypes 6, 7, and 8 exhibited the highest mortality rates, surpassing other genotypes. In contrast, genotype 5 demonstrated the lowest mortality rate among the experimental conditions. Genotype 5 displayed the highest recovery rate, followed closely by genotype 2. Notably,

genotypes 6, 7, and 8 exhibited the least pronounced recovery, indicating a diminished capacity to recover from submergence-induced stress. With the exception of genotypes 6, 7, and 8, all other genotypes demonstrated survival. This suggests that these specific genotypes experienced a considerable impact from submergence stress, resulting in diminished survival rates. These findings underscore the genotype-specific responses to submergence stress, with certain genotypes exhibiting heightened susceptibility (genotypes 6, 7, 8) and others demonstrating resilience and adaptability (genotype 5).

Table 3. Survival summary of the rice germplasm after submergence stress.

Genotype	T	Mortality%	Recovery%	Survival%
1	1	3.0000±0.707BC	2.0000±0CD	2.5000±0BC
2		2.5000±0CD	2.5000±0.707BC	3.0000±0.707B
3		2.0000±0.707DE	3.0000±0AB	3.5000±0AB
4		2.5000±0CD	2.5000±0.707BC	3.0000±0.707B
5		1.5000±0.707E	3.5000±0.707A	4.5000±0.707A
6		5.0000±0.707A	0.0000±0E	1.5000±0C
7		5.0000±0.707A	0.0000±0E	1.5000±0C
8		5.0000±0.707A	0.0000±0E	1.5000±0C
9		3.5000±0.707B	1.5000±0.707D	2.5000±0.707BC
10		3.0000±0BC	2.0000±0CD	3.0000±0B
1	2	3.0000±0A	4.0000±0BC	1.0000±0CD
2		1.5000±0.707BC	3.5000±0.707CD	1.5000±0.707BC
3		3.0000±0A	3.5000±0.707CD	1.5000±0.707BC
4		2.5000±0.707AB	3.0000±0DE	2.0000±0AB
5		2.5000±0.707AB	2.5000±0.707E	2.5000±0.707A
6		1.0000±0C	5.000±0A	0.0000±0E
7		1.0000±0C	5.0000±0A	0.000±0E
8		1.5000±0.707BC	5.0000±0A	0.0000±0E
9		2.000±0ABC	4.5000±0.707AB	0.5000±0.707DE
10		1.5000±0.707BC	4.0000±0BC	1.0000±0CD

SSR Marker-Based Genotyping: In this study, the RM23877 simple sequence repeat (SSR) marker was employed to evaluate the genetic capacity of rice genotypes during the seedling stage concerning submergence tolerance. Among the examined rice genotypes, the submergence-tolerant gene was detected in 07 lines (Figure 6) out of a total of 10 lines. Within these lines, the marker-linked gene associated with submergence tolerance was successfully amplified, signifying the potential suitability of these genotypes in combating submergence-induced stress. Hence, the incorporation of this locus into high-yielding cultivars via marker-assisted selection will result in the advanced generation of novel submergence-tolerant germplasm with reduced time requirements. The presence of marker allele in genotypes GSR-5, GSR-61, GSR-13, GSR-2, GSR-4, IR-6, and Chenab Basmati was indicated as "1", while the absence of the allele as in GSR-16, GSR62 and GSR-59 was indicated by "0" (Table 4). Genotypic screening involves the use of molecular markers and genetic analysis to identify tolerance genes and alleles associated with submergence tolerance. Molecular markers, such as Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSRs), allow for precise detection of specific genetic variations linked to submergence tolerance traits. Genetic analysis further elucidates the inheritance patterns and mechanisms underlying tolerance traits, providing insights into the genetic basis of submergence tolerance in rice genotypes

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Table 4. Marker-assisted selection with RM23877 identifies submergence stress-tolerant genotypes.

Sr No.	Genotypes	RM23877	Sr No.	Genotypes	RM23877
01	GSR-5	1	06	GSR-16	0
02	GSR-61	1	07	GSR-62	0
03	GSR-13	1	08	GSR-59	0
04	GSR-2	1	09	IR-6	1
05	GSR-4	1	10	Chenab Basmati	1

Figure 6. Agarose gel electrophoresis picture of rice genotypes with RM 23877.

Discussion

The observed variations in mortality, recovery, and survival rates emphasize the importance of genotype selection and highlight potential avenues for further investigation into the molecular and physiological mechanisms underlying these distinct responses. Although rice is widely recognized for withstanding flooded situations because of its capacity to sprout without CO_2 and escape slowly rising waters via aerobic means, flash flooding poses challenges to rice's capacity to flee and survive abrupt and total submersion (Jackson & Ram, 2003; Shyamalee & Ranawake, 2023). When rice coleoptiles, leaves, or stems are unable to escape flooding circumstances and endure complete submergence, severe harm has been documented (Sánchez Lozano, 2022). According to the reports, the rice may grow by 25 cm per day in situations of abrupt flooding, but significantly higher water levels can impair plant survival (Vergara, Jackson, & De Datta, 1976). Rice seedlings have shown the ability to withstand complete submergence for up to 20 days, but the rate of seedling survival is highly dependent on age. The majority of rice varieties elongate their shoot when completely submerged. This reaction is only seen in newly emerging leaves in young seedlings. This is one of the submergence escape mechanisms that encourages the return of certain foliage to the atmosphere (Nagai & Ashikari, 2023). This contributes to ensuring sufficient oxygen and carbon dioxide supplies to allow active photosynthesis and aerobic respiration (Nurrahma, Putri, & Syahadat, 2023; Vartapetian & Jackson, 1997). The degree of shoot elongation during submergence is influenced by the submergence environment or stage of seedling growth before submergence and depends on the genetic characteristics of the cultivar. Hormones like ethylene, which interacts with other hormones including auxin, abscisic acid (ABA), gibberellins (GA), and auxin, govern the lengthening of shoots during submergence (Jackson, 2008; Jia, Ma, Chen, & Wu, 2021).

DNA markers exhibit significant promise in enhancing the efficacy and accuracy of traditional plant breeding methods through the application of marker-assisted selection (MAS) (Collard & Mackill, 2008). In contrast to morphological characteristics, molecular markers can reveal significant distinctions at the DNA level. This makes them a valuable tool for germplasm profiling, as they are not affected by environmental factors. Among the various types of markers available, SSR markers are preferred in rice due to their high informativeness, co-dominance, and cost-effectiveness (Bashir *et al.*, 2022; Mukherjee, Das, Alam, Nath, & Dasgupta, 2013). Submergence tolerance is an infrequent characteristic governed by genetic factors, exhibiting relatively substantial heritability, and under the control of one or a small number of genes with significant impact, along with minor regulatory elements (Toojinda, Siangliw, Tragoonrung, & Vanavichit, 2003). A pivotal milestone in the realm of submergence tolerance breeding involved the discovery of quantitative trait loci (QTL) responsible for submergence tolerance, specifically labeled as the SUB1 gene. The genomic segment housing the SUB1 QTL was subsequently meticulously refined to a compact chromosomal locus. Subsequent efforts led to the successful cloning and characterization of this gene, revealing it to be an ethylene response factor (ERF). Further genetic mapping efforts pinpointed this genomic region to an exceedingly narrow span of 0.16 centimorgans (cm) on chromosome 9, involving the analysis of approximately 3000 F2 progeny (Oladosu *et al.*, 2020). Further studies on quantitative trait loci have also confirmed that the primary contributor to submergence tolerance is the Submergence 1 (SUB1) locus located on chromosome 9. The marker RM23877 is tightly linked with the QTL/SUB1 (Tran Dang Khanh, Le Hung Linh, Ta Hong Linh, Le Huy Ham, & Tran Dang Xuan, 2013). The successful incorporation of the SUB1 region through marker-assisted introgression has effectively enhanced submergence tolerance across a diverse array of high-yielding varieties, with no adverse effects on development, crop

yield, or grain quality (Bailey-Serres *et al.*, 2010). There is a need for more comprehensive screening methods that integrate both phenotypic and genotypic data to enhance the accuracy and reliability of submergence tolerance assessments in rice genotypes. Integrating phenotypic traits, such as survival rate and growth recovery after submergence, with genotypic markers associated with tolerance genes can provide a more holistic understanding of submergence tolerance mechanisms. Additionally, further research is needed to explore the genetic basis of submergence tolerance and the interaction between different tolerance genes and alleles, which will contribute to the development of more resilient rice varieties.

Conclusion

This study successfully identified submergence-tolerant genotypes using the RM23877 SSR marker for genotyping. These findings lay the groundwork for marker-assisted selection in breeding programs to develop rice varieties with enhanced submergence tolerance. Such advancements are crucial for promoting sustainable rice production, particularly in South Asia, where climate change is expected to exacerbate the frequency and severity of flooding events. Future research should focus on expanding the genetic base by exploring additional markers associated with submergence tolerance to further refine and enhance the selection process. Integrating genomic selection with traditional breeding methods could accelerate the development of high-yielding, resilient rice varieties. Moreover, implementing field trials across diverse agro-climatic zones will be essential to validate the performance and adaptability of these genotypes under real-world conditions. Investments in infrastructure for precision breeding and capacity-building among local researchers and farmers are also recommended to ensure the effective adoption and dissemination of submergence-tolerant varieties. By continuing to advance our understanding and application of genetic tools in rice breeding, we can better equip agricultural systems to withstand the challenges posed by climate change, thereby ensuring food security and the sustainability of rice production.

Acknowledgments

The authors acknowledge the financial facilitation provided by the Rice Research Program, CSI, and NARC, for the perfect execution of the research.

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