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Characterizing CRCIL-464 Rice Diversity Panel for Using as Multi-Trait Genetic Resources for Breeding Climate-Resilient Varieties Pertaining to Salinity, Flood, and Cold Stress Tolerance

M S Rahman[1*], A A Shoily[1], J Jannaty[1], A Rahman[2], M S Pervin[1] and K M Iftekharuddaula[2]

ABSTRACT

Rice (*Oryza sativa* L.) is highly vulnerable to abiotic stresses, particularly in climate-sensitive countries such as Bangladesh, where salinity, submergence, and cold pose major threats to sustainable production. To identify genetic resources for climate-resilient breeding, a multi-trait diversity panel of 464 rice genotypes (CRCIL-464), comprising advanced breeding lines, released varieties, and diverse germplasm, was evaluated for tolerance to these stresses. Salinity screening at the seedling stage using hydroponics identified 32 tolerant and 25 moderately tolerant genotypes based on SES scores, with selected lines further evaluated under soil-based reproductive-stage salinity. Three genotypes (BR11715-4R-13, BR11921-4R-100, and BR12567-5R-91) outperformed the tolerant check IR58443 in grain yield, though yield reductions highlighted differential tolerance mechanisms between stages. Submergence screening revealed wide variability in recovery and survivability, with one genotype (IR14T156) showing complete survival (100%), although most entries were highly sensitive. Cold stress assessment at the seedling stage identified 12 genotypes with partial recovery, and two lines (SVIN 297 and IRRI154-Hd9+Pi9) displayed the highest survivability (83.3%). Collectively, the CRCIL-464 panel demonstrated substantial genetic variation across multiple stresses. The identified tolerant lines represent promising candidates for incorporation into BRRI's breeding program to develop multi-stress-tolerant rice varieties, thereby enhancing resilience and securing rice production under climate change.

Keywords: Rice, abiotic stress tolerance, salinity, submergence, climate-resilient breeding, multi-trait screening.

INTRODUCTION

Rice (*Oryza sativa* L.), a member of the Poaceae family, is one of the most important staple foods worldwide, supplying dietary energy for more than half of the global population, particularly in Asia, Africa, and Latin America (Fitzgerald *et al.*, 2009). Its remarkable adaptability across diverse ecosystems-including flooded lowlands, irrigated fields, and upland systems-highlights its central role in global food security (Khush, 2005). Despite its resilience, rice productivity is increasingly threatened by abiotic stresses such as drought, salinity, extreme temperatures, and flooding. These environmental constraints not only reduce grain yield but also deteriorate grain quality, with particularly severe impacts in regions experiencing rapid and unpredictable climate change (Zhu, 2016).

A multi-trait diversity panel is a collection of

[1]Plant Physiology, Division, BRRI, Gazipur and [2]Plant Breeding Division, BRRI, Gazipur
Corresponding author's E-mail: sazzadur.phys@brri.gov.bd (M S Rahman)

rice genetic resources characterized by a wide range of inherited traits. These panels are designed to maximize genetic variation, enabling the study and simultaneous selection of multiple traits within a single population. They provide researchers with valuable insights into the genetic basis of important traits and support efforts to enhance adaptation to environmental challenges. The CRCIL-464 panel was developed to capture a broad spectrum of genetic diversity. Once trait variation and genetic diversity are well understood, breeders can apply strategies such as selection indices to simultaneously select multiple traits and improve breeding populations. In this context, the panel was characterized for three major abiotic stress tolerance traits: salinity, submergence, and cold tolerance.

Salinity poses one of the greatest threats to rice cultivation. Salt accumulation in soils interferes with water and nutrient absorption, resulting in reduced germination, impaired seedling growth, stunted plant development, poor tillering, and lower yields (Munns & Tester, 2008). While improved soil and water management practices have been introduced to mitigate salinity effects (Singh *et al.*, 2018), genetic improvement through the development of salt-tolerant rice varieties remains essential for sustainable crop production. Salinization is the accumulation of water-soluble salts in the soil to a level that impacts on agricultural production. Soil is considered saline if the electrical conductivity of its saturation extract (ECe) is above 4 dSm-1 (USDA-ARS, 2008), which is equivalent to approximately 40mM NaCl and generates an osmotic pressure of approximately 0.2 MPa. This definition of salinity derives from the ECe that significantly reduces the yield of most crops (Munns and Tester, 2008). Loss of arable land via salinization is a major factor undermining the productivity of modern agricultural systems (Galvani, 2007). Salinization of agricultural soils occurs primarily due to agricultural practices, including poor water management, high evaporation, heavy irrigation and previous exposure to sea water (Pitman and Lauchli, 2002).

In Bangladesh, more than 30% of the cultivable land is in the coastal areas. Soil salinity has mainly formed from sea water flooding or capillary rise from shallow ground water close to the coast. Soil Resource Development Institute (SRDI) has temporal and spatial data on regular monitoring of soil and water salinity since 1989 besides reconnaissance survey data of 1973. It was estimated in 1973 and 2009 that the area coverage of soils with different degrees of salinity is about 0.833 and 1.056 million hectares respectively. Total spatial increase of saline area was about ~26% in 2009 over 1973 (SRDI, 2010).

Defining salt tolerance of rice is very difficult because of the complex nature of salt stress and the wide range of plant responses. Rice responses to salinity also vary in different growth stages. Seedling and flowering stages of rice are more likely to be affected by salinity, with reduction in seedling growth and yield. Tolerance during seedling stage seems to correlate poorly with tolerance during reproduction, suggesting different sets of traits are probably involved at each stage (Moradi *et al.*, 2003). Reproductive stage is another developmental stage when rice is sensitive to salinity stress, more specifically this stage is the booting stage (7-10 days before and after booting stage) (Singh and Flowers, 2010). The reproductive stage is crucial as it ultimately determines grain yield, but the importance of the seedling stage cannot be underestimated as it determines crop establishment. There are few studies that address the effects of salinity on yield. Most research has been limited to the seedling or early vegetative stages or only reports parameters such as fresh or dry weight although the ultimate aim has been to increase grain yield with limited resources (Moradi and Ismail, 2007; Cheng *et al.*, 2008; Jain *et al.*, 2008; Zang *et al.*, 2008). Hence, to know the response of the rice plant to salinity as a whole, it is imperative that the effects be observed in all the various stages of its development, that is at early seedling, vegetative and reproductive stages (Gregorio *et al.*, 1997).

Flooding is another major constraint in

rice-growing regions, especially in monsoon-prone areas of South and Southeast Asia. Although rice has greater inherent tolerance to waterlogging compared to other cereals, extended periods of submergence can cause oxygen deprivation, reduce photosynthesis, and delay crop maturity (Bailey-Serres *et al.*, 2010). Breeding progress has been made through the incorporation of the Sub1 gene into high-yielding varieties, conferring enhanced survival under flash flooding (Xu *et al.*, 2006). However, the need for broader submergence tolerance and post-flood recovery traits remains a pressing challenge in breeding programs. Submergence is one of the main obstacles to growing rice, particularly during years and in regions with considerable precipitation. More than sixteen percent of rice fields worldwide are susceptible to flooding. (Mackill *et al.*, 1996., Setter *et al.*, 1997). Rice farming in rainfed lowlands of south and south-east Asia is sometimes hampered by flash floods, which submerge the plants completely in water for roughly two weeks. (Septiningsih *et al.*, 2009).

Cold stress, particularly at the seedling stage, is also a critical barrier to rice establishment in temperate and high-altitude ecosystems. Exposure to temperatures below 20°C adversely affects root development, delays germination, reduces seedling vigor, and lowers survival rates, ultimately resulting in poor plant establishment and yield losses (Sharma *et al.*, 2012). Cold tolerance is particularly important in the northern regions of Bangladesh and other areas where early-season low temperatures limit the success of direct-seeded and transplanted rice. Reduced temperatures effects on rice crops vary depending on the genetic makeup of variety, developmental stage, exposure duration, and cold intensity (Díaz *et al.*, 2006., Sravan *et al.*, 2016). The most sensitive phase of low temperature damage in rice is the booting stage, followed by flowering (Matsuo *et al.*, 1995). Low temperatures during the booting and flowering periods reduce plant growth, significantly reduce spikelet fertility, and increase vulnerability to disease (Zeng *et al.*, 2017). The extent to which fertility is reduced varies by variety and the duration of exposure to cold (Satake *et al*., 1983).

With the global population projected to surpass 9 billion by 2050, rice demand is expected to increase by 70–85% (FAO, 2017; Ray *et al.*, 2013). Meeting this rising demand under climate change requires the development of climate-resilient cultivars that can withstand multiple abiotic stresses. Recent advances in molecular genetics, physiology, and adaptive agronomy provide opportunities to accelerate this process (Zhang *et al.*, 2022). Against this backdrop, the present study was designed to evaluate a diverse panel of 464 rice genotypes (CRCIL-464) for tolerance to salinity, submergence, and cold at the seedling stage. The findings aim to identify promising lines for use in early-generation breeding programs in Bangladesh, thereby contributing to the development of climate-resilient rice varieties capable of sustaining yields, reducing environmental risks, and promoting food security in stress-prone environments.

MATERIALS AND METHODS

A multi-trait rice diversity panel was formed in the Plant Breeding Division of BRRI under the USAID funded project Climate Resilient Cereal Innovation Lab (CRCIL). The panel comprised 464 genotypes including advanced breeding lines of BRRI and IRRI origin, released varieties and important local and exotic germplasm (see List of germplasm for CRCIL-464 multi-trait diversity panel in supplementary file). The following panel was evaluated independently for three different abiotic stresses i.e. salinity, submergence and cold including respective standard tolerant and sensitive checks. The investigations were carried out in Plant Physiology Net house & cold-water tank and submergence tank of the Plant Breeding Division, BRRI, Gazipur, during T. Aman 2024 and Boro 2024-25 season.

Phenotypic characterization for salinity tolerance at seedling stage

Sprouted seeds of the CRCIL-464 panel, along with tolerant (IR58443) and sensitive (IRRI154) checks including 12 BRRI's salt tolerant HYVs were sown on a nylon net attached to a Styrofoam sheet, floating on Yoshida's full-strength culture solution, as described by Gregorio *et al.* (1997). The experiment followed an RCB design with two replications. Application of salt stress started at 18 days after sowing with a level of EC 6 dS/m and increased by 2 dS/m each day until reaching a maximum of 12 dS/m. The Yoshida solution was replaced weekly throughout the experiment. The pH and electrical conductivity (EC) of the solution were monitored daily and maintained at 5.0 and 12 dS/m, respectively. Two weeks after stress application, progenies were scored through the SES scale (IRRI, 2013) for classification according to their overall tolerance based on leaf damage symptoms (Table 1).

Table 1. The Standard Evaluation System for rice for salinity scoring at seedling stage (IRRI, 2013).

Score	Observation	Remarks
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants drying	Moderately sensitive
9	Almost all plants are dead or drying	Sensitive

Phenotypic characterization for salinity tolerance at reproductive phase

A total of 34 seedling stage salinity tolerant CRCIL-464 genotypes along with 2 checks were evaluated for reproductive stage tolerance under soil-based system. Sprouted seeds were sown in puddled soil placed in perforated plastic pots, which were then placed inside plastic bowls. Each bowl contained six pots, consisting of four test lines along with tolerant and sensitive checks. Seedlings were grown in the perforated pots for 21 days under tap water, submerging the pots to the brim in a net house. Afterwards, the tap water was replaced with saline water at 10 dS/m. The water level was maintained at the soil surface by adding tap water daily. A separate set without salinity served as control. BRRI-recommended cultural practices were followed until maturity. Grain weights and other yield component traits were measured and recorded and analyzed.

Phenotypic characterization for submergence tolerance at vegetative phase

The CRCIL-464 panel germplasm, including checks, were sown in a standard seedbed to raise seedlings. Twenty-one-day-old seedlings from each entry were transplanted into the submergence tank of the Plant Breeding Division. Each entry consisted of 6 seedlings per line, with two lines per entry, arranged randomly. A single seedling was planted per hill with a spacing of 20 cm x 20 cm. Fourteen days after transplanting, the tank was filled with normal tap water to a height of 1 meter above the soil surface, ensuring complete submergence of the plants. To replicate the turbidity of floodwater in Bangladesh, muddy soil was mixed into the tank water daily. Water quality data recorded before and after creating turbidity. Recovery and survivability scores were noted 7 days after recovery.

Phenotypic characterization for cold tolerance at seedling stage

All genotypes of the CRCIL-464 panel, along with three check varieties (BRRI dhan28, BRRI dhan36, and Hbj B-VI), were evaluated for seedling-stage cold tolerance under artificial conditions using cold-water tanks. Seeds were sown in plastic trays (60 cm × 30 cm × 2.5 cm) filled with granular soil and grown until the three-leaf stage. The trays were then placed in cold-water tanks maintained at a constant temperature of 13 °C. After seven days of cold-water treatment, seedlings were scored for leaf discoloration using the SES scale (IRRI, 2013) (Table 2). Recovery and survival were assessed one week after the removal of cold stress.

Table 2. The Standard Evaluation System for rice for cold scoring at seedling stage (IRRI, 2013).

Score	Observation	Remarks
0-1	No damage to leaves, normal leaf color	Strongly tolerant
2-3	Tip of leaves slightly dried, folded and light green	Tolerant
4-5	Some seedlings moderately folded and wilted, 30-50% seedlings dried, pale green to yellowish leaves	Moderately tolerant
6-7	Seedlings severely rolled and dried, reddish-brown leaves	Sensitive
8-9	Most seedlings dead or drying	Highly sensitive

Data analysis

Data from all experiments were organized using Microsoft Excel. Stress response data under three different abiotic stresses were analyzed using RStudio (https://posit.co/download/rstudio-desktop/) and the Statistical Tool for Agricultural Research (STAR) (http://bbi.irri.org). Graphical representations were generated in RStudio.

RESULTS AND DISCUSSION

Bangladesh is widely recognized as one of the country's most vulnerable to climate change, despite its minimal contribution to global emissions. Rice, the country's staple food crop, is particularly sensitive to climatic variability. Consequently, even slight changes in climate increase the uncertainty of rice production, as climate is a major driver of year-to-year fluctuations in productivity. To ensure sustainable rice production, the development of climate-resilient varieties has become a central objective of BRRI's breeding program.

Salinity tolerance at seedling stage

The breeding successes observed for developing existing salt tolerant varieties follows a long-term goal to combine different traits conferring salt tolerance into an elite background. However, the development is not straightforward because tolerance is controlled by multiple loci or QTLs. Grouping of the genotypes based on the inherent physiological mechanism responsible for salinity tolerance, inter-mating of the genotypes with high degree of expression of the contrasting salinity tolerance mechanism and identifying/screening of the recombinants for pooling of the mechanisms is being followed to enhance the level of salt tolerance further.

At the seedling stage salinity tolerance under hydroponics system the tested CRCIL-464 genotypes showed a multi-modal distribution (Fig. 1) revealing the panel was not normal. Out of the 464 genotypes tested, 32 entries with an average SES score ranging from 3.0 to 4.4 were classified as tolerant, while 25 entries with an average SES score ranging from 4.5 to 5.8 were considered moderately tolerant (Table 3). The remaining genotypes, which had visual scores between 6 and 9, were classified as sensitive to highly sensitive.

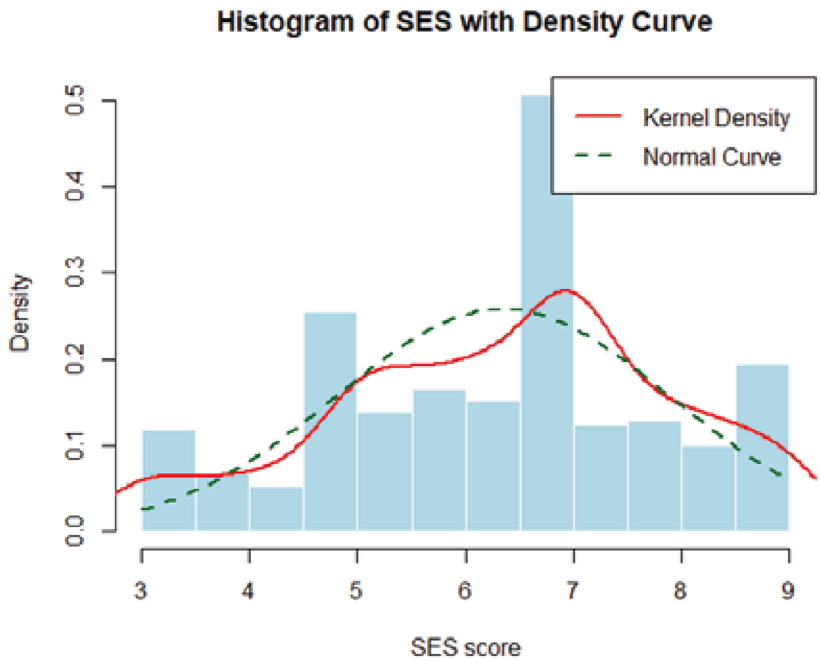

Fig. 1. Histogram of SES with density curve represented distribution of tolerances at seedling stage under salinity stress.

Table 3. List of tolerant and moderately tolerant germplasm identified at seedling stage salinity at 12 dS/m.

SL	Designation	SES	Salinity class	SL	Designation	SES	Salinity class
1	BR10604-5R-58	4.2	T	33	BRRI dhan33	4.8	MT
2	BR11715-4R-13	3.6	T	34	IR96184-24-1-1-AJY2	4.6	MT
3	BR11607-4R-184	4.4	T	35	BR11920-4R-521	4.6	MT
4	BR12459-4R-49	4.0	T	36	IR13F478-3	4.6	MT
5	BR12459-4R-3	3.2	T	37	BR11921-4R-100	5.0	MT
6	BR12557-5R-80 (835)	3.8	T	38	BR11894-R-R-R-R-329	5.0	MT
7	Pi21+Pb1 (34)	4.4	T	39	BR12899-4R-169	4.8	MT
8	BR11723-4R-172	4.0	T	40	BR13157-4R-174	4.6	MT
9	IR127152-3-22-2-1B	4.4	T	41	BR11714-4R-74	4.5	MT
10	BRRI dhan98	4.0	T	42	BR10212-4-3-1	4.8	MT

SL	Designation	SES	Salinity class	SL	Designation	SES	Salinity class
11	BR13169-4R-307	3.8	**T**	43	SVIN 352	4.8	MT
12	IR14F550	4.3	**T**	44	BRRI dhan74 (Ck)	5.0	MT
13	BR9536-2-1-17	4.2	**T**	45	IRRI154-Pi9	5.0	MT
14	IR13F458	3.6	**T**	46	BR11940-4R-167	5.0	MT
15	IR13F478	3.2	**T**	47	BR12465-4R-223	5.2	MT
16	Acc. no. 1630	3.0	**T**	48	TP30649	4.5	MT
17	BR10490-1-2-3-87	4.1	**T**	49	BR12274-4R-46	4.8	MT
18	BR11887-5R-368	3.6	**T**	50	BR12423-6R-38	5.0	MT
19	BR12459-4R-103	4.3	**T**	51	BR13171-4R-207	4.6	MT
20	BR12567-5R-91	3.4	**T**	52	IR16F1019	5.0	MT
21	IR16F1148 (Ck)	3.8	**T**	53	Pokkali	5.0	MT
22	BR12459-4R-214	3.5	**T**	54	IR87870-6-1-1-1-1-B	5.0	MT
23	IR14T156	3.8	T	55	BR12096-4R-25	5.5	MT
24	IR100158-B-2-AJYI	3.6	**T**	56	IRRI154-Hd9+Pi9	4.6	MT
25	BR12890-5R-32	4.0	**T**	57	IR64-Pi9 (E)	5.8	MT
26	IR16F1063	3.8	**T**	**T. ck**	**IR58443 (Tolerant)**	**4.12**	T
27	IR4630	4.3	**T**	**S. ck**	**IRRI154 (Sensitive)**	**7.12**	S
28	Acc. no. 234	4.2	**T**				
29	BR13169-4R-227	4.0	**T**				
30	BR11723-4R-322	3.6	**T**				
31	BR12902-4R-257	4.2	**T**				
32	IR 127152-3-22-18-1-B	4.3	T				

Based on the above scoring system the genotypes having average score <4.4 were treated as tolerant (T) and average score 4.5-5.8 were treated as moderately tolerant (MT).

Understanding and manipulating physiological processes related to salt tolerance is crucial for breeding new salt-tolerant rice varieties. The CRCIL-464 diversity core panel demonstrates genetic variation based on SES scores. It is clear from the results that all the materials showed assorted responses to different salt stress conditions. Among these rice genotypes, 32 lines were found to be tolerant, 25 moderately tolerant, and the rest sensitive to highly sensitive. This evaluation helps identify promising rice varieties for breeding salt-tolerant cultivars.

Salinity tolerance at reproductive phase

A total of 35 seedling-stage salinity-tolerant lines from the CRCIL panel, along with two checks, were evaluated for reproductive-stage tolerance under a soil-based system (Gregorio et al., 1997). Among these, three genotypes BR11715-4R-13, BR11921-4R-100, and BR12567-5R-91 produced higher yields than the tolerant check IR58443 (Table 4, Fig. 3). In terms of yield reduction, only BR12557-57-5R-80(835) exhibited less than 50% reduction. The highest-yielding genotype under salinity, BR11715-4R-13, showed a yield reduction of about 52%, which is close to the threshold for declaring tolerance under stress (Fig. 2). Notably, two genotypes scored 3.8 and 3.6 under seedling-stage salinity stress, suggesting their potential for release and use as whole-growth salinity-tolerant varieties.

Table 4. Phenotypes of selected seedling stage tolerant genotypes at the reproductive phase under salinity stress @10 dS/m.

Genotype	Plant height (cm)	Tiller No.	Filled Grain No.	Spikelet Fertility (%)	Straw Weight (g)	Growth duration (d)
BR13171-4R-125	121.00	13.00	385.00	56.48	26.40	124.00
Acc. no. 1684	123.50	11.00	266.00	38.52	14.15	123.00
BR11303-5R-156	99.50	16.00	211.50	64.35	17.37	120.50
BR11715-4R-13	111.00	13.50	557.00	35.86	26.10	122.50
BR11921-4R-100	89.50	13.50	449.00	52.18	23.81	112.00
BR12459-4R-3	101.00	13.00	458.50	48.19	22.96	125.00
BR12557-57-5R-80(835)	104.50	7.50	324.50	40.76	14.00	124.00
IR17A1211	90.50	9.00	55.57	80.62	7.14	114.00
IR11723-4R-172	92.00	8.50	234.50	59.39	18.51	119.00
IR127152-3-33-2-1B	77.50	12.50	170.00	49.27	14.20	124.00
BRRI dhan98	96.50	11.50	111.00	76.77	24.74	118.00
BR13169-4R-307	105.00	15.00	522.50	31.62	16.81	124.50
BR9536-2-1-17	104.50	12.50	409.50	48.85	20.30	118.00
IR13F458	39.50	4.50	12.50	41.38	5.01	64.50
Acc. no. 1630	106.00	19.50	285.50	68.83	25.68	138.00
BR10490-1-2-3-87	90.00	11.00	256.50	65.58	24.58	125.00
BR11887-5R-368	98.00	10.00	84.50	70.22	23.20	114.50
BR12567-5R-91	89.50	13.00	486.00	26.50	20.52	114.50
IR16F1148	95.00	13.50	165.00	78.89	24.05	117.00
Pi21+Pbi (34)	87.00	12.50	361.00	41.21	16.79	112.00
BRRI dhan67	93.00	16.00	295.50	44.88	20.24	112.00
BRRI dhan97	88.50	16.50	299.00	52.87	24.53	113.00
BRRI dhan99	90.50	16.50	425.50	35.20	24.54	117.00
BR11715-4R-186	126.50	13.50	154.00	77.62	25.23	125.00
IR14T156	106.50	12.50	300.00	52.14	19.15	124.00
BR12459-4R-214	113.00	12.00	461.50	48.77	21.01	126.50
IR100158-B-2-AJY1	107.50	12.50	154.00	71.87	19.36	126.50
Acc. no. 2276	67.50	16.50	396.00	56.37	20.69	112.00
BR12890-5R-32	86.50	16.50	219.00	53.05	22.84	119.50
IR16F1063	47.50	6.50	138.00	33.41	11.16	60.00
Acc. no. 234	131.00	6.00	31.00	45.71	11.17	60.50

Genotype	Plant height (cm)	Tiller No.	Filled Grain No.	Spikelet Fertility (%)	Straw Weight (g)	Growth duration (d)
BR13169-4R-227	94.00	12.50	298.00	52.71	18.48	118.00
BR11723-4R-322	94.00	14.50	276.00	65.56	23.93	124.00
BR12902-4R-257	103.50	12.50	144.34	74.62	17.40	118.00
BR11714-4R-182	98.50	13.00	418.00	22.08	18.53	119.50
IRRI154 (Sen. ck.)	90.29	8.93	146.21	70.05	11.68	124.07
IR58443 (Tol. ck.)	101.50	17.61	450.28	39.68	40.60	121.89
$LSD_{0.05}$	*2.03*	*5.96*	*5.96*	*5.96*		*5.96*
CV (%)	*9.93*	*17.56*	*31.79*	*23.28*		*4.04*

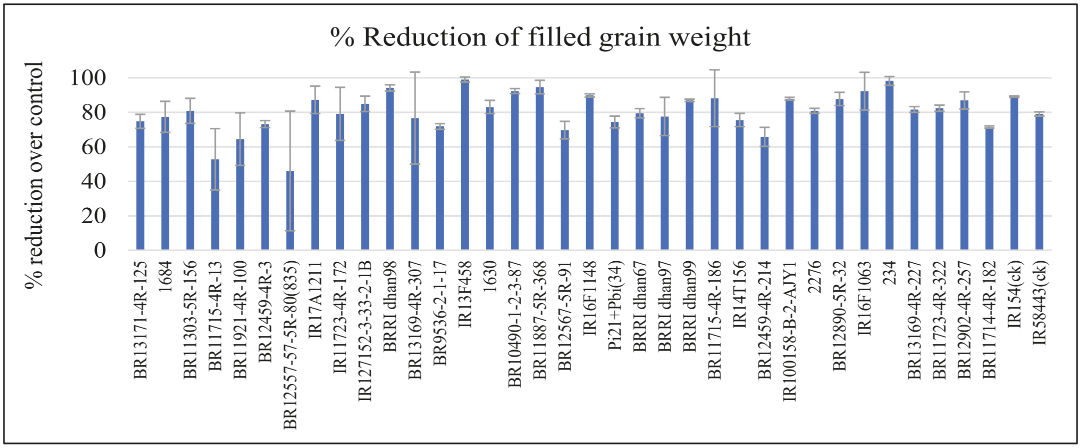

Fig. 2. Percent reduction of filled grain weight under stress condition (salinity 10 dS/m) from 21 DAS to maturity. Error bar indicates standard deviation (*n = 5*).

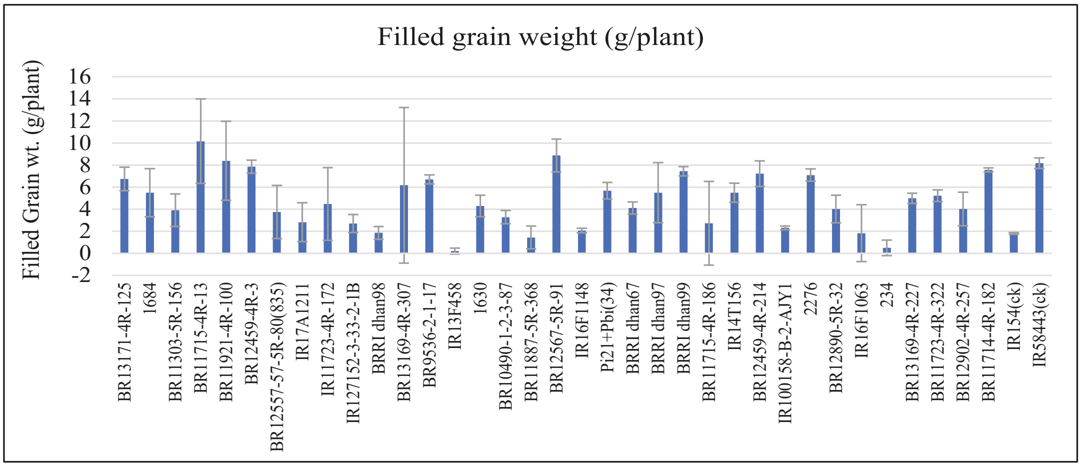

Fig. 3. Filled grain weight under stress condition (salinity 10 dS/m) from 21 DAS to maturity. Error bar indicates standard deviation (*n = 5*).

Submergence tolerance at vegetative phase

The CRCIL-464 panel germplasm, along with standard checks (FR13A and IR42) and four BRRI submergence-tolerant high-yielding varieties (HYVs), were assessed for submergence tolerance at the vegetative stage in the concrete submergence tank at the Plant Breeding Division. Seedlings were raised under normal field conditions in a standard seedbed and transplanted into the concrete tank at 22 days old. Six seedlings per line and two lines per entry were planted using an Augmented Block (RCB) design. Two weeks after transplantation, the plants were completely submerged for 14 days under 1 meter of floodwater. To simulate typical Bangladeshi flood conditions, the water was made turbid daily. Daily measurements of floodwater temperature, pH, dissolved oxygen, and turbidity were taken during the submergence period (Table 6). Seven days after the water receded, plant survivability and recovery scores were recorded. After submergence stress among the CRCIL-464 germplasm, 25 genotypes, including 4 checks, exhibited a survivability range of 8.33% to 100% after recovery, with SES scores ranging from 1 to 9. One genotype, IR14T156, had six planted plants, all of which survived, achieving 100% survivability. Three entries received an SES score of 7, while the remaining scored 9 (Table 5).

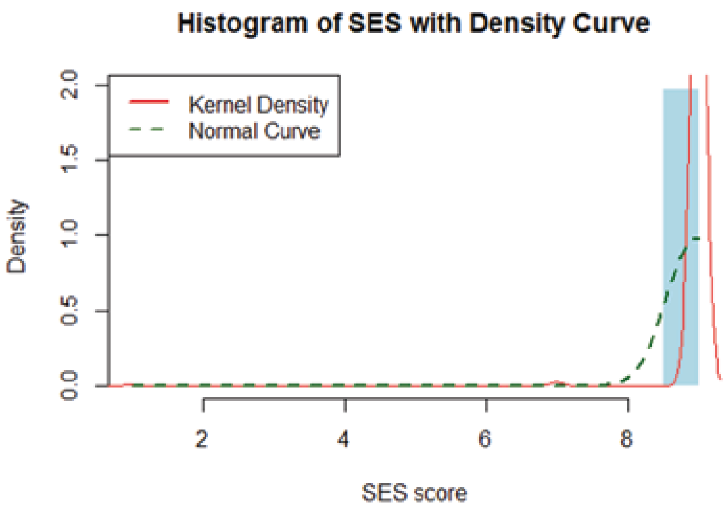

Fig. 4. Histogram of SES with density curve represented distribution of tolerances at seedling stage under submergence stress.

Table 5. Percent survivability (%) with SES of tested germplasm under two weeks of complete submergence.

SL	Entry	Survivability (%) after recovery	SES
1	Capsul	8.33	9
2	BRRI dhan74 (Ck)	8.33	9
3	BR12459-4R-209	8.33	9
4	BR12459-4R-103	8.33	9
5	IR16F1148 (Ck)	50.00	7
6	BR12900-4R-177	16.67	9
7	BR11715-4R-186	8.33	9
8	Acc. no. 2006	60.00	7

SL	Entry	Survivability (%) after recovery	SES
9	Sorsoria	45.45	9
10	Ashful	8.33	9
11	**IR14T156**	**100.00**	**1**
12	BR12905-4R-61	8.33	9
13	BR13169-4R-227	44.44	9
14	BR11894-5R-77	16.67	9
15	IR126952-41-58-26-4-12-12-1	8.33	9
16	BRRI dhan52	25.00	9
17	BRRI dhan108	25.00	9
18	IR16F1019	45.45	9
19	BR12520-5R-75	8.33	9
20	IR126952-28-55-9-9-53-1-6	16.67	9
21	BRRI dhan74 (Ck)	33.33	9
22	BR10211-5-5-7	50.00	7
23	BR11196-5R-83	16.67	9
24	BR11604-4R-84	10.00	9
25	BR11196-5R-445	10.00	9

The results of the submergence screening of the CRCIL-464 panel show significant genetic variability for submergence tolerance among rice genotypes. The distribution for SES was completely skewed towards sensitive indicating less representation of tolerant and moderately tolerant germplasm into the CRCIL-464 panel (Fig. 4). There is a board range of survivability (8.33%–100%), with 100% survival rates for all six seedlings. IR14T156 was the best performer and showed strong resilience in flooding conditions. Most genotypes received SES scores of 9, indicating poor recovery and stress symptoms in post-submergence. Even though certain survivability rates are excellent, the low recovery scores show the importance of post-submergence recovery ability as a breeding trait. Despite the results showing that some genotypes may be able to withstand submergence, they also show the challenge it is to breed for this feature due as survival, recovery, and yield need to be taken into account. Trials should be scaled up, agronomic attributes should be assessed after the flood, and promising lines should be incorporated into crossing or breeding programs with selected elite varieties.

Table 6. Flood water quality data before and after mud mixing in the concrete tank. All data were recorded at 12.00 noon.

	Before mud mixing				After mud mixing			
Day	pH	Temp	Dissolve O_2 mg/L	Turbidity (FNU)	pH	Temp	Dissolve O_2 mg/L	Turbidity (FNU)
Day-1 (08/9/2024)	7.6	33.1	6.5	13.0	7.9	33.0	10.1	128.3
Day-2 (09/9/2024)	8.1	33.4	44.8	15.7	8.1	34.0	44.9	100.1
Day-3 (10/9/2024)	8.3	33.5	68.7	14.8	8.2	33.7	62.3	79.9
Day-4 (11/9/2024)	8.3	33.4	57.9	15.5	8.4	33.9	47.9	93.9
Day-5 (12/9/2024)	8.2	34.4	36.5	16.6	8.4	34.7	33.2	323.4
Day-6 (13/9/2024)	8.3	33.4	34.2	57.3	8.5	33.7	28.1	137.3

Day	Before mud mixing				After mud mixing			
	pH	Temp	Dissolve O_2 mg/L	Turbidity (FNU)	pH	Temp	Dissolve O_2 mg/L	Turbidity (FNU)
Day-7 (14/9/2024)	*Data not recorded due to rain*							
Day-8 (15/9/2024)	*Data not recorded due to rain*							
Day-9 (16/9/2024)	8.3	28.7	25.6	15.2	8.6	29.9	28.6	69.2
Day-10 (17/9/2024)	8.6	30.7	30.6	6.5	8.7	31.2	28.4	48.6
Day-11 (18/9/2024)	8.7	31.2	30.8	9.1	8.9	32.2	24.3	194.0
Day-12 (19/9/2024)	8.8	32.6	26.2	11.8	8.9	34.1	22.3	182.7
Day-13 (20/9/2024)	8.8	34.1	22.4	15.8	8.9	35.5	17.6	103.3
Day-14 (21/9/2024)	8.8	33.7	19.4	25.9	8.9	34.7	12.3	94.1
Average	8.4	32.7	33.6	18.1	8.5	33.4	30.0	129.6

Note: FNU stands for Formazin Nephelometric Units and also signifies that the instrument is measuring scattered light from the flood water at a 90-degree angle from the incident light. FNU is most often used when referencing the ISO 7027 (European) turbidity method.

Cold tolerance at seedling stage

CRCIL-464 panel germplasm with standard checks BRRI HYVs (4) were evaluated for cold tolerance at the seedling stage. Leaf discoloration scores were assessed 10 days after exposure to cold water treatment, using a 1 to 9 scale. The distribution of leaf discoloration SES follows a multi-modal distribution revealing an uneven inclusion of cold tolerant germplasm (Fig. 5). The recovery ability of rice seedlings was also evaluated two weeks after being removed from the cold-water tank. After cold stress among the CRCIL-464 germplasm, 12 genotypes exhibited a survivability range of 16.7% to 83.3% after recovery, with SES scores ranging from 1 to 9. Two genotypes SVIN 297 and IRRI154-Hd9+Pi9 showed 83.3% survivability which was highest survivability percentage. These genotypes require further evaluation.

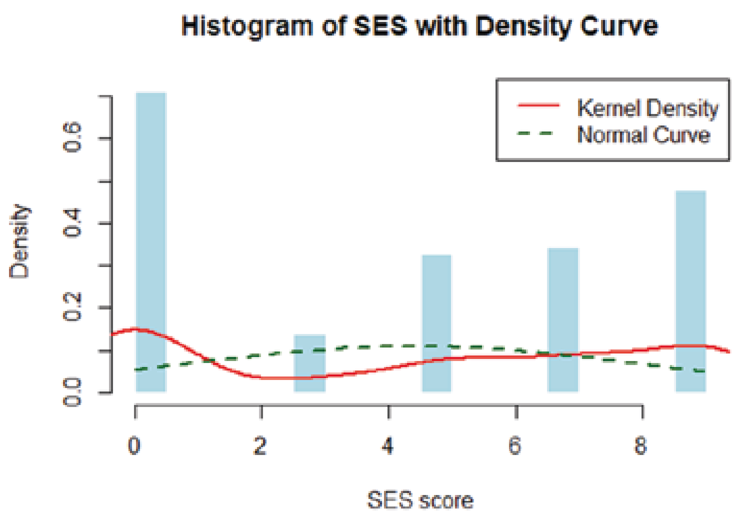

Fig. 5. Histogram of SES with density curve represented distribution of tolerances at seedling stage under cold stress.

Table 7. Effects of cold stress (water temperature 13 ℃ for 10 days) at the seedling stage of CRCIL-464 panel.

SL	Designation	SES Score	Survivability (%)
1	BR10540-4-1-2-4-1	5	50.0
2	**SVIN 297**	**5**	**83.3**
3	BR12557-5R-80 (835)	5	66.7
4	BRRI dhan98	5	33.3
5	BRRI dhan74 (Ck)	3	33.3
6	BR10317-5R-25	3	50.0
7	BRRI dhan92	7	16.7
8	**IRRI154-Hd9+Pi9**	**7**	**83.3**
9	Binashail	7	16.7
10	BRRI dhan81	7	16.7
11	BRRI dhan52	7	16.7
12	BR12902-4R-48	5	50.0
13	**BRRI dhan28 (Ck)**	**9**	**0.00**
14	**BRRI dhan67 (Ck)**	**9**	**0.00**
15	**Hbj-B-VI (Ck)**	**9**	**0.00**

The wide range of SES scores and survival rates represents the genetic diversity present in the CRCIL-464 panel and emphasizes the potential for identifying cold-tolerant lines suitable for breeding programs. The strong performance of SVIN 297 and IRRI154-Hd9+Pi9 recommends their further evaluation under multilocation trials and molecular analysis to confirm the stability of their cold tolerance.

Overall, this study provides promising genetic resources for enhancing cold tolerance in rice, which is particularly critical for direct-seeded rice systems and high-altitude or temperate regions, especially for northern part of Bangladesh where low temperatures at the seedling stage can severely affect crop establishment.

CONCLUSION

The evaluation of the CRCIL-464 multi-trait diversity panel under salinity, submergence, and cold stress revealed substantial genetic variation for abiotic stress tolerance in rice. A set of genotypes demonstrated consistent tolerance at both the seedling and reproductive stages under salinity stress, highlighting their potential for whole-growth-stage salt tolerance. Submergence screening identified a few highly resilient lines, though the overall panel was skewed toward sensitivity, underscoring the need for introgression of stronger submergence tolerance alleles. Cold tolerance assessment identified several promising genotypes with high recovery and survivability, warranting further validation. Collectively, these results provide a valuable genetic resource for breeding climate-resilient rice varieties. The identified tolerant lines offer strong candidates for use in BRRI's breeding pipeline to develop varieties with enhanced resilience to multiple abiotic stresses, thereby supporting sustainable rice production in stress-prone environments such as Bangladesh.

AUTHORS' CONTRIBUTION

MSR and KMI conceived the study and designed the research. AAS, JJ, and MSP assisted in conducting the experiments and contributed to the initial preparation of the manuscript. KMI and AR developed the multi-trait diversity panel and provided financial and technical support. MSR performed the data analysis and led the manuscript writing.

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DECLARATION OF INTERESTS

The authors declare that there are no ethical or financial conflicts of interest.

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