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EVALUATING SALINITY TOLERANCE IN RICE: PHENOTYPIC AND GENOTYPIC ANALYSIS AT THE REPRODUCTIVE STAGE

P.N.M. Marma[1], M.S.R. Khanom [2*], R. Sultana [2], M.W. Akram[3], F. Yasmine[2] and F. Khatun[1]

Abstract

Salinity stress is a major constraint to rice production in the salt-affected coastal regions of Bangladesh. The study aimed to screen eighteen rice genotypes, including checks to find the potential genetic resources for salinity tolerance. A completely randomized design (CRD) with two replications was used at the reproductive stage under controlled conditions using a sustained water bath, where salinity levels ware maintained at 8, 10 and 12 dS/m following the standard IRRI protocol at the Bangladesh Institute of Nuclear Agriculture (BINA). Standard Evaluation Scoring (SES; 1-9 scale) was utilized to assess genotype responses. At 8 dS/m, eleven genotypes, including IRSSTN 1, IRSSTN 11, IRSSTN 21, BRRI dhan97, and Binadhan-10 exhibited strong tolerance. At 10 and 12 dS/m, IRSSTN 1, IRSSTN 11, BRRI dhan97, and Binadhan-10 consistently maintained superior performance. Genetic diversity was assessed using five SSR markers (RM336, RM585, RM1287, RM337, RM3412b), and cluster analysis based on Nei's genetic distance grouped the genotypes into four distinct clusters. Notably, the genotypes identified as salt tolerant based on SES were also grouped together in the molecular clustering, indicating a genetic basis underlying their shared tolerance traits in breeding efforts. The integration of phenotypic and molecular data showed IRSSTN 1, IRSSTN 11, and IRSSTN 21 as promising candidates for developing salt tolerant rice varieties. These findings highlight the importance of using IRSSTN 1, IRSSTN 11, and IRSSTN 21 genotypes in future breeding programs aimed at improving rice resilience in saline environments.

Key words: Breeding programs, phenotypic and Genetic diversity, Rice genotypes, Salinity tolerance and SSR markers,

Introduction

Rice (*Oryza sativa*) is one of the most important staple food crops worldwide, providing a substantial portion of the daily calorie intake for millions of people, particularly in Asia where rice consumption is highest (Bin Rahman and Zhang, 2022). In Bangladesh, rice production contributes about 16% to the country's GDP, and about 70% to its agricultural GDP (Ali, 2023). The country's population of about 174.22 million depends mainly on rice as their primary source, making it central to national food security (Hossain, 2023).

However, rice cultivation in Bangladesh is increasingly challenged by the adverse impacts of climate change (van der Putten *et al.*, 2013). These include salinity, drought, flooding, high-temperature, and declining soil fertility which threaten sustainable crop production. Among these, salinity is one of the most severe environmental constraints that have a detrimental impact on the growth, development, and overall productivity of plants.

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[1] Department of Biotechnology, Bangladesh Agricultural University, Mymensingh-2202,
[2] Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2202
[3] PhD student, Department of Entomology, Bangladesh Agricultural University, Mymensingh-2202
***Corresponding author's E-mail**: sifatbau@gmail.com

(Hoang *et al*., 2016). Its effect is most detrimental during the reproductive stage, where it significantly reduces grain yield compared to the vegetative phase. Therefore, screening rice genotypes for salinity tolerance specifically at the reproductive stage provides a more reliable and effective approach for identifying and developing salinity resilient rice varieties.

Of the 2.85 million hectares of coastal and offshore land in Bangladesh, approximately 1.8 million hectares are affected by varying degrees of salinity (Valjarević *et al.*, 2020). Southern part of Bangladesh is facing this salinity problem which affects the rice crop production in those areas. Also, coastal salinity will continue to increase due to sea level rise because of global warming (Li *et al*., 2023). Vast regions of otherwise arable land remain fallow or unproductive due to salinity. To meet these challenges, the development of salt-tolerant rice varieties is the best solution to increase rice production and ensure the food security of people living in saline-prone areas (Mondal, *et al.*, 2025).

The development of salt-tolerant rice varieties relies on both precise phenotypic screening methods and the integration of molecular marker technologies (Gregorio *et al*., 2002). Molecular characterization enhances understanding of genetic variation and the biological processes linked to salt tolerance. Among molecular tools, microsatellite markers (SSRs) analysis have shown strong potential for detecting key gene loci associated with salinity resistance, thereby offering valuable support to breeders aiming to marker-assisted selection and accelerating the breeding of improved rice cultivars (Hossen *et al.*, 2017).

By evaluating a diverse set of advanced rice breeding lines, the present study aims to deepen the understanding of phenotypic and genetic variation involved in reproductive stage salinity tolerance. Therefore, the objective of this research was to identify the salt tolerant rice genotypes based on both phenotype and molecular marker analysis, with goal of facilitating their use in marker assisted selection in rice breeding programs.

Materials and methods

Experimental Site and Plant Materials

This study was conducted using trays under a polythene shade near the farmyard of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh-2202, Bangladesh. The objective was to screen rice genotypes at the reproductive stage for salinity tolerance using a sustained water bath following IRRI standard protocol (Gregorio *et al.*, 1997). A total of eighteen genotypes were tested, including BRRI dhan28 (salt-susceptible), Binadhan-10, and BRRI dhan97 (salt-tolerant) as checks, collected from BRRI and BINA. The experiment was laid out in a completely randomized design (CRD) with two replications.

Salinity treatments and crop Management

Four (04) salinity treatments were used: 1. Control - normal water 2. EC: 08 dS m^{-1}3. EC: 10 dS m^{-1}4. EC: 12 dS m^{-1}. The pre-germinated seeds were sown (3/4 seeds/pot) on the soil surface of the perforated pot and finally one healthy seedling was kept in each pot after emergence. The pots were irrigated with tap water for the first 60 days. After 60 days of seed sowing, saline water solutions corresponding to the four treatments were added in the tray. The modified standard evaluation system (SES) of IRRI was used to score visual symptoms of salt injury scale 1 to 9 (IRRI, 1996). Data were recorded umder both control and saline conditions for the traits including days to first flowering, days to maturity, plant height (cm), total tillers per hill, effective tillers per hill, panicle length (cm), filled grains per panicle, unfilled grains per panicle, grain yield per hill (g), and 100-grain weight (g).

DNA extraction and molecular marker analysis

Genomic DNA was extracted using the mini preparation CTAB method (Bhowmik *et al.*, 2021) from 21 days old seedling. Out of 19 SSR primers initially tested, five primers (RM336, RM585, RM1287, RM337 and RM3412b) were selected based on polymorphisms and clear banding patterns. Polymerase chain reaction (PCR) amplification was performed in a 10 µl reactions containing: 5 µl PCR Master Mix, 1 µl forward primer, 1 µl reverse primer, 2 µl Nuclease free water and 1 µl of template DNA for each sample. The PCR profile was maintained as initial denaturation at 94℃ for 5 min, followed by 34 cycles of denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min and polymerization at 72℃ for 2 min; and final extension at 72℃ for 7 min. PCR products were separated on 8% polyacrylamide gel **(**PAGE), and stained in ethidium bromide, and visualized using a ultraviolet gel documentation system (Brand and country). Banding patterns of the 18 genotypes were compared with the tolerant control and susceptible control. Genotypes showing similar banding pattern with Binadhan-10 and BRRI dhan97 were considered as salt tolerant and BRRI dhan28 as salt susceptible.

Statistical Analysis

Phenotypic data were analyzed by statistical programs using RStudio with required packages, Minitab 18 and Microsoft Excel. Alpha-Ease 5.5 software was used to determine the molecular weight of each SSR marker allele. Using the software Power Marker (version 3.25), alleles of SSR markers were analyzed. Based on the unweighted pair group method with arithmetic averages (UPGMA) were constructed using the same program and viewing in Tree View using MEGA-X software.

Table 1. List of SSR markers used in this study (Mention position of the marker: at least Chromosome number in the table)

Marker name	Location on Chromosome	Primer size (bp)	Primer sequence (5'-3')	Annealing temperature (°C)
RM336	7	154	Forward: CTTACAGAGAAACGGCATCG Reverse: GCTGGTTTGTTTCAGGTTCG	55
RM585	6	233	Forward: CAGTCTTGCTCCGTTTGTTG Reverse: CTGTGACTGACTTGGTCATAGG	55
RM1287	1	162	Forward: GTGAAGAAAGCATGGTAAATG Reverse: CTCAGCTTGCTTGTGGTTAG	55
RM337	8	192	Forward: GTAGGAAAGGAAGGGCAGAG Reverse: CGATAGATAGCTAGATGTGGCC	55
RM3412b	1	110	Forward: TCATGATGGATCTCTGAGGTG Reverse: GGGAGGATGCACTAATCTTTC	55

Results and Discussion

Phenotypic screening

The evaluation of genotypic responses to varying levels of salinity stress (EC: 8-12 ds m^{-1}) during the reproductive stage revealed significant variations in their tolerance levels. The assessment was carried out using a scale ranging from 1 to 9, where each score represented a specific category of tolerance or susceptibility. At an electrical conductivity (EC) of 8 ds m^{-1}, eleven genotypes (IRSSTN 1, IRSSTN 3, IRSSTN 4, IRSSTN 5, IRSSTN 6, IRSSTN 11, IRSSTN 18, IRSSTN 19, IRSSTN 21, BRRI dhan97, Binadhan-10) demonstrated notable salt tolerance, while seven genotypes (IRSSTN 2, IRSSTN 4, IRSSTN 10, IRSSTN 16, BARI-28, SAL-73, SAL-52) exhibited moderate tolerance (IRRI, 1996). Upon exposure to slightly higher salinity levels at 10 dS m^{-1}, five genotypes (IRSSTN 1, IRSSTN 11, IRSSTN 21, BRRI dhan97, Binadhan-10) maintained their tolerance status. However, seven genotypes (IRSSTN 3, IRSSTN 6, IRSSTN 10, IRSSTN 18, IRSSTN 19, SAL-52, SAL-44) transitioned to the moderately tolerant category (Table 2). At this salinity level, six genotypes (IRSSTN 2, IRSSTN 4, IRSSTN 5, IRSSTN 16, BRRI dhan28, SAL-73) shifted to susceptible group. Under the highest salt stress of 12 dSm^{-1}, only four genotypes (IRSSTN 1, IRSSTN 11, BRRI dhan97, Binadhan-10) retained their tolerance, while other four genotypes (IRSSTN 3, IRSSTN 6, IRSSTN 21, SAL-52) exhibited a moderately tolerant response (Table 2). A notable finding was the increased susceptibility of ten genotypes (IRSSTN 2, IRSSTN 4, IRSSTN 5, IRSSTN 10, IRSSTN 16, IRSSTN 18, IRSSTN 19, BARI-28, SAL-73, SAL-44) which displayed substantial salt injury, indicating increased susceptibility. These symptoms were also observed by several scientists (Bhuiyan, 2005). In reproductive stage, the genotypes of the salt tolerant lines showed relatively better growth and lower symptoms than susceptible genotypes after salinization.

Table 2. Visual salt injury ratings at the reproductive stage using the modified standard evaluation system (SES, IRRI, 1996**)**

Genotypes	Treatments					
	08dS m^{-1}	Tolerance	10dS m^{-1}	Tolerance	12dS m^{-1}	Tolerance
IRSSTN 1	3	Tolerant	3	Tolerant	3	Tolerant
IRSSTN 2	3	Moderately Tolerant	5	Moderately Tolerant	5	Moderately Tolerant
IRSSTN 3	3	Tolerant	5	Moderately Tolerant	5	Moderately Tolerant
IRSSTN 4	5	Moderately Tolerant	7	Sensitive	7	Sensitive
IRSSTN 5	3	Tolerant	7	Sensitive	7	Sensitive
IRSSTN 6	3	Tolerant	5	Moderately Tolerant	5	Moderately Tolerant
IRSSTN 10	5	Moderately Tolerant	5	Moderately Tolerant	7	Sensitive
IRSSTN 11	3	Tolerant	3	Tolerant	3	Tolerant
IRSSTN 16	3	Moderately Tolerant	5	Sensitive	5	Sensitive
IRSSTN 18	3	Tolerant	5	Moderately Tolerant	7	Sensitive
IRSSTN 19	3	Tolerant	5	Moderately Tolerant	7	Sensitive
IRSSTN 21	3	Tolerant	3	Tolerant	5	Moderately Tolerant
BRRI dhan28	5	Moderately Tolerant	7	Sensitive	7	Sensitive
BRRI dhan97	3	Tolerant	3	Tolerant	3	Tolerant
Binadhan-10	3	Tolerant	3	Tolerant	3	Tolerant
SAL-73	5	Moderately Tolerant	7	Sensitive	7	Sensitive
SAL-52	5	Moderately Tolerant	5	Moderately Tolerant	5	Moderately Tolerant
SAL-44	3	Tolerant	5	Moderately Tolerant	7	Sensitive

Here, in 1-9 scale, score 1 indicates highly tolerant (HT), score >1-3 indicates tolerant (T), score >3-5 indicates moderately tolerant (MT), score >5-7 indicates susceptible (S), and score >7-9 indicates highly susceptible (HS)

Results showed significant differences in phenotypic data. In case of days to flowering, minimum value was found in Binadhan-10 (116 days) under control condition, while the maximum value was observed in IRSSTN 6 (133 days) at 12 dS m^{-1} salinity. Days to maturity were recorded minimum 133 and maximum 151 days in IRSSTN 5 and IRSSTN 11, respectively (under what level). Plant height decreased with increased salinity level. The maximum and minimum plant height was observed in IRSSTN 5 (105.50 cm) and IRSSTN 16 (61.00 cm) under control and 12 dS m^{-1}, respectively. Maximum and minimum total tiller was observed in IRSSTN 1 (20.50) and IRSSTN 4 (7.00) under control and 10 dS m^{-1}, respectively. Maximum and minimum (rewrite frequent use of maximum and minimum) number of effective tiller was observed in IRSSTN 1 (18.50) and IRSSTN 1 (4.00) under control and 12 dSm^{-1}, respectively. Maximum and minimum panicle length was observed in

IRSSTN 10 (26.56) and IRSSTN 11 (17.78) under 10 ds m^{-1} and 8 dS m^{-1}, respectively. Maximum and minimum filled grain was observed in IRSSTN 4 (88.00) and IRSSTN 2 (2.50) under control and 12 dS m^{-1}, respectively. Maximum and minimum unfilled grain was observed in IRSSTN 4 (94.45) and BRRI dhan97 (11.80) under control and 12 dS m^{-1}, respectively. Maximum and minimum yield per hill was observed in IRSSTN 1 (39.59) and IRSSTN 4 (0.23) under control and 12 dS m^{-1}, respectively. Maximum and minimum 100 SW was observed in IRSSTN 5 (2.58) and IRSSTN 4 (0.32) under control and 12 dS m^{-1}, respectively (Table 3).

Table 3. Effects of genotypes and salinity treatments on different morphological and reproductive traits related to grain yield at the reproductive stage

Genotypes	Treatment	DF (days)	DM (days)	PH (cm)	Total tiller	Effective tiller	Panicle length (cm)	FG	UFG	100 SW (gm)	Yield per hill (gm)
IRSSTN 1	Control	116.00	134.00	96.50	20.50	18.50	26.26	73.60	25.70	2.21	39.59
	8 dS/m	119.00	137.00	91.00	15.00	14.00	23.52	60.50	29.70	1.86	15.95
	10 dS/m	119.00	138.00	86.50	16.00	11.50	26.27	16.70	67.35	1.84	12.89
	12 dS/m	122.00	142.00	82.00	13.50	12.00	25.53	47.10	49.05	1.97	12.29
IRSSTN 2	Control	123.50	142.50	98.50	8.50	6.00	24.57	48.55	62.35	2.18	5.17
	8 dS/m	125.50	147.00	98.00	8.50	6.00	25.50	22.70	52.70	1.93	2.35
	10 dS/m	127.00	146.50	97.00	7.00	5.00	22.95	11.55	53.45	1.05	1.05
	12 dS/m	128.50	148.50	86.00	7.00	7.00	23.95	2.50	70.30	0.36	0.74
IRSSTN 3	Control	118.50	136.00	102.00	15.50	15.00	22.37	55.60	32.40	2.16	16.98
	8 dS/m	120.50	137.00	104.50	12.00	12.00	24.95	46.30	17.30	2.11	13.78
	10 dS/m	124.00	134.50	97.00	10.00	10.00	22.57	41.50	27.00	0.91	9.04
	12 dS/m	126.00	137.50	84.50	8.50	7.50	22.81	34.70	41.20	1.74	6.96
IRSSTN 4	Control	125.50	142.50	85.00	12.50	12.00	23.38	88.00	52.60	1.21	8.58
	8 dS/m	127.00	144.50	74.00	10.50	8.50	22.25	32.43	54.70	1.07	4.60
	10 dS/m	128.50	147.50	65.00	7.00	5.50	21.70	29.84	69.32	0.91	1.70
	12 dS/m	131.50	147.50	66.50	10.00	6.50	23.24	5.00	94.45	0.32	0.23
IRSSTN 5	Control	118.50	133.00	105.50	15.00	15.00	23.32	66.70	32.30	2.58	22.82
	8 dS/m	121.50	134.50	90.50	14.00	14.00	23.26	17.20	66.40	2.22	3.93
	10 dS/m	123.00	137.00	89.50	10.50	10.00	22.38	4.70	49.20	0.71	0.56
	12 dS/m	124.50	138.00	90.00	10.50	9.00	23.12	14.00	76.71	1.47	1.52
IRSSTN 6	Control	125.00	141.00	96.50	12.50	10.50	22.35	45.50	38.80	2.02	13.89
	8 dS/m	127.00	145.00	95.50	9.50	8.50	22.02	39.90	60.60	2.07	5.75
	10 dS/m	128.50	147.50	87.00	7.00	4.50	20.45	16.83	62.80	1.99	1.84
	12 dS/m	133.00	151.00	85.00	10.00	4.00	22.00	10.95	38.58	0.60	0.61
IRSSTN 10	Control	122.00	141.00	100.50	17.00	13.00	23.78	59.55	39.55	2.47	15.68
	8 dS/m	125.50	146.00	98.00	13.00	8.00	21.39	42.80	34.20	1.83	7.74
	10 dS/m	127.00	146.00	88.00	12.00	7.00	26.52	60.25	57.65	1.86	10.37
	12 dS/m	128.00	148.50	86.00	10.50	7.50	20.68	16.00	73.00	2.14	2.91
IRSSTN 11	Control	124.50	141.50	88.50	12.50	12.50	19.90	58.70	30.50	2.28	13.35
	8 dS/m	126.00	144.00	86.50	12.00	11.00	17.78	58.90	25.80	1.96	14.04
	10 dS/m	127.00	147.00	78.50	10.50	9.50	18.12	47.00	41.70	1.88	7.89
	12 dS/m	128.50	151.00	82.50	9.00	6.50	19.28	60.60	32.20	2.05	7.87
IRSSTN 16	Control	123.00	143.00	89.00	12.00	12.00	23.48	43.20	47.30	1.76	7.09
	8 dS/m	124.00	145.00	90.00	11.50	7.00	21.99	19.40	59.20	1.38	1.67
	10 dS/m	126.50	146.00	72.00	13.00	7.00	21.46	12.75	52.60	1.09	1.14
	12 dS/m	129.00	148.50	61.00	9.50	5.00	19.98	8.85	55.00	0.80	0.80

Table 3. Continued

	Treatment	DF (days)	DM (days)	PH (cm)	Total tiller	Effective tiller	Panicle length (cm)	FG	UFG	100 SW (gm)	Yield per hill (gm)
IRSSTN 18	Control	122.00	143.00	97.50	15.50	12.50	25.10	75.10	44.30	1.99	14.53
	8 dS/m	124.00	145.00	90.50	13.50	12.50	22.70	29.60	40.10	1.39	5.64
	10 dS/m	126.50	146.50	80.00	10.50	10.00	21.15	25.20	59.40	1.21	2.69
	12 dS/m	127.00	149.00	74.00	11.50	5.00	20.65	25.60	53.10	1.24	1.24
IRSSTN 19	Control	119.00	135.00	96.50	13.00	12.50	23.49	75.50	23.94	2.23	16.34
	8 dS/m	121.00	137.50	99.50	13.00	12.50	20.39	43.10	37.30	1.91	9.86
	10 dS/m	124.00	142.00	95.00	13.00	10.50	22.34	31.85	35.60	1.84	8.29
	12 dS/m	128.50	143.50	93.50	9.00	6.50	20.85	13.50	57.00	1.06	1.06
IRSSTN 21	Control	121.50	136.00	88.50	14.50	13.50	22.55	56.90	40.20	1.84	17.65
	8 dS/m	123.00	139.00	80.00	14.00	12.50	21.69	75.90	27.50	1.61	15.70
	10 dS/m	125.00	141.50	81.00	14.50	11.00	21.40	77.10	37.20	1.77	13.18
	12 dS/m	128.50	143.00	82.00	9.50	5.00	19.99	33.40	27.00	1.83	3.43
BRRI dhan28	Control	118.00	135.00	83.00	16.50	15.50	21.79	59.10	11.80	2.06	19.39
	8 dS/m	119.50	136.00	82.50	13.50	13.50	20.53	31.40	19.70	1.83	10.44
	10 dS/m	121.00	138.00	91.00	10.00	8.50	22.71	37.50	18.20	1.91	9.30
	12 dS/m	124.00	142.50	77.50	9.50	6.50	22.63	12.53	36.70	1.64	3.85
BRRI dhan97	Control	124.50	141.00	97.00	12.50	12.50	20.94	51.10	30.80	2.46	18.56
	8 dS/m	126.00	142.00	90.50	11.00	10.00	20.47	26.10	63.10	2.28	4.71
	10 dS/m	127.00	143.50	93.00	12.50	6.00	19.08	17.50	66.80	1.84	3.63
	12 dS/m	129.00	146.00	82.50	12.00	4.00	20.93	9.10	77.90	1.74	1.24
Binadhan-10	Control	116.00	135.50	100.50	12.00	12.00	22.15	64.50	15.80	2.51	25.02
	8 dS/m	118.00	139.00	90.50	10.50	10.00	21.15	63.00	18.80	2.50	20.22
	10 dS/m	119.00	141.50	92.50	10.00	9.00	21.75	48.60	31.50	2.27	12.09
	12 dS/m	121.00	142.00	94.00	8.50	8.00	21.16	58.60	22.30	2.20	13.66
SAL-73	Control	125.50	140.00	104.00	8.00	8.00	23.60	58.50	55.30	2.34	11.92
	8 dS/m	126.50	141.00	86.50	8.50	5.50	23.84	11.50	71.05	1.58	1.58
	10 dS/m	127.00	142.00	87.50	8.00	7.00	25.70	49.75	51.10	1.99	6.94
	12 dS/m	128.00	144.00	86.50	10.00	6.00	21.83	20.70	69.10	1.81	2.28
SAL-52	Control	126.00	141.00	84.50	12.50	12.50	22.35	39.80	49.80	2.29	12.79
	8 dS/m	127.00	141.50	78.00	12.00	12.00	19.90	53.70	38.60	2.11	11.57
	10 dS/m	128.50	143.00	75.00	8.50	8.50	20.01	33.15	38.10	2.04	7.64
	12 dS/m	130.00	147.00	74.50	10.50	8.00	19.42	27.70	57.45	1.96	4.65
SAL-44	Control	120.00	135.50	82.00	17.00	17.00	22.65	55.50	51.80	2.32	23.46
	8 dS/m	121.00	136.50	73.50	15.50	14.50	20.73	54.00	47.00	2.04	17.77
	10 dS/m	122.00	138.00	72.50	16.00	10.50	21.15	48.70	62.70	2.21	6.81
	12 dS/m	123.00	139.50	76.50	12.00	6.50	20.42	49.13	29.10	1.75	3.86
LSD$_{0.05}$		1.81	1.96	5.31	2.17	2.24	1.86	4.08	9.22	0.35	2.63
Mean		124.33	141.79	87.33	11.69	9.64	22.14	39.78	45.71	1.78	9.11
Standard Error		0.44	0.53	1.14	0.33	0.40	0.22	2.50	2.07	0.06	0.87
Standard Deviation		3.74	4.52	9.67	2.78	3.38	1.89	21.21	17.60	0.52	7.42
Minimum		116.00	133.00	61.00	7.00	4.00	17.78	2.50	11.80	0.32	0.23
Maximum		133.00	151.00	105.50	20.50	18.50	26.52	88.00	94.45	2.58	39.59
Level of sign.		**	**	**	**	**	**	**	**	**	**
CV%		0.73	0.69	3.05	9.29	11.64	4.21	5.15	10.12	9.83	14.48

**Indicates significant at 0.01 probability level.

Here, DF= Days to flowering, DM= Days to maturity, PH=Plant height (cm), TT/P= No. of total tillers per hIll, ET/P= No. of effective tillers per hill PL= Panicle length (cm), FG/P= No. of filled grains per panicle, UG/P= No. of unfilled grains per panicle, HGW= Hundred grains weight (g), and GY/H-Grain yield per hill. SD and SE means standard deviation and standard error respectively

ANOVA conducted on various morphological traits of rice genotypes subjected to different salinity stress levels reveals significant variations in their performance (Kaufmann and Schering, 2007). Salinity stress leads to a noteworthy increase in both DF and DM (Goswami and Deka, 2020). This delay in flowering and maturity can have adverse effects on rice growth and yield, as it prolongs the reproductive phase, potentially resulting in yield reduction. Under salinity stress, plant height generally experiences a decrease. This reduction in height, while seemingly negative in terms of plant size, could be seen as a positive adaptation strategy to conserve water resources, ultimately contributing to improved water-use efficiency. Salinity stress tends to decrease both total and effective tiller number. This reduction can be detrimental as it often results in decreased grain yield due to fewer tillers contributing to overall panicle formation (Per Schjønning and Mathieu Lamandé, 2018). Salinity stress typically leads to shorter panicles. This reduction in panicle length can negatively impact grain production as it may limit the space available for grain development (Zhang and Lu, 2020). Salinity stress exerts negative influences on both 100 seed weight and yield per hill. Lighter grains and reduced yield are among the most significant negative impacts of salinity stress on crop productivity. Both FG and UFG tend to decrease under salinity stress, implying a negative impact on the photosynthetic capacity of rice plants. Reduced photosynthesis can lead to lower grain yield (Zörb, Geilfus and Dietz, 2018). Salinity stress tends to have predominantly negative impacts on various morphological traits, including delayed flowering and maturity, reduced plant height, fewer tillers, shorter panicles, lighter grains, and lower yield. However, it is crucial to acknowledge that certain genotypes may exhibit positive adaptations under stress conditions (Hartman and Tringe, 2019). These findings underscore the importance of rigorous genotype selection and breeding efforts to minimize the adverse effects of salinity on rice production, ultimately contributing to more resilient and productive crop systems.

Genotypic Screening

Initially nineteen primers were used for polymorphism survey. Five primers (RM336, RM585, RM1287, RM337, and RM3412b) showed clear polymorphism out of 19 which were linked to the *Saltol* QTL on Chromosome 1 and other QTL on chromosome 6 & 8, were finally used to evaluate nineteen rice genotypes for salinity tolerance. (Gimhani *et al*., 2014*)*. The major allele frequency ranged from 0.158 to 0.211 across the markers. RM336 and RM3412 exhibited the highest major allele frequency at 0.211. In contrast, RM585, RM1287, and RM337 showed a major allele frequency of 0.158 (Table 4). The number of alleles varied among the markers, ranging from 8 to 15 alleles. RM337 presented the highest allele number with 15 alleles, followed by RM1287 with 13 alleles, RM585 with 12 alleles, RM336 with 11 alleles, and RM3412b with 8 alleles. Genetic diversity, as measured by genetic diversity index (GD), was found to be high across all markers, with values ranging from 0.853 to 0.920. RM337 exhibited the highest genetic diversity index of 0.920, followed by RM1287 (0.909), RM585 (0.903), RM336 (0.886), and RM3412b (0.853) (Table 4).

Polymorphic Information Content (PIC) values ranged from 0.836 to 0.914. RM337 showed the highest PIC value of 0.914 indicating a high level of polymorphism within the studied genotypes. This was followed by RM1287 (0.901), RM585 (0.895), RM336 (0.876), and RM3412b (0.836) (Table 4). The SSR markers employed in this study demonstrated substantial polymorphism and genetic diversity among the rice genotypes, providing valuable insights into their genetic architecture and potential breeding applications.

Table 4. Amplification results of five SSR markers on genetic diversity analysis of the rice genotypes

Markers	Major Allele Frequency	Allele No.	Genetic Diversity	PIC
RM336	0.211	11	0.886	0.876
RM585	0.158	12	0.903	0.895
RM1287	0.158	13	0.909	0.901
RM337	0.158	15	0.920	0.914
RM3412b	0.211	8	0.853	0.836
Mean	0.179	11.8	0.894	0.884

The banding patterns of 19 different rice genotypes were analyzed molecularly using five different SSR markers, and the results are shown in Figure 1and Figure 2. Binadhan-10, BRRI dhan28, and BRRI dhan97 were references for comparing the banding patterns. Genotypes that produced bands identical to or very close to those of the salt-tolerant Binadhan-10 and BRRI dhan97 were expected to exhibit tolerance to salt and those who are close to BRRI dhan28 were considered as susceptible (Fig. 1 & 2). Although there was a limitation to using Markers, the banding pattern aligned with Standard Evaluation Scoring (SES), and cluster pattern.

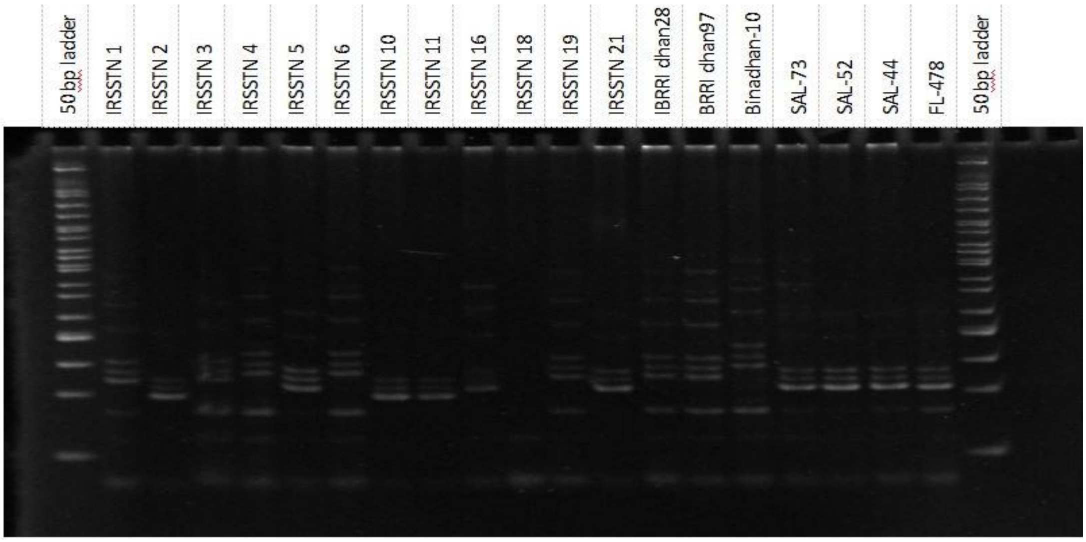

Fig. 1. Banding pattern of 19 rice genotypes with RM3412b primer.

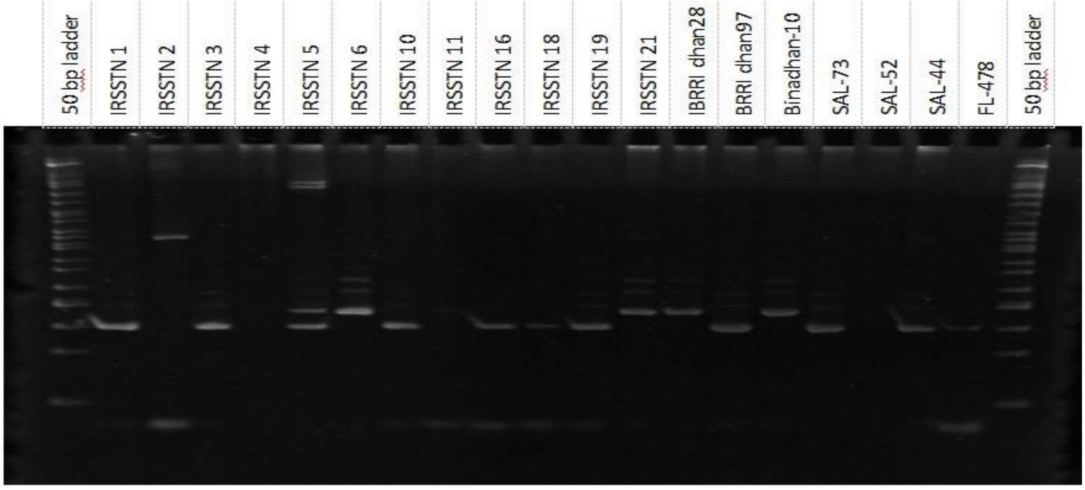

Fig. 2. Banding pattern of 19 rice genotypes with RM337 primer.

The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram, constructed based on molecular marker data and analyzed using the Jaccard similarity coefficient, revealed the genetic relationships among the rice genotypes under study (Fig. 3). The dendrogram classified the genotypes into four main clusters, each cluster further subdivided into several distinct subclusters reflecting the genetic diversity present among them. Fig. 3 illustrated that, most of the salinity tolerant and moderately tolerant genotypes were grouped in Cluster I and Cluster II except for IRSSTN 02, IRSSTN 04 and IRSSTN

19 which were susceptible. Cluster I was divided into two subclusters. Subcluster 1 comprised Binadhan-10 and IRSSTN 02, while Subcluster 2 consisted of FL-478 and IRSSTN 06. Cluster II exhibited three subclusters. Subcluster 1 included BRRI dhan97 and IRSSTN 03, Subcluster 2 comprised IRSSTN 01 and SAL-52. Subcluster 3 consisted of IRSSTN 11, IRSSTN 04, and IRSSTN 19. Cluster III formed a susceptible group and displayed genetic dissimilarity to the other genotypes except for IRSSTN 21& IRSSTN 10 which were tolerant. This cluster was also divided into three subclusters. Subcluster 1 consisted of BRRI dhan28, IRSSTN 05, and SAL-73. Sub cluster 2 contains only IRSSTN 21 and Sub cluster 3 comprised IRSSTN 10, IRSSTN 18, and SAL-44. Cluster IV contained only one genotype, IRSSTN 16, indicating its genetic distinctiveness from the other studied genotypes (Figure 3). All the genotypes in a particular cluster showed higher distance with the genotypes in another cluster. This also indicates the homogeneous nature of the rice genotypes present in the same cluster. The results were supported by the findings of Rahman et al. (2012) and Islam et al. (2018). The subdivision of clusters and formation of distinct subclusters within the dendrogram elucidate the intricate genetic diversity present among the rice genotypes, providing valuable insights for future breeding strategies and genetic resource management.

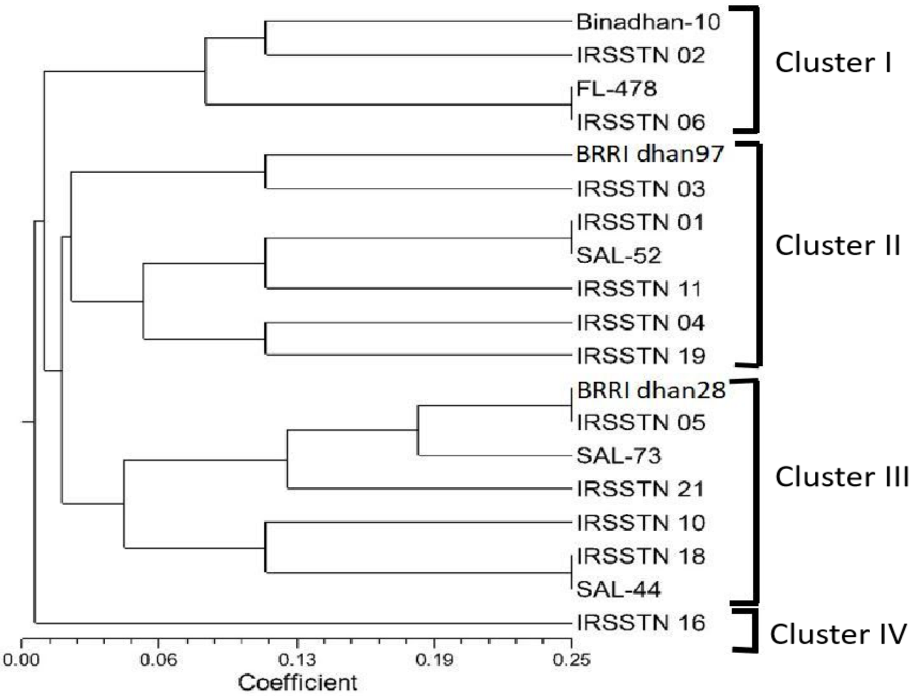

Fig. 3. **Molecular marker based genetic relationship between the rice genotypes shown by UPGMA dendrogram analyzed through Jaccard similarity coefficient.**

Conclusion

This study evaluated the salt tolerance of eighteen distinct rice genotypes under varying salinity levels (8 dSm^{-1}, 10 dSm^{-1}, and 12 dSm^{-1}). At the highest salinity level of 12 dSm^{-1}, only four genotypes, namely IRSSTN 1, IRSSTN 11, BRRI dhan97, and Binadhan-10, displayed notable salt tolerance. In addition to evaluating salt tolerance through morphological traits, this study also incorporated molecular characterization using SSR markers (RM336, RM585, RM1287, RM337, RM3412b). The SSR analysis revealed distinct genetic relationships among the rice genotypes, which complemented the phenotypic observation of salt tolerance. Clustering based on Nei's genetic distance highlighted the genetic diversity within the genotypes and identified clusters of closely related varieties with shared salt tolerance traits. The integration of molecular data with phenotypic screening provided a comprehensive understanding of the genetic and morphological mechanisms underlying salt tolerance in rice. This combined approach is valuable for the development of targeted breeding strategies aimed at improving salt tolerance in rice, thereby enhancing the resilience of rice cultivation in saline-prone areas.

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