Bangladesh J. Nuclear Agric, 39(2): 17-34, 2025 *ISSN 0258-7130*
DOI: https://doi.org/10.3329/bjnag.v39i2.87279

MORPHO-MOLECULAR ANALYSIS OF COASTAL RICE GERMPLASM USING *SALTOL*-SPECIFIC MARKERS AT THE SEEDLING STAGE

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Abstract

Rice is the staple food for 3.5 billion people worldwide, and soil salinity poses a major threat to its production in countries like Bangladesh. This study, conducted at the Plant Breeding Division of BINA, aimed to identify salinity-tolerant rice genotypes. Fourteen landraces, three advanced lines, with three check varieties were hydroponically screened at the seedling stage under EC 8, 12, and 14 dS m^{-1} following IRRI standard protocols. After 21 days of salinization, six genotypes were identified as tolerant, five as moderately tolerant, and the remainder as susceptible at EC 14 dS m^{-1} based on SES score. ANOVA revealed significant differences among genotypes for shoot length, root length, shoot and root fresh and dry weights, and standard evaluation score (SES), with most traits significant at 0.1% probability. Path coefficient analysis indicated that root fresh weight, root dry weight, and shoot length had direct positive effects on SES at the genotypic level, while shoot fresh weight and shoot length were positively influential at the phenotypic level. All traits showed higher phenotypic than genotypic coefficients of variation, indicating environmental influence, and heritability ranged from 80.84% to 99.10%, highest for shoot length. Molecular confirmation of *Saltol* QTL with three SSR markers (RM7075, RM1287, RM10772) detected an average of 10 alleles per locus, with PIC values from 0.700 to 0.914. Combining morphological and molecular data, Khasrael, Kajolsael, Khusi, Patnai, Rajasael, and Kasfulbalam were identified as salt-tolerant, providing valuable material for breeding programs.

Keywords: *Oryza sativa*, Salinity tolerance, *Saltol* QTL, SSR marker, UPGMA dendrogram

Introduction

Rice is a key staple crop for global food security, cultivated on over 16 million hectares in more than 100 countries and producing around 800 million tonnes annually to feed nearly half of the world's population. Globally, rice contributes almost 85% of cereal consumption and occupies about 5% of the world's arable land (BRKB, 2025; USDA, 2025). Bangladesh is the third-largest rice producer (FAO, 2024), and rice is cultivated across almost the entire country.

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Despite its importance, rice production is increasingly constrained by abiotic stresses, which significantly reduce yield potential. Among these, salinity stress remains one of the most severe challenges, especially in coastal agro-ecosystems. Globally, soil salinity now affects hundreds of millions of hectares of agricultural land; for example, topsoil salinization currently impacts about 424 million hectares, with subsoil salinity extending over 833 million hectares, and this area is expected to expand further under ongoing climate change trends (Sarkar *et al.,* 2025). Salinity levels in affected fields often exceed 4 dS/m, a threshold associated with significant reductions in rice growth and yield, and projections suggest salinity increases of around 26 % by 2050 in many coastal areas due to sea-level rise and reduced freshwater inflow (World Bank Group, 2015). A meta-analysis of 58 studies found that salinity can reduce rice grain yield by about 64.5 % on average across different conditions (Zheng *et al.,* 2023).

Previous studies have demonstrated that genetic improvement is one of the most effective and economically viable strategies for enhancing salinity tolerance in rice, with traditional landraces, wild relatives, and induced mutants serving as valuable sources due to their broad genetic diversity (Reddy *et al.,* 2017; Thomson *et al.,* 2010). While conventional breeding and mutation breeding approaches have successfully identified tolerant genotypes, progress is often slow because salinity tolerance is a complex trait controlled by multiple genes. Phenotypic screening at the seedling stage, particularly under hydroponic conditions, has therefore been widely employed to evaluate tolerance, as it allows uniform stress application, rapid assessment, and reproducible measurement of morphological responses. Although early-stage screening effectively identifies salt-tolerant genotypes for further breeding, reliance solely on phenotypic selection may be unreliable due to environmental variability (Tabassum *et al.,* 2021).

To overcome these limitations, molecular characterization has increasingly been integrated with phenotypic screening. The *Saltol* quantitative trait locus (QTL) on chromosome 1 is one of the most extensively studied genomic regions associated with seedling-stage salinity tolerance in rice (Marè *et al.,* 2023). Marker-assisted selection (MAS) using Saltol-specific markers has been successfully applied to introgress salinity tolerance from tolerant landraces into elite high-yielding cultivars, thereby improving selection efficiency and reducing breeding time.

Therefore, the present study aimed to conduct a morpho-molecular analysis of coastal rice germplasm at the seedling stage using *Saltol*-specific markers, alongside phenotypic evaluation under salinity stress. The study further aimed to examine the association between key morphological traits and salinity tolerance-related attributes. The findings are expected to facilitate the identification of superior germplasm and provide a scientific basis for developing salt-tolerant rice genotypes.

Materials and Methods

Experimental setup

The study was conducted in the glasshouse of the Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, from 27 November 2022 to 15 January 2023. Twenty rice genotypes, including 14 germplasm from saline-prone areas, three advanced lines, and three checks (Binadhan-10 as tolerant, Binadhan-7 and BRRI dhan28 as susceptible (Haque *et al.,* 2012; Kibria *et al.,* 2017; Tahjib-Ul-Arif *et al.,* 2018), were evaluated for seedling-stage salinity tolerance following the modified IRRI protocol (IRRI, 2013). The experiment was arranged in a Completely Randomized Design (CRD) with two replications under four treatments: control (T_1), 8 dS m^{-1} (T_2), 12 dS m^{-1} (T_3), and 14 dS m^{-1} (T_4). Hydroponic culture used Peter's nutrient solution (200 g Peter Professional + 42 g $FeSO_4$ per 200 L water; pH 5.10-5.15, adjusted with 1 N HCl/NaOH), which was stirred three times daily and replaced every five days. Salinity treatment was initiated 14 days after germination and maintained continuously in the hydroponic system for the following 21 days. Data were collected from five plants per genotype per treatment, including shoot and root lengths, fresh weights, and dry weights after oven-drying at 60 °C for seven days.

Evaluation of salt stress symptoms

The modified standard evaluation score (SES) presented in Table 1 was used in rating the visual symptoms of salt toxicity following IRRI, 2013. This scoring discriminated between the susceptible and tolerant, as well as the moderately tolerant, genotypes. Scoring was done at 14 and 21 days after salinization.

Table 1. Modified Standard Evaluation Score (SES) for visual salt injury at the seedling stage

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

Molecular characterization

Fresh, young leaves from 21-day-old seedlings were collected, washed, dried, and stored at –80 °C for DNA extraction using the CTAB method. Genomic DNA (50 ng/µl) was amplified in 10 µl PCR reactions containing 1 µl DNA, 1 µl forward primer, 1 µl reverse primer, 5 µl master mix, and 2 µl nuclease-free water, using three polymorphic SSR markers (RM7075, RM1287 and RM10772) were used to evaluate 20 rice genotypes for salinity tolerance (Babu *et al.*, 2014; Gimhani *et al.*, 2014; Kumari *et al.*, 2019; Rashid *et al.*, 2018; Suprayogi *et al.*, 2021) (Table 2). Reactions were run in a thermocycler for 35 cycles: initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, respective annealing temperature (Table 2) for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were resolved on 8% polyacrylamide gels, stained with ethidium bromide, visualized under UV light, and documented, following strict aseptic precautions.

Table 2. SSR markers linked to the Saltol QTL on Chromosome 1 used for molecular characterization.

Locus name	Amplicon size range (bp)	Repeat motif	Sequence	Annealing temperature (°C)	Reference
RM7075	155	$(ACAT)_{13}$	F: TATGGACTGGAGCAAACCTC R: GGCACAGCACCAATGTCTC	50	(Babu *et al.*, 2014; Rashid *et al.*, 2018)
RM1287	162	$(AG)_{17}$	F: GTGAAGAAAGCATGGTAAATG R: CTCAGCTTGCTTGTGGTTAG	55	*(Kumari et al., 2019; Suprayogi et al.,* 2021)
RM10772	173	$(CTT)_{16}$	F: 5′GCACACCATGCAAATCAATGC3′ R: 5′CAGAAACCTCATCTCCACCTTCC3′	55	*(Gimhani et al., 2014; Kumari et al.,* 2019)

Data analysis

The Unweighted Pair Group Method with Arithmetic Averages (UPGMA) dendrogram was used to cluster the twenty genotypes according to the genetic similarity matrix. NTSYSpc version (2.10) was the computer package used to do the cluster analysis and create the dendrogram. Data analysis included correlation visualization using GGally::ggpairs and multivariate assessment through PCA plotted with ggplot2 to explore trait associations and genotype clustering.

Results

Phenotypic Evaluation

Rice is highly sensitive to salinity, which negatively impacts germination, growth, and yield. In this study, twenty genotypes were screened morphologically and molecularly, revealing that salinity caused leaf growth suppression and premature senescence (Zeng *et al.*, 2001), while tolerant genotypes showed comparatively less damage; control seedlings exhibited normal growth, facilitating the identification of tolerant lines.

Salinity injury at the seedling stage was evaluated at 8, 12, and 14 dS m^{-1} using IRRI’s SES (1 = highly tolerant, 3 = tolerant, 5 = moderately tolerant, 7 = susceptible and 9 = highly susceptible), with final scoring 21 days after salinization. Among the 20 genotypes evaluated at 14 dS m^{-1}, six were classified as tolerant (Kasrael, Kajolsael, Patnai, SAL-45, Talmugur, Binadhan-10), five as moderately tolerant, and nine as susceptible. The susceptible group included the check varieties BRRI dhan28 and Binadhan-7 (Table 3).

(a) (b) (c) (d)

Fig. 1. Comparison of phenotypic variations under different salinity levels in rice genotypes (a) Control, (b) EC- 8 dS m^{-1}, (c) EC- 12 dS m^{-1} and (d) EC-14 dS m^{-1}, respectively.

Table 3. SES score of 20 rice genotypes under salinized condition grown in hydroponic culture at the seedling stage

Genotypes	EC- 8dS/m		EC-12dS/m		EC-14dS/m	
	SES score	Tolerance	SES score	Tolerance	SES score	Tolerance
Khasrael	3	T	3	T	3	T
Kajolsael	3	T	3	T	3	T
Khunsi	3	T	3	T	5	MT
Daksael	3	T	5	MT	5	MT
BRRI dhan28	3	T	5	MT	7	S
Rupeshsor	3	T	5	MT	7	S
Patnai	3	T	3	T	3	T
Rajasael	3	T	3	T	5	MT
Binadhan-10	3	T	3	T	3	T
Bohorimota	5	MT	5	MT	7	S
Sadagotal	5	MT	3	T	5	MT
SAL-73	3	T	3	MT	7	S
Khasfulbalam	3	T	3	T	5	MT
Hatibejor	3	T	5	MT	7	S
Dudkolom	3	T	5	MT	7	S
SAL-52	3	T	5	T	7	S
SAL-45	3	T	3	T	3	T
Talmugur	3	T	3	T	3	T
Binadhan-7	3	T	5	MT	7	S
Holdegotal	3	T	3	T	7	S

1-9 Scale, where 1 = highly tolerant (HT), 3 = tolerant (T), 5 = moderately tolerant (MT), 7 = susceptible (S) and 9 = highly susceptible (HS)

Hydroponic evaluation for seedling stage salinity tolerance

Analysis of variance

The ANOVA (Table 4) revealed highly significant ($p < 0.01$) differences among genotypes for all traits, with the highest mean sum of squares observed for percentage of live leaves (1446.9) and shoot length (670.35), indicating substantial genetic variability (Burdi *et al.,* 2025). Treatment effects were also highly significant ($p < 0.01$), explaining most of the variation, particularly for % live leaves (52696.5) and shoot length (2369.34), confirming the strong influence of salinity stress on plant performance (Haq *et al.,* 2009). Moreover, the genotype × treatment interaction was significant ($p < 0.01$) for all traits (e.g., 6.42 for root length, 14.39 for shoot length), indicating differential genotype responses under varying salinity levels and suggesting the potential to identify salt-tolerant genotypes (Burdi *et al.,* 2025) through screening.

Table 4 Mean sum of squares from the ANOVA

Source of variation	df	Fresh wt. (g) roots	Fresh wt. (g) shoots	Root length	Shoot length	Dry wt. roots	Dry wt. shoots	% Live leaves	SES
Genotypes	19	0.14376**	1.3210**	31.946**	670.35**	0.02183**	0.03891**	1446.9**	4.3829**
Treatment	3	1.84373**	9.9420**	265.985**	2369.34**	0.05599**	0.15985**	52696.5**	32.9583**
G x T	57	0.03028**	0.0929**	6.424**	14.39**	0.02017**	0.00247**	263.6**	1.4496**
Error	80	0.01023	0.0170	1.799	3.04	0.00010	0.00126	153.3	0.3750

Level of significance" *** '0.01'; ** '0.05'; * '0.1

Percent changes in the morphological trait

Salinity clearly reduced root and shoot growth, as well as fresh and dry biomass, and the proportion of live leaves, while increasing visual injury (SES) in most genotypes. The magnitude of reduction increased with salinity levels (8, 12, and 14 dS m^{-1}) (Fig. 3). The results are presented in Table 2.

Shoot fresh weight decreased by 0.53–2.24 g (8 dS m^{-1}), 0.45–1.61 g (12 dS m^{-1}), and 0.22–1.53 g (14 dS m^{-1}), with most genotypes performing better under control conditions. At 8 dS m^{-1}, most genotypes showed a reduction of 15-40% relative to the control. However, at 14 dS m^{-1}, the reduction exceeded 60% in genotypes like SAL-52 and Binadhan-10. Khasrael showed the highest tolerance, maintaining the largest absolute SFW across all salinity levels.

Root fresh weight reductions ranged from 0.45–1.16 g (8 dS m^{-1}), 0.33–0.70 g (12 dS m^{-1}), and 0.20–0.67 g (14 dS m^{-1}). Compared to control conditions, the percentage reduction in RFW was approximately 20–50% at 8 dS m^{-1}, reaching over 75% at 14 dS m^{-1} for sensitive genotypes. Talmugur and Khasrael maintained the highest RFW under stress, while SAL-52 and Binadhan-7 exhibited the most drastic proportional reductions (Fig. 2).

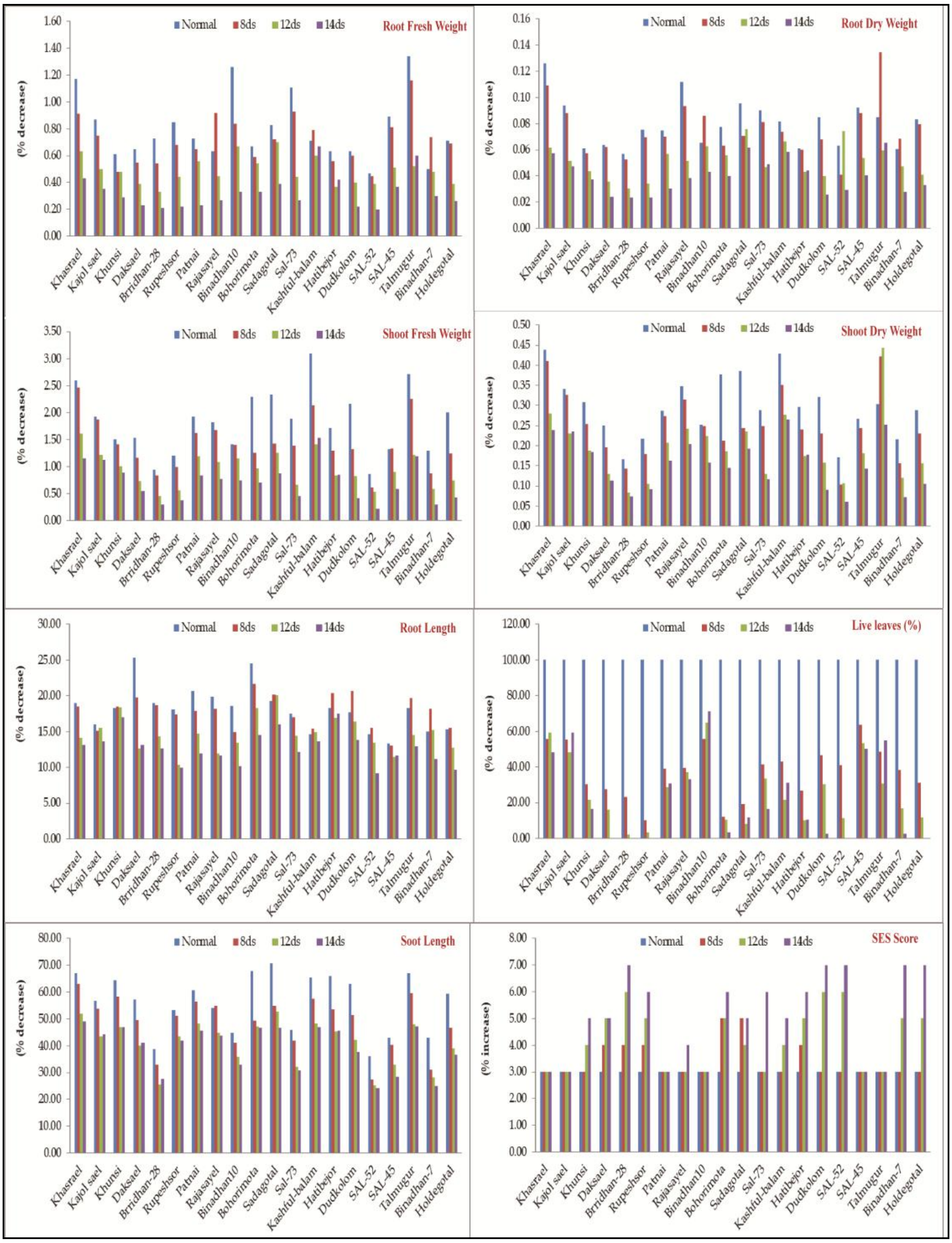

Fig. 2. Bar plots showing the reduction of studied traits in different levels of stress

Root length decreased by 13.08–21.70% at 8 dS m^{-1}, 10.00–20.03% at 12 dS m^{-1}, and 9.17–17.50% at 14 dS m^{-1}, with most genotypes maintaining greater root length under control conditions. The maximum root lengths were recorded in Bohorimota and Sadagotal, while the most significant proportional decreases occurred in SAL-52 and Dudkolom (Fig. 2).

Shoot dry weight declines ranged 0.10–0.42 g (8 dS m^{-1}), 0.08–0.44 g (12 dS m^{-1}), and 0.06–0.26 g (14 dS m^{-1}). This reflects a proportional reduction of approximately 25–60% relative to control conditions. Khasrael and Sadagotal maintained the highest dry biomass (minimum reduction), while SAL-52 and Binadhan-10 exhibited the maximum proportional reduction across all salinity levels.

Root dry weight decreased by 0.04–0.13 g at 8 dS m^{-1}, 0.03–0.08 g at 12 dS m^{-1}, and 0.02-0.07 g at 14 dS m^{-1}, with most genotypes maintaining higher values under control conditions. This represents a proportional reduction of up to 75% compared to control conditions in sensitive genotypes like SAL-52 and Binadhan-7 (maximum reduction). Conversely, Khasrael and Talmugur exhibited the minimum proportional reduction, maintaining the highest dry biomass across all stress levels.

Under control most plants retained 100% live leaves. Under salinity %LL ranged 10.32–63.56% (8 dS m^{-1}), 2.38–65.05% (12 dS m^{-1}) and 2.78–71.07% (14 dS m^{-1}). Khasrael and Kajol sael showed the highest tolerance by maintaining approximately 50–60% green foliage at 14 dS m^{-1} (minimum reduction). In contrast, genotypes such as SAL-52, Dudkolom, and Holdegotal experienced the greatest reduction, with survival rates dropping below 10% (Fig. 2).

Trait association

Correlation analysis revealed that root fresh weight (FRW) was positively and significantly associated with most traits under salinity stress, but not under control conditions (Fig. 4). FRW showed strong positive associations with shoot fresh weight (FSW, 0.769*), root dry weight (DRW, 0.847*), and shoot dry weight (DSW, 0.697*) across salinity levels. Traits such as root length (RL) and shoot length (SL) were significantly correlated with FRW overall, but these associations were observed as non-significant under individual salinity treatments. FSW was consistently positively associated with DSW, DRW, and SL under all salinity levels. FSW and RL were only significantly correlated at 14 dS m^{-1}, but their overall correlation under all salinity levels was strong (0.558*). RL was positively associated with SL (0.613*) and LL (0.385*) at 14 dS m^{-1} and 8 dS m^{-1}, respectively. SL exhibited a strong positive correlation with DSW (0.815*) under all salinity levels. DRW showed a strong association with DSW (0.809*) across salinity levels, except at 12 dS m^{-1}, and LL was positively correlated with DSW overall (0.612*), but non-significant at 12 dS m^{-1}.

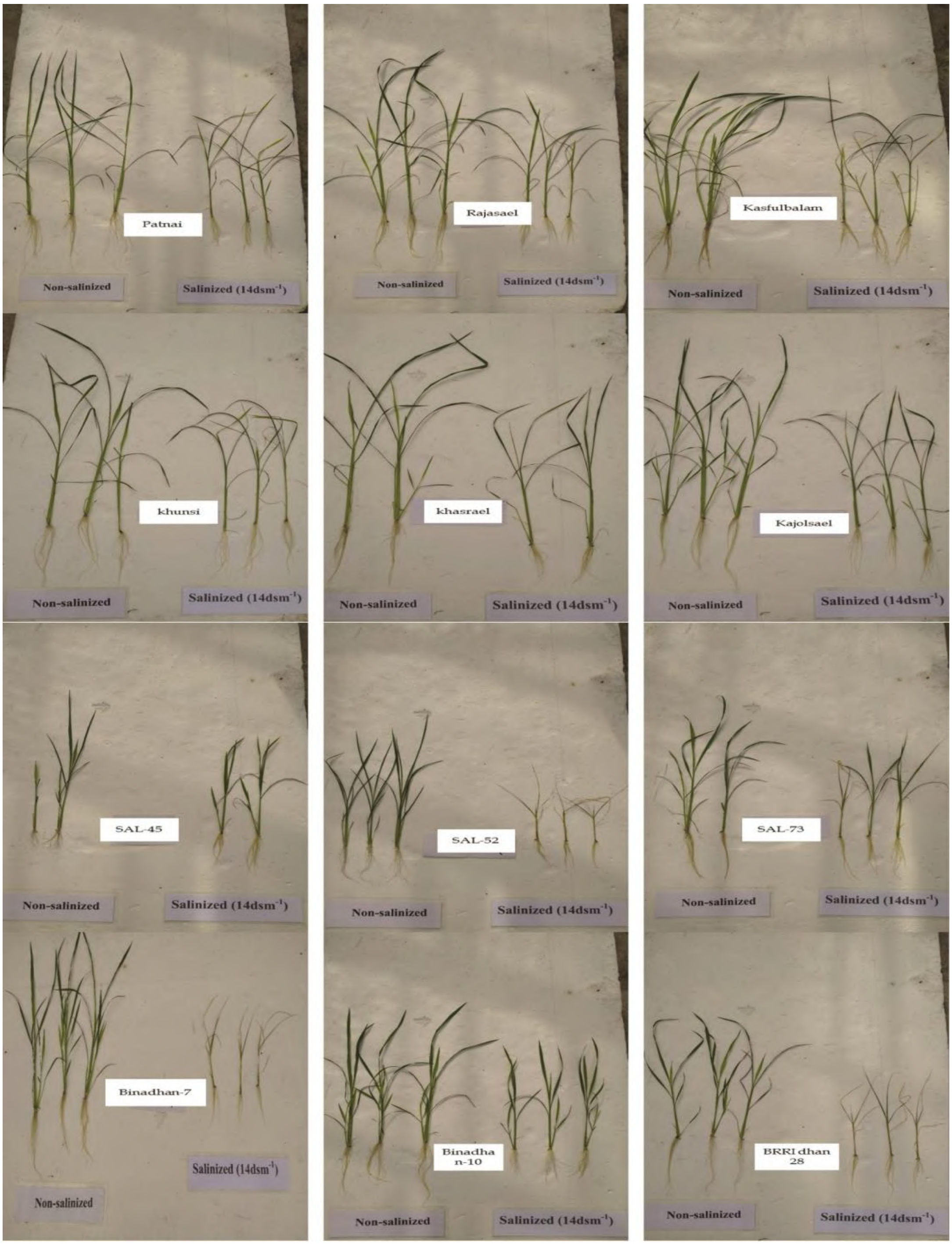

Fig. 3. Morphological changes of promising genotypes with checks at 14 dSm^{-1} salinity stress

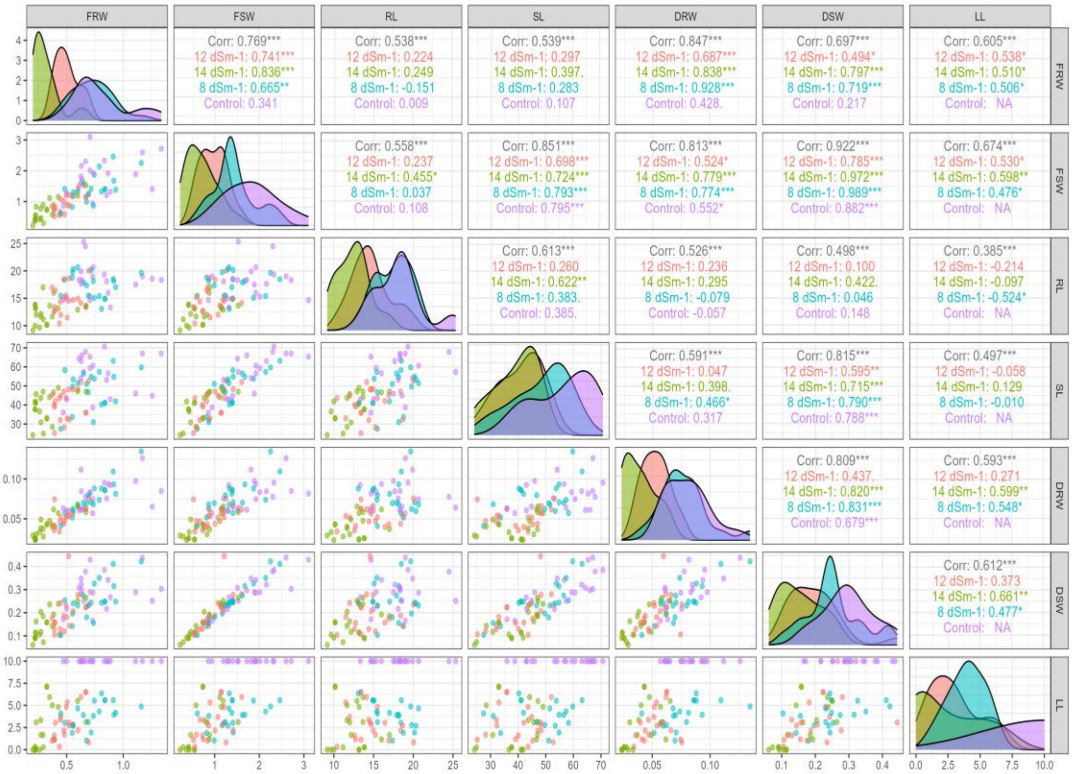

Fig. 4. Correlation studies among morphological and physiological traits of 20 rice genotypes under different salinity stress and control.

Estimation of genetic parameters for morphological characters

The genetic parameters of the studied morphological traits, including genotypic and phenotypic variances, coefficients of variation (GCV and PCV), heritability, genetic advance (GA), and GA as a percentage of the mean (GA%), are presented in Table 5. Significant variability was observed for all traits, with PCV consistently higher than GCV, indicating environmental influence. Root dry weight showed the highest variability (PCV 144.13; GCV 143.48), followed by shoot fresh weight (66.63; 65.78) and shoot dry weight (63.37; 61.35), while root length (25.90; 24.48), SES (39.42; 36.18), and shoot length (39.56; 39.39) had the lowest. Heritability ranged from 80.84% to 99.10%, highest for shoot length (99.10%) and lowest for percent live leaves (80.84%). Genetic advance was greatest for percent live leaves (47.10) followed by shoot length (37.46) and lowest for root dry weight (0.21) and shoot dry weight (0.27). GA%, reflecting the contribution of additive gene action, was highest for root dry weight (294.21%) followed by shoot fresh weight (133.77%) and lowest for root length (47.66%) and SES (68.41%), indicating strong potential for selection in breeding programs (Table 5).

Table 5. Estimation of genetic parameters for morphological characters related to salt stress

Characters	Phenotypic variance (δ^2p)	Genotypic variance (δ^2g)	PCV (%)	GCV (%)	Heritability (h^2b)	GA	GA (%)
Fresh wt (g) roots	0.077	0.067	47.61	44.34	86.71	0.50	85.05
Fresh wt (g) shoots	0.669	0.652	66.63	65.78	97.46	1.64	133.77
Root Length	16.87	15.07	25.90	24.48	89.34	7.56	47.66
Shoot Length	336.70	333.66	39.56	39.39	99.10	37.46	80.77
Dry wt. roots	0.0110	0.011	144.13	143.48	99.09	0.21	294.21
Dry wt. shoots	0.0201	0.019	63.37	61.35	93.73	0.27	122.35
% live leaves	800.10	646.80	60.92	54.78	80.84	47.10	101.45
SES	2.38	2.00	39.42	36.18	84.24	2.68	68.41

** indicates significant at 0.01 probability level.

Principal component analysis (PCA)

Principal component analysis (PCA) was conducted to assess the effect of salt stress on key rice traits. The first four principal components (PCs) had eigenvalues greater than one, collectively explaining 100% of the total variation. PC1 accounted for 71.2% and PC2 for 9.9% of the variation, with the first two PCs capturing over 81.1% of the total variation. With the four different levels of stress, PCA formed four distinct clusters Fig. 5).

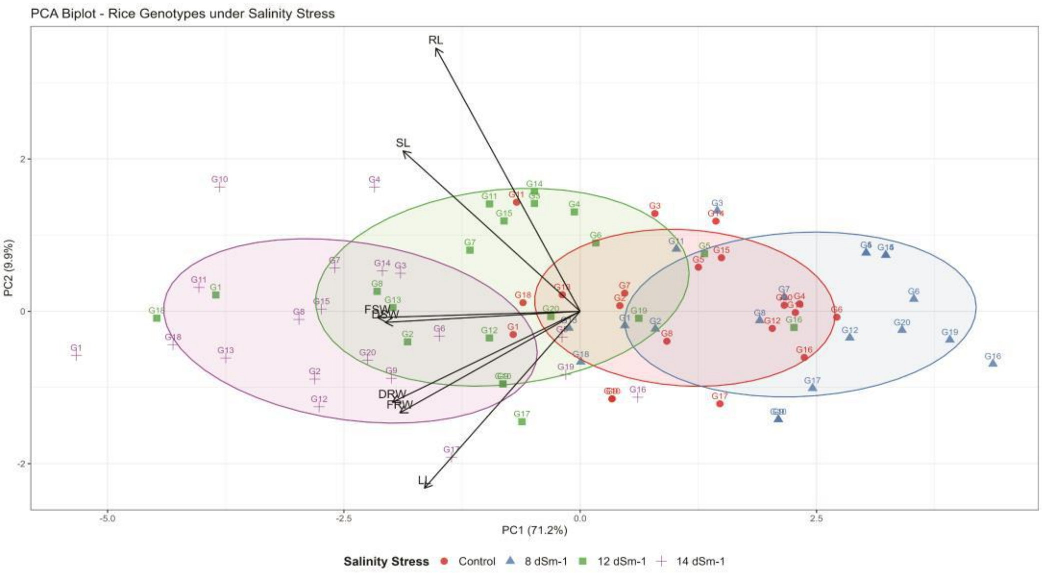

Fig. 5. Principal Components Analysis (PCA) in different levels of stress

G_1 = Khasrael, G_2 = Kajolsael, G_3 = Khunsi, G_4 = Daksael, G_5 = Brridhan-28, G_6 = Rupeshsor, G_7 = Patnai, G_8 = Rajasayel, G_9 = Binadhan10, G_{10} = Bohorimota, G_{11} = Sadagotal, G_{12} = SAL-73, G13= Kashful-balam, G_{14} = Hatibejor, G_{15} = Dudkolom, G_{16} = SAL-52, G_{17} = SAL-45, G_{18} = Talmugur, G_{19} = Binadhan-7, G_{20} = Holdegotal (G: genotype, SES: Standard evaluation score)

The PCA biplot reveals a strong positive correlation between biomass traits, as Root and Shoot weights (Fresh and Dry) cluster together, indicating they decline in unison under stress. Growth vectors for Shoot and Root Length point toward the Control and 8 dSm^{-1} groups, confirming these parameters are highest at lower salinity levels. As stress reaches 14 dSm^{-1}, genotypes shift to the left, opposite the growth vectors, demonstrating maximum reduction. Live Leaves (LL) serves as a distinct survival indicator, separating the most resilient genotypes from those experiencing severe physiological decline.

Molecular characterization of rice genotypes for salinity tolerance

To assess the presence of the Saltol QTL, ten SSR markers known to be associated with salinity tolerance were initially screened. Three polymorphic markers are linked to the Saltol QTL on Chromosome 1 (RM7075, RM1287, and RM10772), were finally used to assess the salt tolerance of 20 genotypes of rice (Babu *et al.*, 2014; Gimhani *et al.*, 2014; Kumari *et al.*, 2019; Rashid *et al.*, 2018; Suprayogi *et al.*, 2021).

Allelic information

Among the three SSR markers evaluated across 20 landraces, RM7075 exhibited the highest number of alleles (14), followed by RM1287 (12), while RM10772 had the lowest (5), totaling 31 alleles. The average number of alleles per marker was 10.33 (Table 6). Genetic diversity was highest for RM7075 (0.92) and RM1287 (0.90) and lowest for RM10772 (0.745). All SSR markers were multi-allelic and co-dominant, highlighting their effectiveness in detecting DNA polymorphism. On average, 18% of the landraces shared a common major allele, ranging from 10% (RM7075) and 15% (RM1287) to 30% (RM10772). Polymorphism information content (PIC), which reflects allele diversity and frequency, ranged from 0.700 (RM10772) to 0.914 (RM7075), with an average of 0.835 (Table 6), indicating substantial polymorphism among the studied landraces.

Table 6. Data on major allele frequency, gene diversity and Polymorphism Information Content (PIC) among 20 rice genotypes using 3 SSR markers

Locus name	Allele Frequency (%)	No. of Allele	Gene Diversity	PIC
RM7075	0.1	14	0.92	0.91425
RM1287	0.15	12	0.9	0.89145
RM10772	0.3	5	0.745	0.70018
Mean	0.18333	10.33333	0.855	0.83529

Banding pattern of 20 landrace genotypes using the SSR markers

The banding patterns of 20 rice genotypes for molecular analysis using three SSR markers were compared with reference to those of Binadhan-7, Binadhan-10 and BRRI dhan28 presented in Fig. 6-8. The genotypes that gave bands with the same position or near with salinity tolerant Binadhan-10 were supposed to be tolerant to salinity (Fig. 6-9). The banding patterns were compared against the tolerant check Binadhan-10 (a Saltol carrier). Genotypes exhibiting bands at the same or similar positions as Binadhan-10 were identified as potentially harboring the Saltol QTL alleles, indicating high salt tolerance.

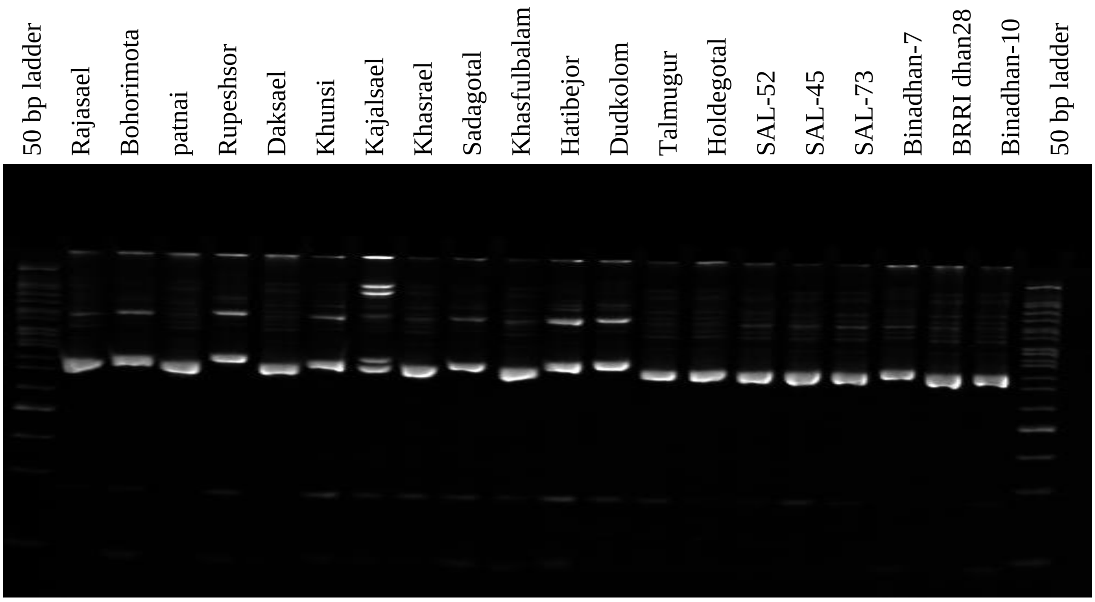

Fig. 6. DNA profile of 20 rice genotypes with RM7075.

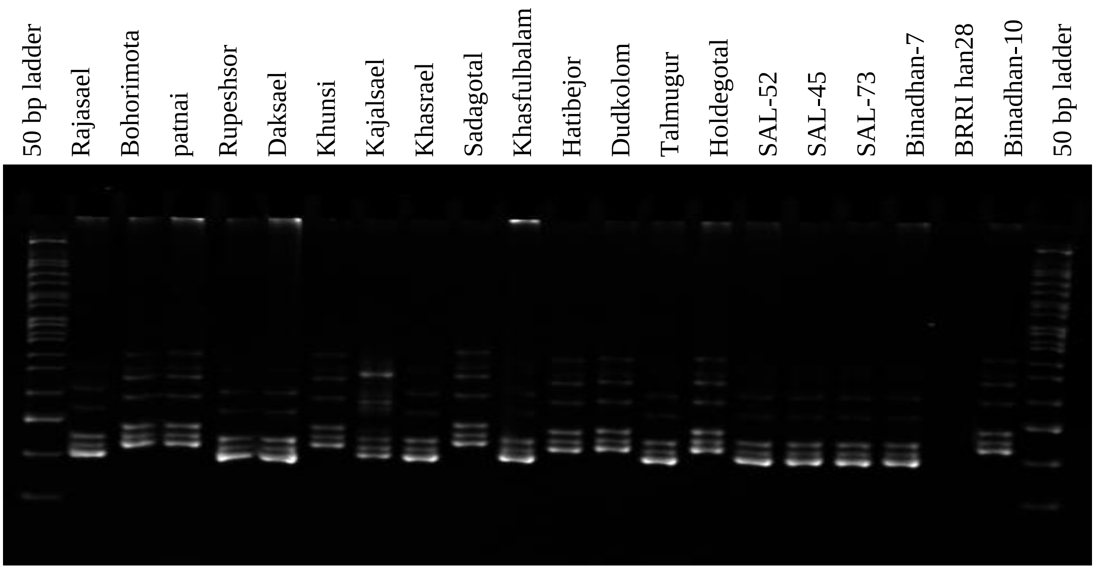

Fig. 7. DNA profile of 20 rice genotypes with RM1287.

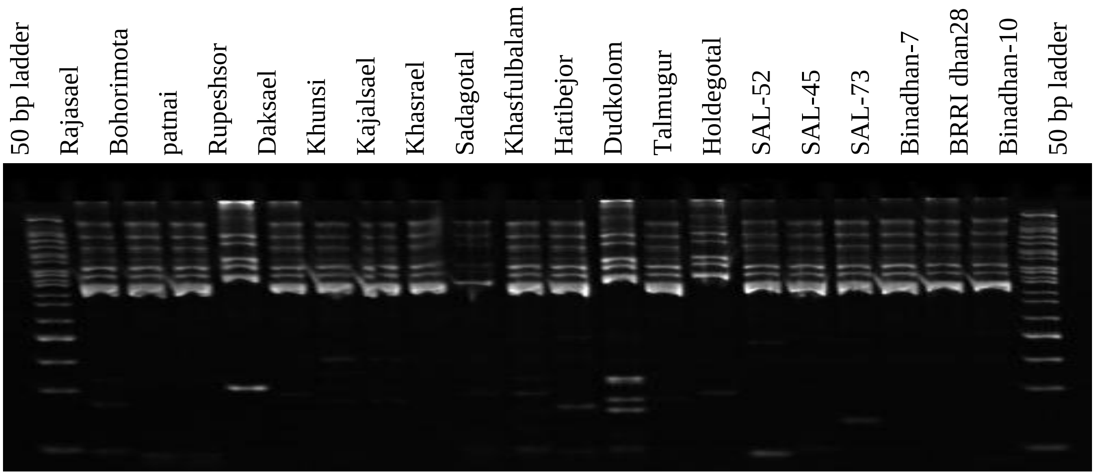

Fig. 8. DNA profile of 20 rice genotypes with RM10772.

UPGMA dendrogram based genetic relationship analysis

Molecular screening revealed that SAL-45 and Binadhan-10, grouped in cluster 1, exhibited superior salinity tolerance, while Khasrael and Kajolsael in cluster 2 showed good tolerance based on combined phenotypic and genotypic analyses; genotypes in cluster 4, including Talmugur and Patnai, were moderately tolerant to salinity stress (Fig. 9).

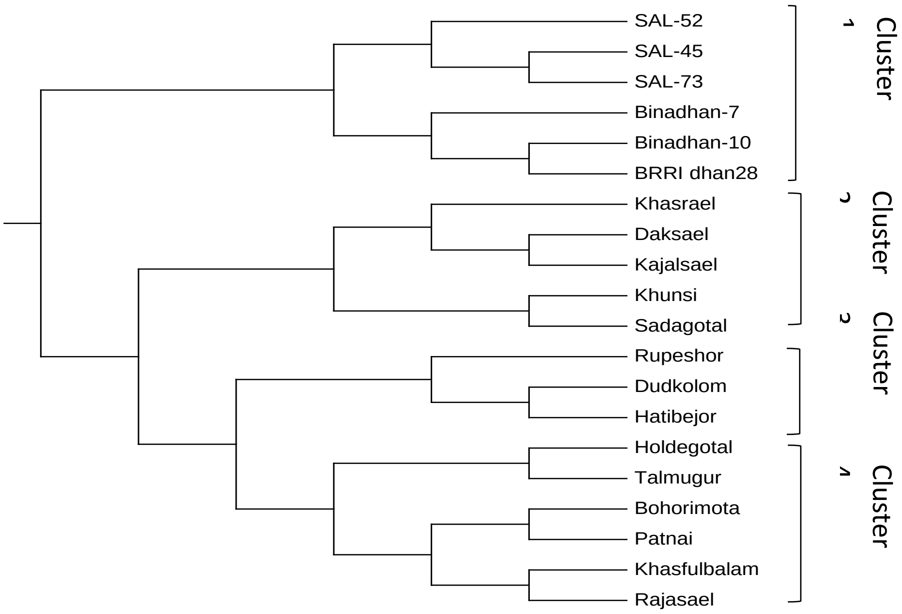

Fig. 9. UPGMA dendrogram showing the genetic relationship among the rice genotypes based on SSR markers.

Discussion

Salinity significantly reduced rice growth in coastal area of Bangladesh, causing stunted shoots, leaf rolling, tip browning, drying, and reduced root and stem development. Seedlings under non-saline conditions remained uniform and healthy, while saline-treated genotypes showed wide variation in tolerance, with SES scores ranging from 3 (tolerant) to 9 (highly susceptible) (Marè *et al.*, 2023) also reported wide variation in phenotypes in rice from tolerant (score 3) to highly susceptible (score 9) using Standard Evaluation Score (SES) of IRRI standard protocol (Prakash *et al.,* 2022). The results of this study also revealed that tolerance level of plant decreases with the increasing of salinity level. Similar findings were reported by (Zeng *et al.*, 2001).

Twenty rice genotypes were evaluated for their genetic potential, and significant differences were observed for all traits, including shoot and root length, fresh and dry weights, percent live leaves (Rahmanzadeh *et al.*, 2008). Shoot growth is generally more sensitive than root growth under high salinity, as osmotic stress reduces leaf area and inhibits calcium transport via the xylem, resulting in stunted shoots (Jenks *et al.,* 2007).

Salinity restricted root growth in all rice genotypes, with tolerant genotypes maintaining longer roots than sensitive ones, consistent with findings reported by (Roy *et al.*, 2014). Under salinity stress, Daksael showed the longest roots and SAL-45 the shortest, with tolerant genotypes maintaining higher shoot and root biomass than sensitive ones, likely due to the presence of Saltol-linked alleles and other adaptive genes controlling ion homeostasis and stress resilience. According to (Sweet *et al.*, 1990)salinity reduces dry weight accumulation by solidifying and altering cell wall structure. Significant genetic variation was observed among the 20 rice genotypes, with PCV exceeding GCV for all traits, indicating some environmental influence. High heritability for most traits, especially shoot length (99.10%), suggests strong genetic control and good potential for selection in breeding programs.

Three SSR markers (RM7075, RM1287, and RM10772) were used to screen rice genotypes for salt tolerance, comparing banding patterns with the tolerant variety Binadhan-10. A total of 31 alleles were detected across 20 genotypes, with RM7075 showing the highest number of alleles (14), RM1287 with 12, and RM10772 the lowest (5), giving an average of 10.33 alleles per marker (Table 6). Giarrocco *et al.* (2007) reported 8.42 alleles per locus; range 3-21 by using 26 SSR loci to estimate genetic relationship among 69 Argentine rice accessions. The highest genetic diversity was observed for RM7075 (0.92) and RM1287 (0.90), while RM10772 showed the lowest (0.745), indicating that markers detecting more alleles exhibited higher gene diversity, consistent with Heenan *et al.* (1988). Similar findings were reported by Dhar *et al.* (2012) where gene diversity was inversely related to major allele frequency. SSR markers, being multi-allelic and co-dominant, are effective in detecting DNA polymorphism. On average, 18% of the 20 landraces shared a common major allele, ranging from 10% (RM7075) to 30% (RM10772). Polymorphism information content (PIC) values, reflecting allele diversity and frequency, varied from 0.700 (RM10772) to 0.914 (RM7075), with an average of 0.835. Molecular clustering showed SAL-45 and Binadhan-10 in cluster 1, indicating strong salinity tolerance. Khasrael and Kajolsael, in cluster 2, also showed good tolerance, while Talmugur and Patnai in cluster 4 displayed moderate tolerance under salinity stress.

Seedlings were screened under three salinity levels (8, 12, and 14 dS m^{-1}) and control. Six genotypes Kasrael, Kajolsael, Patnai, SAL-45, Talmugur, and Binadhan-10 were identified as salt tolerant based on SES and superior shoot/root growth, biomass, and live leaf retention. Genetic analysis revealed significant variability with high heritability (80.84–99.10%) and substantial genetic advance for key traits, indicating additive gene action suitable for selection. Molecular screening using three SSR markers (RM1287, RM7075, and RM10772) detected 31 alleles, with RM7075 and RM1287 exhibiting the highest diversity, highlighting their utility in identifying tolerant lines. The selected tolerant genotypes, which combine favorable morphological performance with Saltol-linked alleles, represent valuable progenitors for future breeding programs aimed at developing high-yielding, salt-tolerant rice for coastal Bangladesh.

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