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Biochemical and growth-based screening for salt tolerance in aromatic aman rice at seedling stage

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ABSTRACT

Early screening at the seedling stage can significantly reduce the time and effort needed to develop new salt-tolerant varieties. With this objective, the Department of Crop Botany, GAU, has developed nearly 100 advanced lines of aromatic rice and evaluated them for salinity tolerance at the seedling stage. These lines were evaluated for salinity tolerance at the seedling stage under pot culture conditions, following a completely randomized design. A salinity level of 8 dSm^{-1} was applied, and relevant physiological and morphological data were systematically recorded. The findings revealed that in control, plant height was in between 34.17 and 56.83 cm. In contrast, under salinity, it varied from 30.17 cm and 55.00 cm. With an average of 87.47%, the relative height of seedlings ranged between 76.28% and 98.54%. The shoot elongation rate of rice seedlings in control ranged 0.38 cm day^{-1} to 2.24 cm day^{-1}, while, under salinity, it varied from 0.1 cm day^{-1} to 2.02 cm day^{-1}. The average shoot elongation rate for control and salinity was 1.26 and 0.53 cm day^{-1}, respectively. The relative elongation rate ranged from 10.23% to 97.22%, averaging 43.40%. The dry weight of seedlings in control ranged from 0.35 g to 1.63 g, averaging 0.79 g, compared to 0.10 g to 1.52 g under salinity, averaging 0.54 g. Relative dry weight ranged between 22.83% to 93.26% with an average of 66.70%. Clustering and additional evaluation of the genotypes' revealed cluster 5 containing PB38, PK37, PK50, PK51, PK52, PK85, PK86 and PK95 had higher proline accumulation (0.322 µmol g^{-1} FW), lower MDA (0.303 µmol g^{-1} FW) and a relatively higher Chl a/ Chl b ratio (2.59) under saline condition. These genotypes might be used for further hybridization program to develop new salt-tolerant rice variety

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Introduction

The crop plants in our earth often have to face a series of myriads of stresses, counting both biotic and abiotic, which can severely disturb their typical growth and productivity (Kazemi and Eskandari, 2011). Salinity stands out as one of the key primary abiotic stressors hampering rice production in many countries (Islam *et al.*, 2021). The detrimental role of salinity induced stress on the growth and development of plant yielded from various factors including nutritional imbalances, ionic stress, and osmotic stress (Ashraf and Wu, 1994; Parida and Das, 2005). Saline soils, characterized by the presence of an excessive amount of sodium ions and dominant anions such as chloride and sulfate, lead to significant challenges with their high electrical conductivity (>4 dS m^{-1}) (Ali *et al.*, 2013; Irakoze *et al.*, 2021). Different stress induced by salinity frequently lead up to toxicity due to ion accumulation, while the overproduction of reactive oxygen species (ROS) further exacerbates the mutilation to plant tissues (Munns *et al.*, 2006).

To challenge the salt stress, salt-tolerant plants deploy several plans of strategy, including modifying the membrane properties included in ion regulation and partitioning (Li *et al.*, 2008). These strategies primarily aim in restricting salt induction inside the plant tissue and reducing cytoplasmic salt concentrations. Salt-tolerant plants also perform more effective vacuolar ion assimilation compared to that of salt-sensitive counterparts (Munns, 2002).

Although rice is generally considered as salt-sensitive, the sensitivity varies across different growth phases. It shows greater salt sensitivity at early vegetative and reproductive stages but exhibits some tolerance during germination and active tillering stage (Lutts *et al.*, 1995; Zhu *et al.*, 2001). The effect of salinity level and stress durations on rice varies, and survival of individual plant is inversely correlated with Na^+ and Cl^- accumulation (Moradi and Ismail, 2007). Physiological parameters such as plant survival, growth rate, and dry weight are commonly applied to evaluate the performance of rice cultivars for salinity tolerance at seedling stage (Haq *et al.*, 2009; Ali *et al.*, 2014, Rana *et al.*, 2019; Iqbal *et al.*, 2024).

The coastal and deltaic regions of our country is facing ever increasing challenges from salinity, necessitating the development of rice varieties resilient to adverse brackish condition. In Bangladesh, with almost 53% of the cultivated area being affected by salinity (Abedin and Hosenuzzaman, 2023), and furthermore lack of fresh water made the cultivation expensive. Considering this, the cultivation of salt-tolerant, high-value fragrant rice varieties could be a suitable and sustainable solution. Aromatic fine grain rice varieties are in high demand due to their taste, palatability, cooking characteristics, and aroma (Ejaz *et al.*, 2020). Over the last decade, aromatic rice has captivated major market share globally (Calpe, 2004), prompting efforts to increase the production through breeding initiatives tailored to regional conditions.

In line with this, the Crop Botany Department of GAU has developed around 100 advanced lines of aromatic rice by crossing Pokkali with Kalozira and Pokkali with Badshabhog. Pokkali, well-known for its salt tolerance, is a valuable genetic asset for breeding programs enhancing salt tolerance (Mohammadi-Nejad *et al.*, 2008), while Kalozira and Badshabhog are well-established aromatic rice varieties in Bangladesh (Verma *et al.*, 2018). Some of these newly developed genotypes exhibit promising capabilities in salt tolerance. Accounting Bangladesh's diverse agroclimatic conditions, the adaptation of rice varieties to changing environments is crucial. Therefore, this experiment was conducted to assess the morphological and quality attributes of aromatic rice lines, and screening them for salt tolerance at the seedling stage.

Materials and Methods

The experiment was conducted during the Aman season of 2022 in a net house at the Department of Crop Botany, Gazipur Agricultural University (GAU), Gazipur-1706, Bangladesh. A total of 97 advanced aromatic rice lines, along with the control variety BRRI dhan34, were used as experimental materials. The advanced lines included two distinct genetic backgrounds: lines designated as PK, developed through hybridization between Pokkali (a salt-tolerant donor) and Kalozira (an aromatic local landrace), and PB lines, derived from a cross between Pokkali and Badshabhog (an aromatic local landrace). Prior to sowing, seeds were visually inspected and selected to ensure they were healthy and free from disease.

The experiment followed a completely randomized design (CRD) with three replications. Seedlings were grown in plastic pots filled with homogenized soil. Salinity stress was imposed at the vegetative stage, 15 days after sowing (DAS), by applying a saline solution prepared with NaCl to achieve a soil electrical conductivity (EC) level of 8 dSm^{-1}. The EC was regularly monitored using a conductivity meter and maintained throughout the stress period by adjusting the saline solution as needed. Control plants were irrigated with non-saline water under otherwise identical conditions.

Morphological data were recorded between 35 and 45 DAS. Traits measured included Seedling height (cm), Shoot elongation rate (cm/day) and Seedling dry weight (g). Representative genotypes from each of the five clusters (identified via cluster analysis described below) were selected for biochemical evaluation. The following biochemical traits were measured:

- Proline content: Estimated using the ninhydrin acid reagent method described by Bates *et al*. (1973).
- Malondialdehyde (MDA) content: Determined following the thiobarbituric acid reactive substances (TBARS) method described by Heath and Packer (1968).
- Chlorophyll content: Quantified according to Arnon's (1949) method using spectrophotometry.

All collected data were subjected to statistical analysis using RStudio software. A non-hierarchical K-means cluster analysis was performed to group the genotypes based on their morpho-physiological responses to salinity stress. Five distinct clusters were identified, representing different levels of salinity tolerance.

Results

Salinity stress (8 dSm^{-1}) imposed at the seedling stage significantly affected morphological traits across the 97 advanced aromatic rice lines. A marked reduction in seedling height, shoot elongation rate, and seedling dry weight was observed in most lines compared to the non-stress control. However, the magnitude of reduction varied considerably, indicating differential tolerance among genotypes. Table 1 presents the expressive data of the parameters studied in response to salinity.

Height of seedlings at control and saline condition

The range of heights for seedlings under saline and control conditions was 30.17 to 55 cm and 34.17 to 56.83 cm, respectively. The average height of seedlings in control condition and saline treated condition was 42.70 and 37.33 cm, respectively (Table 1). Among the ninety-eight rice genotypes, the plant height of most (49) genotypes under control condition ranged from 40 to 45 cm whereas under saline condition plant height of most (65) genotypes ranged from 35 to 40 cm (Fig. 1). The frequency distribution of seedling plant height under saline and control conditions revealed a skewed distribution towards the right (a = 1.71 and 0.34, respectively), indicating that the majority of genotypes were around the median and a small percentage were above it. The

Table 1. Descriptive statistics of different studied parameters of the seedlings under salinity

Plant characters	Minimum	Maximum	Mean	Sd. Deviation	CV (%)	Skewness
Plant height at control (cm)	34.17	56.83	42.70	3.68	8.62	0.34
Plant height at salinity (cm)	30.17	55.00	37.33	3.73	9.98	1.71
Relative plant height (%)	76.28	98.54	87.47	5.08	5.81	0.18
Elongation rate at control (cm day^{-1})	0.38	2.24	1.26	0.34	26.77	0.18
Elongation rate at salinity (cm day^{-1})	0.10	2.02	0.53	0.37	70.4	2.47
Relative elongation rate (%)	10.23	97.22	43.40	21.44	49.4	0.85
Dry weight at control (g)	0.35	1.63	0.79	0.27	33.77	0.81
Dry weight at salinity (g)	0.10	1.52	0.54	0.25	47.38	1.5
Relative dry weight (%)	22.83	93.26	66.70	14.31	21.45	-0.83

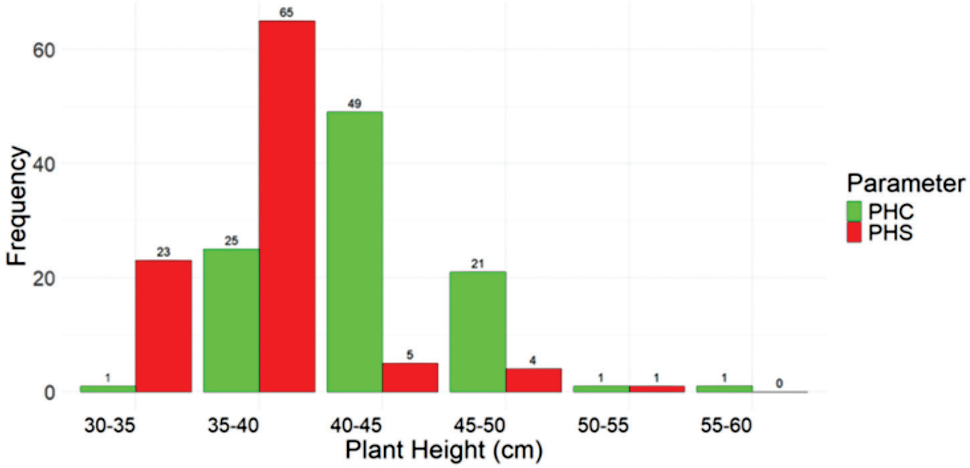

Fig. 1. Frequency distribution of plant height at seedling stage in control (PHC) and saline condition (PHS)

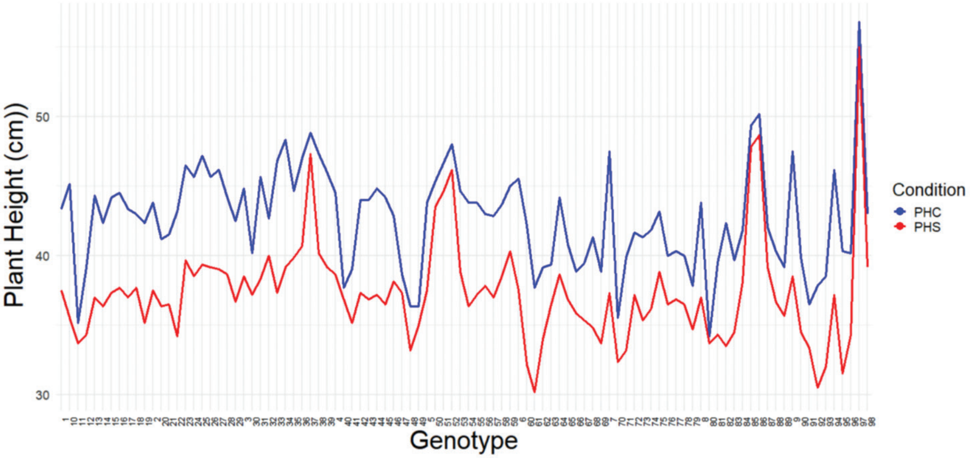

Fig. 2. Distribution of plant height in control vs. salt-treated rice seedlings of ninety-eight genotypes/ variety

decline in plant height of the genotypes under saline condition was evident (**Fig. 2**).

Relative height of seedlings

Relative plant height of seedlings ranged from 76.28 to 98.54 % with an average of 87.47 % (**Table 1**). The frequency distribution of relative plant height showed almost normal distribution with skewed towards right (a = 0.24). Relative plant height of most of the genotypes were around the median and few of them were more than median (**Fig. 3**). Among the ninety-eight rice genotypes, relative plant height of thirty-six genotypes ranged from 85

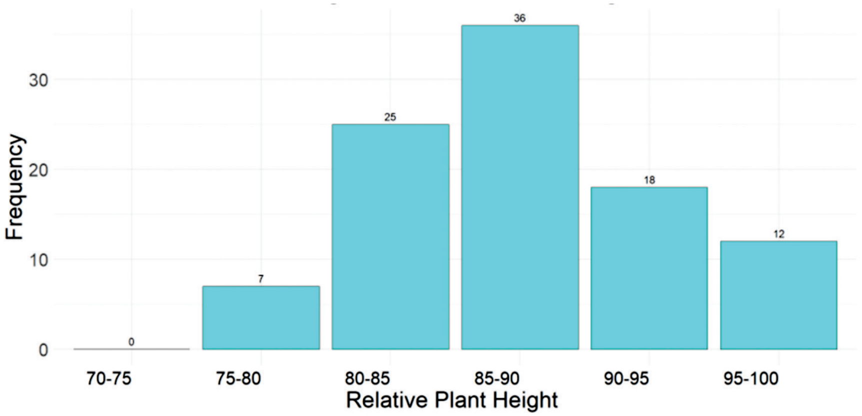

Fig. 3. Frequency distribution of relative plant height at seedling stage of ninety-eight genotypes/variety

to 90 %, twenty-five genotypes ranged from 80 to 85 %, and only twelve genotypes were found to be above 95 % (**Fig. 3**).

Elongation rate of seedlings at control and saline condition

There was a noticeable difference between the seedlings under control and the saline condition in terms of elongation rate (**Fig. 4**). The elongation rate of seedlings at control condition ranged from 0.38 to 2.24 cm day^{-1} with an average of 1.26 cm day^{-1} whereas the elongation rate of seedlings at saline condition ranged from 0.1 to 2.02 cm day^{-1} with an average of 0.53 cm day^{-1} (**Table 1**). The frequency distribution of elongation rate at both control and saline condition showed almost normal distribution with skewed towards right (a = 0.18 and 2.47, respectively) indicating that most of the genotypes were around the median and few of them were more than median (**Fig. 5**). At control condition, among the ninety-

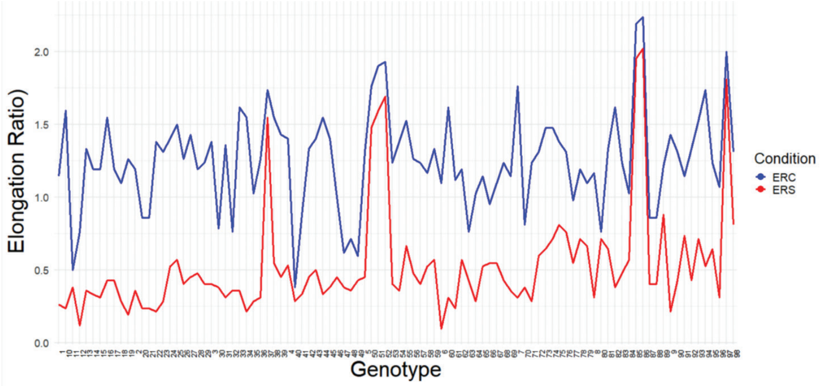

Fig. 4. Frequency distribution of elongation rate at seedling stage in control (ERC) and saline condition (ERS)

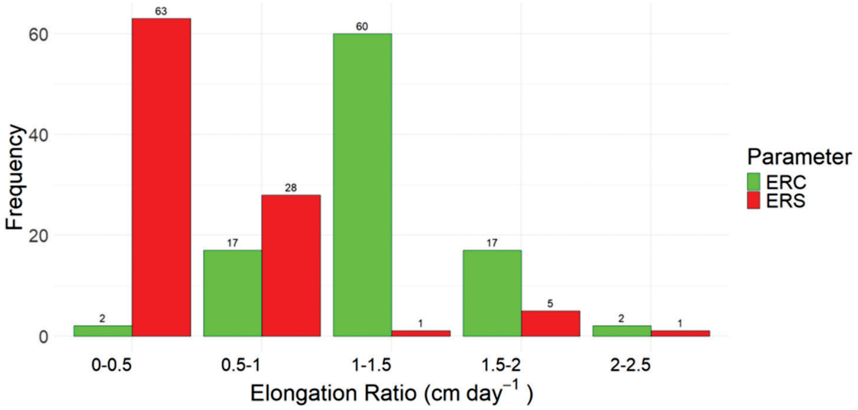

Fig. 5. Distribution of elongation rate in control vs. salt-treated rice seedlings of ninety-eight genotypes/variety

eight rice genotypes, elongation ratio of sixty genotypes was found between 1.0 to 1.5 cm day^{-1} whereas, most (63) number of genotypes were found to fall between 0.1 to 0.5 cm day^{-1} range in saline treated seedlings (**Fig. 5**).

Relative elongation rate of seedlings

Relative elongation rate (RER) of seedlings ranged from 10.23 to 97.22 % with an average of 43.4 % (**Table 1**). The frequency distribution of relative elongation rate showed almost normal distribution with slightly skewed towards right (a = 0.85) indicating that the majority of genotypes fell within the median range, with some genotypes outperforming the median and vice versa (**Fig. 6**). Among the ninety-eight rice genotypes, relative elongation rate of most genotypes (45) was found between 20 to 40 %, nine of them ranged below 20 % and ten genotypes were found to range above 80 % (**Fig. 6**).

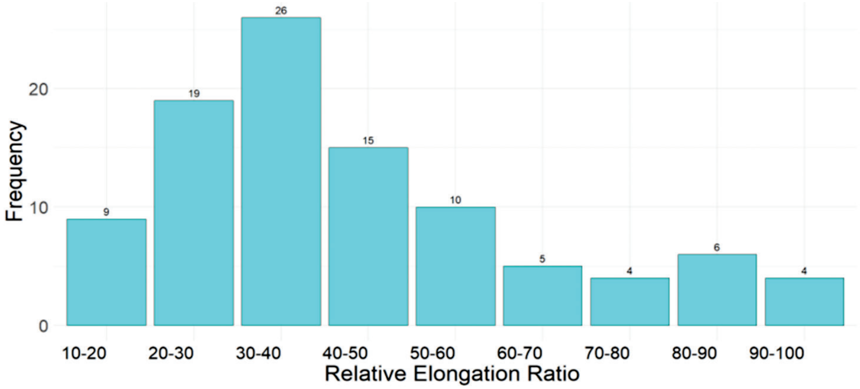

Fig. 6. **Frequency distribution of relative elongation rate at seedling stage of ninety-eight genotypes/variety**

Dry weight of seedlings at control and saline condition

It was evident that the control and saline-treated seedlings differed in dry weight and the dry weight of the genotypes under salinity was found to be lower (**Fig. 7**). Dry weight of seedlings at control condition ranged from 0.35 to 1.63 g with an average of 0.79 g while dry weight of seedlings at saline condition ranged from 0.1 to 1.52 g with an average of 0.54 g (**Table 1**). The frequency distribution of dry weight of seedlings at both control and saline condition showed almost normal distribution with skewed towards right (a = 0.81 and 1.5) indicating that most of the genotypes were around the median and few of them were more than median **(Fig. 8)**. Among the ninety-eight rice genotypes, dry weight of most (64) number of genotypes ranged from 0.5 to 1.0 g whereas most (54) number of genotypes ranged between 0.1 to 0.50 g in case of salt

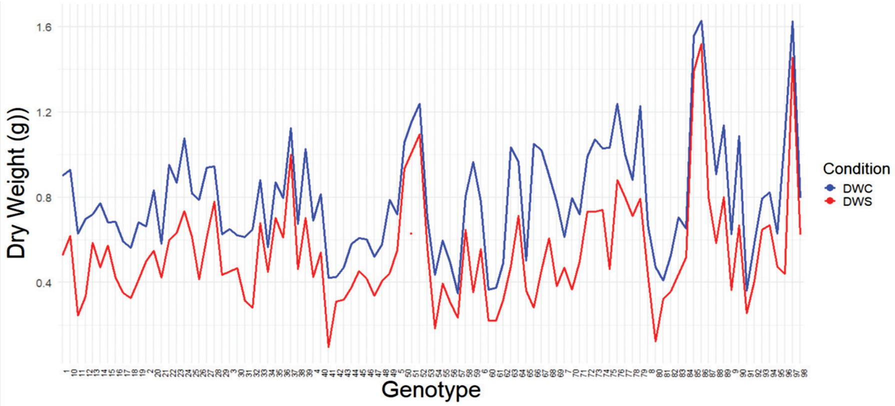

Fig. 7. Distribution of dry weight in control vs. salt-treated rice seedlings of ninety-eight genotypes

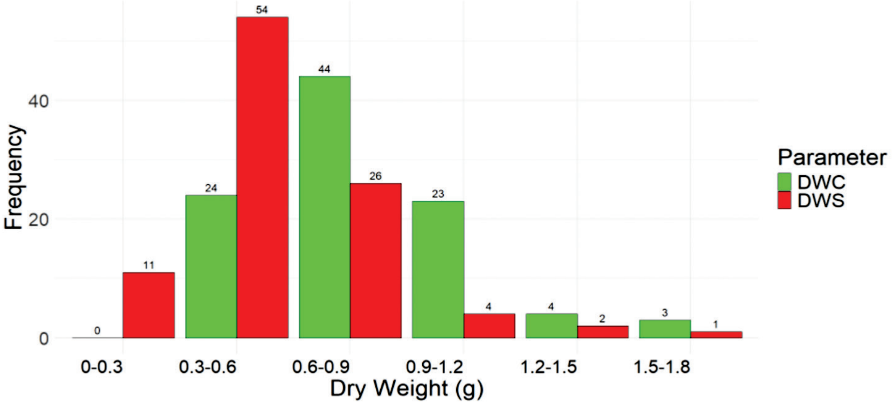

Fig. 8. Frequency distribution of dry weight at seedling stage in control (DWC) and saline condition (DWS)

treated seedlings. Dry weight of very few (5) genotypes under saline condition were found to be above 1.0 g in contrast to twenty-one genotypes under control condition (Fig. 8).

Relative dry weight of seedlings

Relative dry weight indicates the reduction in dry weight of the genotypes under salinity compared to control. The genotypes varied greatly in their relative dry weight. The relative dry weight of seedlings ranged from 22.83 to 93.26 % with an average of 66.7 % (**Table 1**). The frequency distribution of the relative dry weight of seedlings showed almost normal distribution with skewed towards left (a = -0.83) indicating that relative dry weight of most of the genotypes were high. Among the ninety-eight rice genotypes, relative dry weight of most genotypes (60) ranged from 60 to 80 %, sixteen genotypes ranged from 80 to 100 % almost maintaining their relative dry weight under salinity (**Fig. 9**).

Grouping of genotypes through cluster analysis

Given the large number of genotypes, it was challenging to interpret the influence of each variable within individual groups. In this context, it was needed to make statistically homogenous group on the basis of variables evaluated. Aiming that a non-hierarchical K-means cluster analysis was conducted to form statistically homogeneous groups based on the measured traits having high correlation coefficients for grouping of ninety-eight genotypes (**Fig. 10**). Based on various plant traits, the genotypes were classified into five distinct clusters (**Table 2**). Cluster 2 contained the largest number of genotypes (49), followed by cluster 1 with 29 genotypes. Clusters 3 and 4 each included eight genotypes, while cluster 5 had the fewest, with only four.

Cluster characterization

The results of the K-means cluster analysis revealed meaningful patterns in the

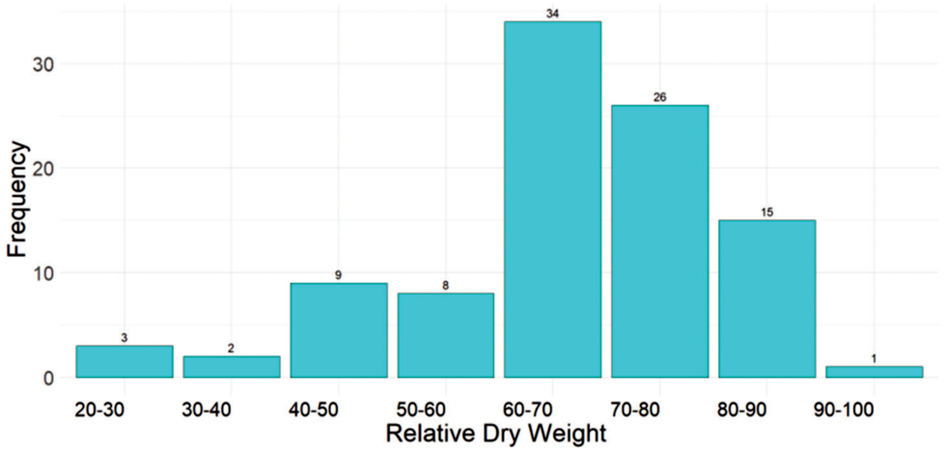

Fig. 9. Frequency distribution of relative dry weight at seedling stage of ninety-eight genotypes/variety

Table 2. List of five clusters of 98 genotypes classified by K- means clustering

Cluster no.	Genotypes
1	PK1, PK2, PK7, PK13, PK15, PK18, PK20, PK21, PK23, PK26, PK31, PK32, PK33, PK35, PK44, PK45, PK53, PK54, PK59, PK61, PK63, PK64, PK67, PK69, PK71, PK75, PK82, PK92, PK96
2	PK3, PK4, PK5, PK8, PK14, PK16, PK17, PK24, PK25, PK27, PK28, PK29, PK30, PK36, PK38, PK39, PK40, PK42, PK43, PK46, PK47, PK48, PK49, PK55, PK56, PK57, PK58, PK62, PK65, PK68, PK70, PK72, PK73, PK74, PK76, PK77, PK78, PK79, PK81, PK83, PK84, PK87, PK88, PK89, PK90, PK91, PK93, PK94, BRRI dhan34
3	PK6, PK9, PK10, PK12, PK19, PK22, PK34, PK60
4	PK11, PK41, PK66, PK80
5	PK37, PK50, PK51, PK52, PK85, PK86, PK95, PB38

distribution of rice genotypes based on their morphological and physiological traits (**Table 3**). This method, which yielded high correlation coefficients, effectively grouped 98 rice genotypes into five distinct clusters These smaller clusters could be of particular interest for targeted breeding programs, as they may harbor rare alleles or specialized adaptations. The detail description of mature plants is not described here.

Cluster 1: The genotypes of this cluster were characterized second lowest relative plant height of seedlings (85.52%), second lowest relative elongation rate of seedlings (31.13%) and relative dry weight of seedlings (62.54%) is also second lowest in this cluster (**Table 3**).

Cluster 2: The genotypes of this cluster were characterized by moderate relative plant height of seedlings (88.01%), moderate relative elongation rate of seedlings (45.99%) and relative dry weight of seedlings (66.84%) is second highest in this cluster (**Table 3**).

Cluster 3: The genotypes associated with this cluster were described by the lowest relative plant height of seedlings (81.16%), the lowest relative elongation rate of seedlings (14.88%) and relative dry weight of seedlings (63.32%) is moderate in this cluster (**Table 3**).

Cluster 4: The genotypes of this cluster were characterized by the second highest relative plant height of seedlings (94.18%), second highest relative elongation rate of seedlings (70.94%) and relative dry weight of seedlings (28.76%) is lowest in this cluster (**Table 3**).

Cluster 5: The genotypes of this cluster were characterized by the highest relative plant height of seedlings (94.20%), highest relative elongation rate of seedlings (86.71%) and relative dry weight of seedlings (87.61%) is also highest in this cluster (**Table 3**).

The clustering pattern of the genotypes revealed that the advanced line under cluster 5 were the best among the 5 clusters in terms of the parameters studied. Cluster 1 and 3 were

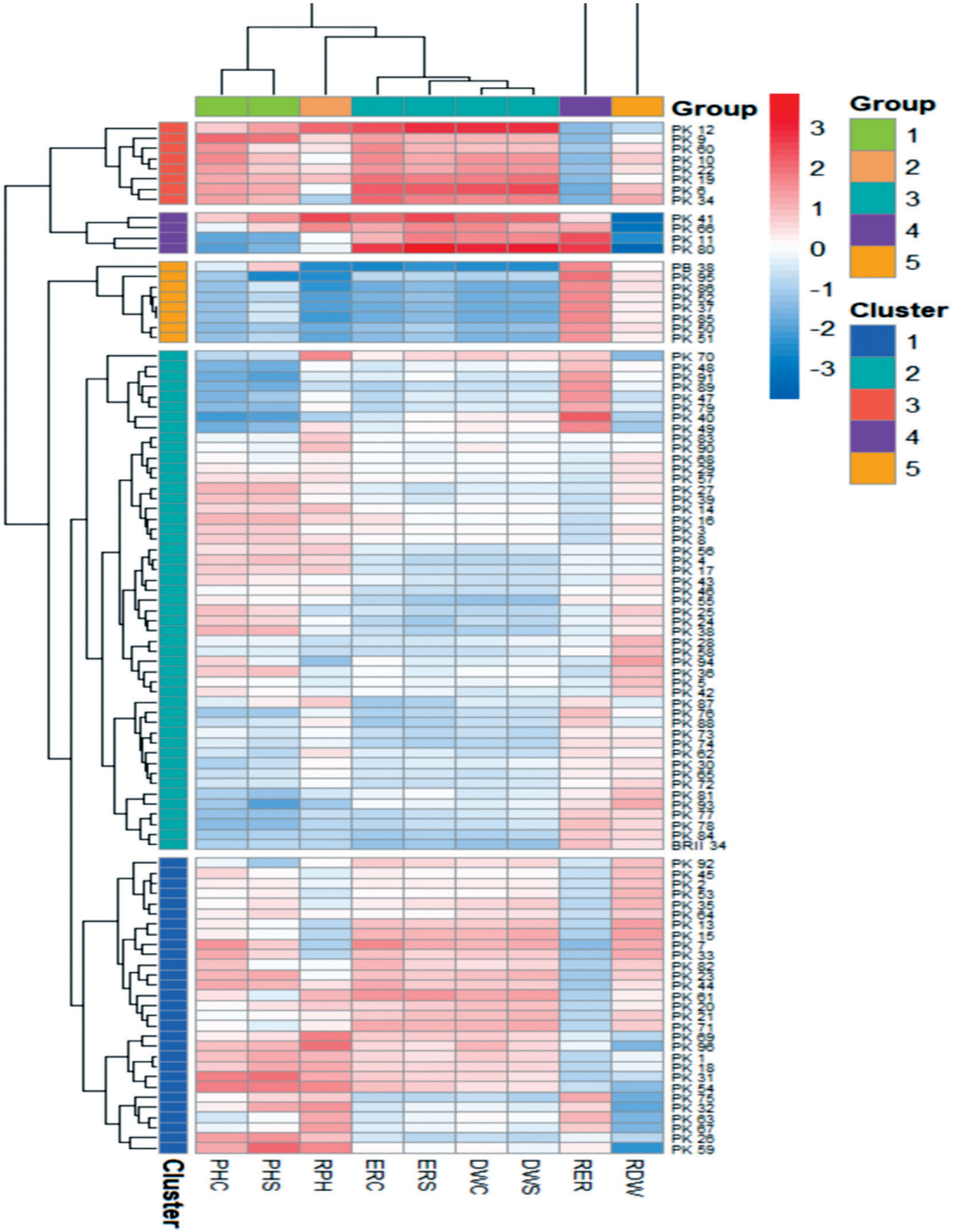

Fig. 10. Tree Diagram Analysis of 98 genotypes/variety under salt stress at seedling stage (Red and blue color indicates positive and negative contributions, respectively)

Table 3. Comparison profile of the five groups of 98 genotypes classified by K-means clustering (Seedlings)

Plant characters	Cluster				
	1	2	3	4	5
Plant height of seedlings at control	42.97	41.89	44.17	36.79	48.19
Plant height of seedlings at saline	36.74	36.80	35.81	34.59	45.58
Relative plant height of seedlings	85.52	88.01	81.16	94.18	94.20
Elongation ratio of seedlings at control	1.22	1.20	1.33	0.77	1.87
Elongation ratio of seedlings at saline	0.36	0.51	0.20	0.49	1.59
Relative elongation ratio of seedlings	31.13	45.99	14.88	70.94	86.71
Dry weight of seedlings at control	0.75	0.77	0.70	0.64	1.26
Dry weight of seedlings at saline	0.46	0.54	0.44	0.19	1.11
Relative dry weight of seedlings	62.54	69.40	63.32	28.76	87.61

characterized by their lowest features whereas, cluster 2 and 4 were characterized by moderate features in most of the plant characters. To further evaluate the salinity tolerance of the genotypes lipid peroxidation (MDA content), osmoprotectants accumulation (proline content) and chlorophyll mechanism from representative samples of each cluster were observed.

Proline content (µmol g^{-1} FW): The five clusters varied significantly in their quantity of proline accumulation. Under controlled condition the clusters didn't vary in proline content but significant variation was found in response to salinity. Among them, the highest (0.322 µmol g^{-1} FW) proline content was observed in cluster 5, followed by cluster 1 (0.279 µmol g^{-1} FW), 3 (0.279 µmol g^{-1} FW) and 2 (0.252 µmol g^{-1} FW), respectively. Samples from cluster 4 showed the least (0.240 µmol g^{-1} FW) amount of proline synthesis under saline condition (**Table 4**).

MDA content (µmol g^{-1} FW): Under saline condition, the increase in ROS leads to the peroxidation of lipids, resulting in higher levels of malondialdehyde (MDA). MDA is a crucial indicator of the extent of cellular damage due to salinity i.e. higher the MDA content greater the cellular damage. Among the clusters, highest (0.677 µmol g^{-1} FW) MDA content was observed in cluster 4 under salt stress. Cluster 5 (0.303 µmol g^{-1} FW) reflected comparatively lower damage than the others (**Table 4**).

Chlorophyll (a and b) concentration: Under saline condition, significant difference was observed in chlorophyll a content among

Table 4. Proline and MDA content of the clusters (average from representative samples)

Cluster	Proline (µmol g^{-1} FW)		MDA (µmol g^{-1} FW)	
	Control	Saline	Control	Saline
1	0.152 (a)	0.279 (b)	0.151 (a)	0.404 (c)
2	0.143 (a)	0.252 (c)	0.153 (a)	0.550 (b)
3	0.156 (a)	0.279 (b)	0.157 (a)	0.571 (b)
4	0.155 (a)	0.240 (c)	0.160 (a)	0.677 (a)
5	0.147 (a)	0.322 (a)	0.156 (a)	0.303 (d)
LSD (0.05)	0.0359	0.0252	0.0306	0.0164
CV (%)	8.46	3.25	6.96	4.34

Note: CV=Coefficient of Variance, LSD: Least Significant Difference, MDA: Malondialdehyde

Table 5. Chlorophyll content of the clusters (average from representative samples)

Cluster	Chl a (mg g^{-1})		Chl b (mg g^{-1})		Chl a/Chl b	
	Control	Saline	Control	Saline	Control	Saline
1	1.82 (b)	1.50 (b)	0.761 (a)	0.701 (a)	2.42 (a)	2.14 (a)
2	1.82 (b)	1.48 (b)	0.749 (a)	0.692 (a)	2.43 (a)	2.15 (a)
3	1.76 (bc)	1.46 (b)	0.782 (a)	0.674 (a)	2.25 (a)	2.18 (a)
4	1.60 (c)	1.21 (c)	0.697 (a)	0.537 (b)	2.29 (a)	2.29 (a)
5	2.02 (a)	1.63 (a)	0.782 (a)	0.710 (a)	2.59 (a)	2.31 (a)
LSD (0.05)	0.199	0.110	0.1545	0.1333	0.558	0.618
CV (%)	3.92	2.67	7.26	7.13	8.26	9.90

Note: CV=Coefficient of Variance, LSD: Least Significant Difference, Chl b: Chlorophyll b, Chl a: Chlorophyll a

the clusters and the highest (1.63 mg g^{-1}) chlorophyll a concentration was observed in cluster 5 followed by cluster 1 (1.50 mg g^{-1}). Similarly, chlorophyll b concentration was found to be lower than control. The ratio of chlorophyll a to chlorophyll b also decreased under salt stress and it was the highest (2.31 mg g^{-1}) in cluster 5 followed by cluster 4 (2.29 mg g^{-1}) and the lowest (2.14 mg g^{-1}) for cluster 1. (Table 5)

Discussions

The development of an effective, reliable, and efficient evaluation system is essential for breeding salt-tolerant rice varieties. An efficient early screening to identify genotypes for salt tolerance would make a leap to the total salt resistant breeding program (Kakar *et al.*, 2019). Currently, indicators used to assess salt tolerance in rice are broadly categorized into two types: morphological and physiological parameters (Krishnamurthy *et al*., 2016; Jaiswal *et al*., 2019). Morphological evaluation typically involves applying salinity stress at specific growth stages—most commonly at the seedling, vegetative, and reproductive stages. The morphological parameters evaluation method involves conducting salinity treatments at different stages of growth and then recording the salt induced damage symptoms on plants, leaves, tillers, and spikelet fertility (Chang., 2019).

The observed reduction in average seedling height under saline conditions indicates that salinity stress negatively impacts early plant growth. A decline from 42.70 cm in the control to 37.33 cm under salt stress reflects the sensitivity of rice genotypes to saline environments. With an average of 87.47%, the relative height of seedlings ranged between 76.28% and 98.54%. Notably, a shift in the height distribution was also evident, with the majority of genotypes falling into lower height categories under salinity. Reduction in shoot length in *O. sativa* cultivars results from replacement of K+ by Na+ as survival mechanism (Marcaida *et al*., 2014).

Among the morphological features, height was remarkably decreased in salt-treated due to a problematic photosynthetic activity, disputed transport of compatible nutrients, and reduced cell division, all of which eventually affect the shoot growth adversely (Islam *et al*., 2018). Reduced photosynthetic efficiency, coupled with lower stomatal conductance, leads to stomatal closure, further contributing to decreased plant height have been reported in several studies (Pandey and Shukla, 2015; Ali *et al*., 2014; Bhuiyan *et al*., 2008). Salt-tolerant genotypes maintain their growth better under salt stress, indicated by higher Relative Plant Height (RPH), indicating their potential for salt tolerance. The variation in growth pattern and rate among varieties under salinity stress have been reported frequently (Hasanuzzaman *et al*., 2009; Puvanitha and Mahendran, 2017).

At the early seedling stage, rice exhibits heightened sensitivity to salinity compared to later developmental stages such as tillering (Shereen *et al*., 2005). This vulnerability is evident in the noticeable reduction in seedling height under salt stress, as reported across multiple studies (Trotti *et al*., 2024; Kakar *et al*., 2019; Ali *et al*., 2014; Bhuiyan *et al*., 2008). The significant decline in shoot length under increasing salt concentrations suggests that salinity disrupts fundamental physiological processes such as cell division and elongation, water uptake, and nutrient transport (Trotti *et al*., 2024; Ali *et al*., 2014; Jiang *et al*., 2010). These disruptions are especially critical during early growth, when plants are establishing their structural and metabolic frameworks.

Seedling growth and fresh weight decrease with increased salt stress from 5 to 7.5 dS m^{-1} (Kazemi and Eskandari, 2011). Various greenhouse and field trials have also shown that salinity stress reduces rice stand density and seedling biomass production (Hussain *et al*., 2017). Thus, root and shoot lengths are critical indicators of rice plant in response to salt (Hussain *et al*., 2017).

The shoot elongation data from the present study clearly illustrates the detrimental effect of salinity on early seedling growth. Under control conditions, elongation rates ranged from 0.38 to 2.24 cm/day, with an average of 1.26 cm/day. However, under salt stress, this range narrowed to 0.10–2.02 cm/day, and the average elongation rate dropped sharply to 0.53 cm/day—representing a 57.94% reduction in growth rate. The relative shoot elongation rate among genotypes varied significantly, from as low as 10.23% to as high as 97.22%, with a mean of 43.40%. Salt-sensitive rice cultivars, especially those belonging to the Japonica group, tend to exhibit greater reductions in plant height and shoot elongation under salinity stress compared to salt-tolerant cultivars such as Nona Bokra and Pokkali (Ferdose *et al*., 2009; Korres *et al*., 2022). The variation observed indicates substantial genotypic differences in salinity tolerance, where some of the genotypes maintained shoot growth even under stress, while others were severely affected (Trotti *et al*., 2024).

This suggests that selection of salt-tolerant genotypes may offer an advantage in maintaining shoot growth and ensuring better early vigor, which contributes to improved crop competitiveness under saline conditions (Liu *et al*., 2019). Nonetheless, while salinity tolerance is crucial, it should not come at the cost of yield potential. The findings align with reports of salinity-induced suppression in shoot growth due to osmotic stress, ion toxicity, and hormonal imbalance, which collectively impair cell division and elongation (Balasubramaniam *et al*., 2023; Ganie *et al*., 2021; Lee *et al*., 2003).

The observed reduction in seedling dry weight under salinity stress highlights the significant inhibitory effect of salt on biomass accumulation in rice. The average dry weight declined from 0.79 g in the control to 0.54 g under saline conditions, reflecting a substantial loss in growth potential. This reduction is likely due to impaired physiological functions such as photosynthesis, nutrient uptake, and protein synthesis, all of which are disrupted by osmotic stress and ion toxicity associated with salinity (Trotti *et al*.,2024; Safitri *et al*., 2018). Salinity directly impacts biomass accumulation in rice affecting the photosynthetic rate by reducing carboxylation in chloroplasts, thus impacting the plant's ability to generate reducing groups, electron transport, and ATP synthesis in thylakoids (Etesami and Glick, 2020). A decrease in chlorophyll content is often the first indication of plant responses to salinity stress (Ismail *et al*., 2007; Roy and Basu, 2008) which ultimately leads to low and faulty photosynthetic activities ultimate resulting less dry matter accumulation in rice seedlings (Xu *et al*., 2024).

Meng *et al*. (2021) reported reduced shoot and root growth of rice under salinity in China, with significant declines in biomass at jointing, heading, and maturity stages, leading to yield loss—consistent with findings by Ali *et al*. (2013) and Wei *et al*. (2020). Similar reductions in shoot biomass were noted by Puvanitha and Mahendran (2017) in Sri Lanka. In Thailand, Santanoo *et al*. (2023) observed nearly a 40% decrease in final biomass in saline fields compared to non-saline ones.

The relative dry weight, which ranged from 22.83% to 93.26% among genotypes, further underscores the variability in salinity tolerance. Sodium salt stress causes dehydration and shrinkage in plant cells, leading to reduced shoot development and elongation, particularly in salt-sensitive rice genotypes under salt stress (Chang *et al*., 2019). Genotypes maintaining a higher percentageage of their biomass under stress conditions demonstrate a stronger capacity to withstand the detrimental effects of salinity, possibly through more efficient osmotic adjustment, ion compartmentalization, or antioxidative defense mechanisms (Che Yah *et al*., 2023; Reddy *et al*., 2017). This genotypic variation is critical for identifying and selecting salt-tolerant lines suitable for breeding programs targeting saline-prone environments (Ganie *et al*., 2021; Qin *et al*., 2020).

The decrease in both fresh and dry weight of rice seedling is accredited to low water uptake in the presence of salt. This phenomenon of physiological drought is very common in the coastal areas. Such growth inhibition of plant is a common response to salinity, and hence, plant growth is a crucial agricultural index of salt stress tolerance (Sen *et al*., 2017; Khan *et al*., 2023). Plant vigor at early stage is the key to salt tolerance in rice, as it allows the crop to avoid toxic salinity effects (Kumar *et al*., 2013). The initial seedling stage along with reproductive stages are considered as the most sensitive stage to salinity stress, with severe stress causing even plant death before maturity (Coca *et al*., 2023). High survival rate of several rice genotypes was considered to be a gift of early vigor and quick growth capability under saline conditions (Sazali *et al*., 2023; Ganie *et al*., 2021).

Under saline conditions, notable variations were observed in the biochemical responses of rice genotypes. The highest proline content (0.322 μmol g^{-1} FW), indicating strong osmotic adjustment, was found in genotypes previously grouped in cluster 5, while the lowest (0.240 μmol g^{-1} FW) was recorded in those formerly grouped in cluster 4. Proline is a well-documented phenomenon, in response to salt stress. As an osmoprotectant, proline helps to maintain cellular osmotic balance under adverse conditions (Hasegawa *et al*., 2000). Maintaining a lower Na^+/K^+ ratio in the cytosol is a crucial strategy for achieving salt tolerance in rice. This balance is regulated through the coordinated action of ion transporters and channels, along with the accumulation of osmo-protectants such as proline. (Trotti *et al*., 2024). It also helps to stabilize proteins and membranes, scavenging ROS. Higher proline accumulation is often seen as a protective mechanism against salt stress (Chutipajit *et al*., 2009).

Lipid peroxidation, as indicated by malondialdehyde (MDA) content, was most severe in cluster 4 (0.677 μmol g^{-1} FW), suggesting greater oxidative damage, whereas cluster 5 showed lower MDA levels (0.303 μmol g^{-1} FW), implying better stress tolerance. Malondialdehyde (MDA), a low molecular weight organic compound produced as a byproduct of lipid peroxidation, significantly increases under salt stress in rice genotypes. Under stressful conditions, free radicals or reactive oxygen species (ROS) damages membrane lipids, causing cellular injury and consequently elevating MDA production (Saddiq *et al*., 2017). Sensitive genotypes exhibited 14% higher MDA levels compared to resistant ones (Xu *et al*., 2024).

Regarding chlorophyll content, genotypes from cluster 5 exhibited the highest chlorophyll a (1.63 mg g^{-1}) and a favorable chlorophyll a/b ratio (2.31), both of which are indicators of maintained photosynthetic efficiency under salt stress. Under salt stress, chlorophyll content declines due to enhanced oxidation and degradation triggered by the accumulation of reactive oxygen species (ROS). Moreover, the extent of chlorophyll reduction is directly proportional to the severity of salinity (Balasubramaniam *et al*., 2023). The ratio of chlorophyll a to b decreases when exposed to salt stress, indicating that chlorophyll a is more negatively impacted by salt stress than chlorophyll b (Trotti *et al*., 2024). Given that chlorophyll a is the primary pigment involved in photosynthetic processes (Balasubramaniam *et al*., 2023; Santos, 2004; Daiz *et al*., 2002), the observed decrease in this ratio may be the primary cause of the lower rate of photosynthesis in rice under salinity stress (Moradi and Ismail, 2007).

The hierarchical clustering is considered to be one of the finest methods for screening purpose, since the similar genotypes based on one or multiple factors are grouped together, leading to a vivid conclusion (Ahmed *et al*., 2023). We identified five distinct advanced line clusters, with Cluster 5 demonstrating superior seedling performance and tolerance indices compared to the other four clusters. It was used to cluster the genotypes based on growth and development parameters. Significant differences were observed among the five clusters. Improved cultivars in cluster 5 exhibited higher plant height, shoot elongation rate, and dry weight at seedling stages under saline conditions.

Conclusion

Salinity stress at 8 dSm^{-1} imposed during the seedling stage of rice significantly hampers plant growth and development. At this salinity level, the plant height, elongation rate and dry matter accumulation of the seedlings of genotypes were all found to be adversely affected. Among the 98 seedlings, initial morphological screening for salinity tolerance, primarily grouped the genotypes in to five separate groups. Further biochemical assessment of these clusters identified cluster 5 to be the best performing cluster at seedling stage under saline stress. In addition, 90 genotypes from other four clusters were found low to moderately salt tolerant. Genotypes PB38, PK37, PK50, PK51, PK52, PK85, PK86, and PK95 from cluster 5 demonstrated better performance under saline conditions, indicating their potential as promising candidates for further experimentation and breeding efforts aimed at enhancing salt tolerance in rice.

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Conflict of Interest

The authors affirm that no financial or commercial relationships that might be construed as a potential conflict of interest existed during the course of the research.

Author Contributions

“Conceptualization, Shayma Parvin, Md. Israr, M A Baset Mia, H I Khan and Nasrin Akter Ivy.; methodology, M A Baset Mia, H I Khan and Nasrin Akter Ivy; resources, M A Baset Mia.; data curation, Shayma Parvin, Md. Israr, M A Baset Mia.; writing—preparation of the initial draft, Shayma Parvin, Md. Israr; writing, review and editing, H I Khan and Nasrin Akter Ivy.; supervision, M A Baset Mia.; project administration, M A Baset Mia.; revenue acquisition, M A Baset Mia. All authors have reviewed the manuscript in its current form and given their approval”.

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