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Salinity-induced variation in physiological and biochemical profiles of aromatic rice genotypes

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ABSTRACT

The aromatic rice is generally low-yielding and prone to abiotic stress, especially the salinity, which hinders yield and quality drastically. This experiment was conducted to assess the physiological and biochemical profiles of aromatic rice genotypes under salt stress. Two aromatic rice genotypes such as PK31 and PK37 were cultured under 0, 4, 6, 8, and 10 dSm^{-1} salinity for both tillering (TS) and panicle initiation (PI) stages. The study employed in a factorial completely randomized design with three replications. The results indicated that salinity stress severely affected the physiological, biochemical profiles, and yield attributes of PK31 than PK37, particularly at the TS. Plant height, stem elongation rate, chlorophyll fluorescence (Fv/Fm), shoot and root dry weight, fertile grains, grain weight, total dry matter, and harvest index decreased significantly with increasing salinity. At 10 dS m^{-1}, plant height declined to 60.00 cm in PK31 and 66.78 cm in PK37 at TS. Proline accumulation increased markedly with salinity, reaching 2.06 µmol g^{-1} in PK37 compared with 1.68 µmol g^{-1} in PK31. Chlorophyll fluorescence decreased from 0.811 under control to 0.614 at 10 dS m^{-1} in PK37, while PK31 declined to 0.519. Grain weight at TS was completely lost at 10 dS m^{-1} in both genotypes. Sodium accumulation in shoots, roots, and grains increased with salinity, with higher accumulation in PK31. Overall, PK37 exhibited greater salinity tolerance than PK31 through better physiological performance, lower sterility, and higher yield stability under saline conditions.

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Introduction

Aromatic or fragrant rice is popular for its pleasant aroma and taste. However, its yield potential is generally low because it is sensitive to biotic and abiotic stresses and shows excessive vegetative growth in fertile soils. Grain quality in aromatic rice mainly depends on aroma, small grain size, and aman season cultivation. Grain yield and quality are influenced by both genetic and environmental factors. The aroma of fragrant rice is mainly due to the synthesis of 2-Acetyl-1-Pyrroline (2AP), and majority of the HYV are nonaromatic in nature. Most HYV rice varieties are coarse-grained, while fine and aromatic rice receive less attention in the earlier times. Aromatic rice varieties usually have lower yield potential and are more sensitive to various biotic and abiotic stresses, resulting in lower overall production (Mia and Shamsuddin, 2011).

Salinity is an abiotic stress that hinders crop yield through the deleterious effects mediated by plant omics. The saline soils of the coastal areas are very critical in physicochemical behavior. Salt stress negatively affects plant growth and development through osmotic stress, ionic stress, and nutrient imbalance (Ashraf and Wu, 1994; Parida and Das, 2005). Generally, osmotic stress caused by salinity is followed by toxicity due to ion accumulation. Excess reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) can damage plants. These harmful compounds accumulate in plant tissues due to ion imbalance and hyperosmotic stress. The build-up of ROS causes lipid oxidation, which impairs cellular metabolism and physiology and severely damages membrane integrity (Munns *et al.,* 2006).

Recently, Bangladesh has been achieved almost self-sufficient in rice production by the adoption of modern high-yielding varieties along with improved agricultural practices (Sultana *et al.,* 2023). The saline-affected coastal areas of Bangladesh, which are fallow throughout the Rabi and late Kharif seasons, can also be utilized to increase rice production. Lower productivity of glycophytic crops is caused by salinity, a severe chronic issue in Bangladesh's coastal districts. The coastal zones of Bangladesh are among the world's most vulnerable areas to climate change. Sea level rise and reduced freshwater flow from upper catchments are the main factors leading to increasing soil and water salinity, causing negative effects on crop production (Feist *et al*., 2023). More than 53% of the country's cultivated area is exposed to salinity, with rice as the main crop (Abedin and Hosenuzzaman, 2023).

Irrigation with fresh water can reduce salinity to a great extent, but it is exorbitant due to the lack of non-saline water resources. Future rice varieties will need higher salinity tolerance due to worsening salinity caused by climate change, especially in coastal tropical regions (Clarke *et al.,* 2015; IPCC, 2014; Nicholls *et al.,* 2016; Ponce *et al.,* 2021).

In Bangladesh, vast areas of land remain fallow in the coastal regions due to salt accumulation,

which intensifies during the winter season due to increased evaporation. In Bangladesh's saline areas, aman rice yield also declines in the late Kharif season due to low rainfall. Crop production is difficult and costly because of freshwater scarcity and expensive irrigation. Ordinary glycophytes are not profitable for the common farmers in Bangladesh. Therefore, the cultivation of high-value, salt-tolerant crop varieties could be a potential alternative for a sustainable crop production system in the country. However, in Bangladesh, there is currently no suitable high-yielding aromatic rice variety that is tolerant to salinity.

Cultivating salt-tolerant high-value crops can be an effective and eco-friendly solution for sustainable crop production in Bangladesh. Aromatic fine rice is a high-value crop that is gaining prominence due to increasing demand in the local market and export potential in foreign countries. The Department of Crop Botany at Gazipur Agricultural University (GAU) has been researching salinity tolerance in crops and developed about 115 advanced aromatic rice lines by crossing Kalojira and Badshabough with Pokkali. These genotypes have gone through multiple screening processes, and some were found capable of tolerating various levels of salinity. In this study, two genotypes, PK31 and PK37, were selected to evaluate salinity tolerance at the tillering and panicle initiation stages through morpho-physiological, biochemical, and yield analyses under salt stress.

Materials and Methods

The experiment was conducted at the shade-house of the Department of Crop Botany, GAU, Gazipur under pot culture condition during *aman* season, 2022. Two aromatic rice genotypes viz. PK31 and PK37 were used as planting material in this experiment. They were collected from the Department of Crop Botany at GAU, Gazipur. These two genotypes were selected as tolerant (PK37) and susceptible (PK31) based on the findings of the earlier experiment (Parvin *et al.,* 2024).

Two aromatic rice genotypes viz. PK31 and PK37 were grown under pot culture condition using 10 kg soil. Five levels of salinity (0, 4, 6, 8, and 10 dSm^{-1}) for both tillering (TS) and panicle initiation (PI) stages. The study employed in a factorial completely randomized design with three replications. Plastic pots were filled up with the soils, collected from the field of Salna series under the Madhupur tract, and had a clay loam texture with a nearly neutral pH (6.5). Fertilizers were applied as per recommendation of Fertilizer Recommendation Guide (FRG, 2018). Urea, triple super phosphate, muriate of potash and gypsum were used as the source of N, P, K and S, respectively. The rates of N, P, K and S were 96, 16, 80, and 12 kg ha^{-1}, respectively. During pot preparation, all fertilizers along with half of the urea were applied, while the remaining urea was top-dressed in two equal splits at 25 and 45 days after sowing. Before being planted, rice seeds were surface sterilized for five minutes with a 0.2% $HgCl_2$ solution and thoroughly rinsed with tap water. Thirty-

days-old seedlings were transplanted to the pots. In each pot, three healthy seedlings were maintained after 10 days of transplanting. Pots were regularly watered with distilled water to avoid moisture stress.

Application of treatments

Commercial NaCl solutions were applied at two growth stages: TS (30 days after transplanting) and PI stage (60 days after transplanting). Five treatment doses were applied at one-day intervals in both stages. Pots were labeled according to salinity level and treatment stage, and electrical conductivity (EC) was regularly monitored using an EC meter.

Data collection

Plant height

Plant height of the advanced aromatic rice lines was measured before and after salinity treatment to assess the effect of salinity stress. Relative plant height was calculated by dividing plant height under salinity by plant height under control conditions. Shoot elongation rate was determined from the increase in plant height over time, while relative shoot elongation rate was calculated as the elongation rate of salt-treated plants relative to the control and expressed as a percentage.

$$\text{Relative plant height} = \frac{\text{Plant height at saline}}{\text{Plant height at control}} \times 100$$

$$\text{Shoot elongation rate (cm day}^{-1}) = \frac{\text{Final plant height - Initial plant height}}{\text{Duration between the two plant heights}}$$

$$\text{Relative shoot elongation rate} = \frac{\text{Shoot elongation rate of plants at salinity}}{\text{Shoot elongation rate of plants at control}} \times 100$$

Tillers and grain production

The total number of tillers plant^{-1} from both TS and PI stage salt treated plants were counted and the data were recorded. Effective tillers are those who exhibit panicle. Also, a count and record of the number of functional tillers were made for each plant. The grains were harvested and oven dried and their weight were measured to see the effect of salinity on grain yield of aromatic rice. The number of grains panicle^{-1} for each treatment were counted and recorded. The number sterile grains panicle^{-1} for each treatment were also counted for each advanced line. To see the effect of salinity on grain sterility the percent grain sterility was determined using the following formulae:

$$\text{Percent sterile grain panicle}^{-1} = \frac{\text{Number of sterile grains panicle}^{-1}}{\text{Total number of grains panicle}^{-1}} \times 100$$

Harvest index

The total dry matter plant^{-1} was determined by adding shoot weight, root weight and grain weight of each plant sample. The harvest index was calculated by dividing the grain weight of a plant by total dry matter of the plant and multiplying it by 100 (Mamun *et al*., 2022).

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Grain + Straw yield}} \times 100$$

Chlorophyll fluorescence (Fv/Fm)

For the purpose of measuring chlorophyll fluorescence (Fv/Fm), potted plants from the Department of Crop Botany at GAU were carefully placed in the testing rooms. An hour was spent dark acclimating the plants before any observations were made. An individual plant's Fv/Fm was determined using a pulse amplitude modulation fluorometer.

Determination of proline (μ mol g^{-1} FW)

Proline content was determined following ninhydrin acid reagent method (Bates *et al.*, 1973). A 0.2 g fresh leaf sample was homogenized at 4^{o}C in 2 mL of 1% sulphosalicylic acid, and the mixture was centrifuged for 15 minutes at 15,500 rpm. One mL of the filtrate was placed in a screw cap, and then one milliliter of the glacial acetic acid and ninhydrin reagent solution were added thereafter the mixture was boiled for thirty minutes. After completing the reaction on ice, 2 mL of toluene were used to extract the reaction mixture. The hydrated phase was separated from the toluene-containing chromophore. Using spectrophotometry, the absorbance was measured at 520 nm wavelength. Using L-proline standard solutions (0, 4, 8, 12, 16, and 20 µg mL^{-1}), a standard curve was established using the same process. The amount of proline (in µg per mL) was calculated using the standard curve. The following formula was used to calculate the amount of proline:

$$\text{Proline (µmol g}^{-1}\text{ FW)} = \frac{\text{µg proline mL}^{-1} \times \text{Vol. of extract (mL)} \times \text{toluene used (mL)}}{115.13 \times \text{g samples}}$$

Determination of malondialdehyde content (µmol g^{-1} FW)

The amount of MDA generated by the thiobarbituric acid reaction was measured in order to evaluate lipid peroxidation, using the procedure outlined by Heath and Packer (1968) and Fatema *et al*. (2023). At 4°C, fresh leaf 0.1 g of each advanced line was homogenized with 2 mL of 0.1% trichloroacetic acid (TCA), and the mixture was centrifuged for 10 minutes at 12000 rpm. The MDA content was calculated by applying the formula given below and expressed as µmol g^{-1} FW.

$$\text{MDA (µmol g}^{-1}\text{ FW)} = \frac{\text{(A532-A600)} \times \text{Vol. of extract (mL)}}{\text{Aco.} \times \text{SW} \times \text{DF}}$$

Here, Aco. = Extinction co-efficient of MDA = 155.

Determination of Na^+ concentration

The Na^+ ion concentration of the grain was determined by wet oxidation method (Singh *et al*., 1999). In this method, a digestion flask containing 1 g of oven-dried powdered materials and 15 mL of a di acid combination (HNO_3: $HClO_4$ = 2:1) was filled and left overnight. The micro-Kjeldahl digestion system was used to carry out the digestion, and it was then heated for two hours at 180–200°C. The Na concentration was ascertained using a flame emission spectrophotometer at a wavelength of 589 nm. A gas flame was used to aspirate the samples, and at 10 psi, the air pressure was fixed. Percent emission was measured using the procedure that Ghosh *et al*. (1983) had outlined.

Statistical analyses

The collected data on various parameters of the selected genotypes were tabulated and analyzed using statistical software RStudio Version: 2023.12.0+369. To find the significant difference between groups, the means for each treatment were computed, and an analysis of variance (ANOVA) was done for each character under discussion using the Least Significant Difference (LSD) method. Regression analysis was applied where needed.

Results and discussion

Effect of salinity on plant height and elongation rate

The interaction between genotypes and salinity treatments was significant. Salinity reduced plant height more severely at the tillering stage (TS) than at the panicle initiation (PI) stage (Table 1). Relative plant height (RPH) decreased with increasing salinity in both genotypes, with the greatest reduction at 10 dSm^{-1}. At TS, RPH declined to 64.46% in PK31 and 66.56% in PK37, indicating greater sensitivity of PK31. At

PI, the reduction was less pronounced, with RPH remaining above 88% in both lines. Similar findings were reported by Mahmood *et al.* (2009), Nozulaidi *et al*. (2015) and Puvanitha and Mahendran (2017). Shoot elongation rate and relative elongation rate (RER) also declined with increasing salinity. The highest values were observed under control conditions, while the lowest occurred at 10 dSm^{-1}. PK37 maintained a higher RER than PK31 and remained statistically comparable to the control up to 8 dSm^{-1}, but decreased sharply at 10 dSm^{-1}. These results agree with previous reports that high salinity significantly suppresses rice growth and elongation rates.

Effect of salinity on the number of tillers plant^{-1}

Salinity had no significant effect on the number of tillers per plant in both genotypes. At 10 dS m^{-1}, tiller numbers (11.44 and 10.44) were similar to the control (12.00 and 10.78). A slight reduction was observed at the PI stage. The decline in the number of tillers of the genotypes may be due to their failure in competing for nutrients, as observed by Ishizuka and Tanaka (1963). The variable effect of salinity on tiller number was also observed by Hussain *et al.* (1989), who noticed that tiller number per hill differed among the varieties. The number of effective tillers per plant in both genotypes decreased significantly with increasing salinity at both

Table 1. Effect of salinity on the plant height of the genotypes of aromatic rice

Rice lines	Salinity (dSm^{-1})	Plant height (cm)		Relative plant height (%)		Elongation ratio	
		TS	PI	TS	PI	SER	RSER
PK31	0	95.67 ab	95.33 a	100.00 a	100.00 a	0.53 ab	100.00 a
	4	90.67 ab	94.33 ab	94.78 ab	98.95 ab	0.46 abc	86.52 ab
	6	86.00 b	94.00 ab	89.92 ab	98.60 ab	0.39 cd	73.59 bc
	8	73.67 c	92.66 ab	76.98 c	97.21 ab	0.31 de	57.59 cd
	10	60.00 d	84.66 b	62.70 d	88.85 b	0.14 f	27.08 e
PK37	0	100.33 a	100.33 a	100.00 a	100.00 a	0.58 a	100.00 a
	4	95.67 ab	99.00 a	95.39 ab	98.66 ab	0.47 abc	82.80 abc
	6	93.00 ab	95.66 a	92.70 ab	95.35 ab	0.47 abc	81.53 abc
	8	89.00 b	93.66 ab	88.71 b	93.36 ab	0.44 bc	76.32 abc
	10	66.78 cd	91.00 ab	66.56 cd	90.71 ab	0.19 ef	32.79 de
CV (%)		4.32	3.74	4.40	3.94	0.143	12.31

CV = Co-efficient of variance, Means bearing the same letter do not differ at 5% level of significance, TS = Tillering stage, PI = Panicle initiation, SER = Stem elongation ratio, RSER = Relative stem elongation ratio.

TS and PI stages. The reduction was more severe at the TS, where no effective tillers were produced at 10 dSm^{-1} in either line. At the PI stage, the decline was comparatively gradual. Overall, PK37 showed a slightly higher effective tiller percentage than PK31 under normal conditions. However, both lines performed similarly under severe salinity stress, with the lowest values recorded at higher salinity levels. This finding was in line with the findings of Bohra and Doerffling (1993), Khatun *et al.* (1995), and Zeng *et al.* (2002), who also recorded similar responses.

Effect of salinity on proline content

Proline content increased markedly with rising salinity at both TS and PI stages. The lowest levels were observed under control conditions, while the highest occurred at 10 dSm^{-1}. Overall, PK37 showed higher proline accumulation than PK31, with the maximum value recorded in PK37 at 10 dSm^{-1} (2.06 µ mol g^{-1} at TS and 0.679 µ mol g^{-1} at PI stage) (Table 3). Lutts *et al.* (1996) reported that salt-resistant rice cultivars accumulate more proline than salt-sensitive ones. Chutipaijit *et al.* (2009 and 2011) also observed a similar response in the proline content of tolerant rice varieties.

Effect of salinity on Malondialdehyde (MDA) content

At the TS, MDA content showed no clear trend with increasing salinity. However, PK31 had a significantly higher mean MDA level (0.040 µmol g^{-1}) than PK37 (0.031 µmol

Table 2. Effect of salinity on total tillers and effective tillers of the genotypes of rice

Rice lines	Salinity (dSm^{-1})	Total tillers (no. $plant^{-1}$)		Effect tillers (no. $plant^{-1}$)		Effect tillers (%)	
		TS	PI	TS	PI	TS	PI
PK31	0	12.00 ab	12.00 a	11.55 a	11.55 a	96.28 a	96.28 a
	4	13.22 ab	12.00 a	9.44a b	10.33 ab	71.5 abc	86.1 abc
	6	13.00 ab	11.45 ab	8.67 ab	8.00 def	66.6 abc	69.8 cde
	8	9.45 b	11.44 ab	3.56 cd	6.22 f	40.38 c	54.41 ef
	10	12.11 ab	11.44 ab	0.00 d	4.44 g	0.00 d	39.03 f
PK37	0	11.00 ab	10.78 b	10.55 ab	10.56 ab	96.02 a	97.94 a
	4	13.33 ab	10.67 b	10.33 ab	10.0 abc	77.38 ab	93.78 ab
	6	14.22 a	10.56 b	9.67 ab	9.33 bcd	67.9 abc	88.40 ab
	8	12.61 ab	10.45 b	7.33 bc	8.28 cde	58.44 bc	79.4 bcd
	10	11.11 ab	10.44 b	0.00 d	7.22 ef	0.00 d	69.17 de
CV (%)		12.65	3.18	18.82	7.07	19.45	7.39

CV = Co-efficient of variance, Means bearing the same letter do not differ at 5% level of significance, TS = Tillering stage, PI = Panicle initiation.

Table 3. Effect of salinity on proline, MDA contents and Fv/Fm of the genotypes PK31 and PK37

Rice lines	Salinity (dSm^{-1})	Proline (μ mol g^{-1})		MDA (μ mol g^{-1})		Fv/Fm	
		TS	PI	TS	PI	TS	PI
PK31	0	0.29 f	0.147 g	0.030 b	0.051 a	0.792 a	0.781 ab
	4	0.42 f	0.198 f	0.036 ab	0.043 a	0.782 a	0.716 cd
	6	0.86e	0.257 e	0.032 b	0.046 a	0.692 b	0.703 cd
	8	1.38 c	0.369 c	0.067 a	0.042 a	0.594 c	0.683 d
	10	1.68 b	0.585 b	0.036 ab	0.043 a	0.519 d	0.596 e
PK37	0	0.28 f	0.138 g	0.037 ab	0.053 a	0.811 a	0.795 a
	4	0.84 e	0.232 ef	0.023 b	0.052 a	0.776 a	0.775 ab
	6	1.13 d	0.299 d	0.040 ab	0.046 a	0.708 b	0.75 abc
	8	1.49 c	0.383 c	0.029 b	0.065 a	0.682 b	0.73 bcd
	10	2.06 a	0.679 a	0.027 b	0.051 a	0.614 c	0.714 cd
CV (%)		4.94	4.08	32.01	26.73	2.24	2.51

CV = Co-efficient of variance, Means bearing the same letter do not differ at 5% level of significance, TS = Tillering stage, PI = Panicle initiation.

g^{-1}), indicating greater membrane damage in PK31. The highest value was recorded in PK31 at 8 dSm^{-1} (0.067 μmol g^{-1}), while the lowest was in PK37 at 4 dSm^{-1} (0.023 μmol g^{-1}) (Table 3). At the PI stage, the genotypes didn't show any significant difference in MDA content with increasing levels of salinity. No particular trend in response to salinity was observed at this stage as well. The highest (0.065 μ mol g^{-1}) and lowest (0.042 μ mol g^{-1}) MDA content was observed in PK37 at 8 dSm^{-1} and PK31 at 8 dSm^{-1}, respectively. Lutts *et al*. (1996) found that the response of MDA content to salt stress varied among different rice cultivars. They observed an increase in MDA content in some cultivars, whereas the others exhibited no significant change or even a decrease.

Effect of salinity on chlorophyll fluorescence (Fv/Fm)

Salinity significantly reduced chlorophyll fluorescence (Fv/Fm) in both genotypes, with a stronger effect at the TS than at PI stage. PK31 showed a greater decline than PK37. The highest Fv/Fm values were recorded under control conditions (0.792 and 0.811), while the lowest values occurred at 10 dSm^{-1} (0.519 and 0.614). PK37 at higher salinity levels showed values comparable to PK31 at moderate salinity, indicating that PK31 was more sensitive to salinity stress (Table 3). Similar results were found at the PI stage as well. The Fv/Fm ratio of both genotypes was found to decrease with increasing levels of salinity. The mean (0.753, 0.696) of the genotypes varied significantly in response to

different salinity levels. The highest (0.781, 0.795) Fv/Fm ratio was found at control for both genotypes, and the lowest (0.596, 0.713) was observed at 10 dSm^{-1}. Likewise, TS, Fv/Fm ratio of the advanced line PK31 at PI stage was more sensitive to salinity than that of PK37 and the Fv/Fm of PK37 at 10 dSm^{-1} (0.713) was statistically similar to the Fv/Fm of PK31 at 4 dSm^{-1} (0.716). Kanmani *et al*. (2017) found similar results that showed a decrease in Fv/Fm content of rice against increasing salinity levels. Majidi-Mehr and Amiri-Fahliani (2020) found that grain yield under stress conditions showed a positive and significant correlation with Fv, chlorophyll, and Fv/Fm, indicating that with increasing leaf chlorophyll content and Fv/Fm, more photosynthesis is carried out, and thus the grain yield increases.

Effect of salinity on shoot dry weight

Shoot weight declined with increasing salinity at both TS and PI stages. At the TS, PK37 showed higher mean shoot weight (12.54 g) than PK31 (7.32 g). Both lines had their highest shoot weight under control conditions (15.93 g in PK37 and 15.45 g in PK31). The lowest values were recorded at 10 dSm^{-1}, with PK31 (2.91 g) and PK37 (7.83 g), showing a clear reduction under high salinity stress (Table 4). At the PI stage, although shoot weight decreased with increasing salinity levels, there was no discernible difference in shoot weight between genotypes. At control conditions, PK37 had the highest (15.99 g) shoot weight, while PK31 had the lowest (10.76 g) at 10 dSm^{-1}. Zeng *et al*. (2001) observed similar results and noted that a reduction in shoot dry weight was significant when plants were treated with salinity at earlier stages. Pushpam and Rangasamy (2002) observed that salinity induced a reduction in shoot weight in susceptible cultivars when compared to the tolerant cultivar. Similar results were observed by Mahmood *et al.* (2009), Haq *et al.* (2009), and Jamil *et al.* (2012).

Effect of salinity on root dry weight

Root weight decreased in both genotypes with increasing salinity at the TS, showing a similar trend. PK37 recorded higher root weight (3.43 g) than PK31 (2.68 g), indicating a clear varietal difference. Under control conditions, both lines had the highest and statistically similar root weights (PK37: 4.00 g, PK31: 4.06 g). The lowest value was observed in PK31 (1.78 g) at 8 dSm^{-1} salinity (Table 4). At the PI stage, the root weight of the genotypes did not differ significantly. There was no significant decrease in root weight with increasing salinity level. With rising salt levels, PK31's root weight decreased; it was maximum (3.93 g) under control conditions and lowest (3.05 g) at 10 dSm^{-1}. Although PK37's root weight decreased at all salinity levels, it did not follow the same pattern as PK31. Rodrigues *et al.* (2002) and Roy *et al.* (1992) observed that the length and dry weight of rice shoots and roots decreased with increasing salinity levels. Lin and Kao (2001) noted that increasing salinity concentrations progressively decreased root growth. Zeng *et al.* (2001) found that rice varieties were

Table 4. Effect of salinity on shoot and root weight, and panicle length of the lines of rice

Rice lines	Salinity (dSm^{-1})	Shoot weight (g)		Root weight (g)		Shoot: root		Panicle length at PI (cm)
		TS	PI	TS	PI	TS	PI	
PK31	0	15.45 a	15.52 a	4.07 a	3.93 a	3.80 ab	3.98 a	22.83 ab
	4	9.32 cd	15.33 a	3.24 ab	3.64 a	2.89 bc	4.24 a	22.50 ab
	6	5.59 e	14.4 ab	2.48 bc	3.63 a	2.37 cd	3.98 a	24.17 a
	8	3.35 f	13.50 ab	1.78 c	3.38 a	1.92 cd	4.03 a	22.50 ab
	10	2.91 f	10.80 b	1.85 c	3.05 a	1.60 d	3.57 a	21.50 ab
PK37	0	15.93 a	15.99 a	4.00 a	4.20 a	3.98 a	3.82 a	20.67 b
	4	15.63 a	15.10 a	3.98 a	3.62 a	3.92 ab	4.25 a	21.33 ab
	6	13.21 b	15.30 a	3.42 ab	3.47 a	3.86 ab	4.42 a	22.00 ab
	8	10.10 c	13.50 ab	3.02 ab	3.90 a	3.43 ab	3.54 a	21.00 b
	10	7.83 d	13.30 ab	2.72 bc	3.79 a	2.89 bc	3.51 a	22.50 ab
CV (%)		5.95	10.13	12.92	13.24	11.69	13.87	4.72

CV = Co-efficient of variance, Means bearing the same letter do not differ at 5% level of significance, TS = Tillering stage, PI = Panicle initiation.

more susceptible to salt stress at early stages of growth. Heenan *et al.* (1998) noted that different varieties show different responses to salinity at different growth stages.

Effect of salinity on shoot-root ratio

The shoot:root ratio decreased with increasing salinity at both TS and PI stages in both genotypes, PK31 and PK37. PK37 generally showed a higher ratio (mean 3.62) than PK31 (mean 2.52) at the TS. The highest value was recorded in PK37 under control conditions (3.98), while the lowest was in PK31 at 10 dSm^{-1} (1.60). Overall, PK31 showed a more pronounced decline under salinity stress compared to PK37 (Table 4). At the PI stage, the genotypes did not differ in their shoot-to-root ratio, and no particular pattern was seen when salinity levels changed. The shoot-root ratio in PK37 at 6 dSm^{-1} was the greatest (4.42) value, and 10 dSm^{-1} for the lowest (3.51) value. Negative impacts of salinity on the root-to-shoot dry weight of rice are reported by Castillo *et al.* (2007), and Shultana *et al.* (2019). Zeng *et al.* (2000) reported that salt stress at the earlier development stage of rice affects the shoot and root biomass production.

Effect of salinity on panicle length

The panicle length of the genotypes at different salinity levels at the PI stage was recorded to observe the effect of salinity on the panicle length of the genotypes. Greater panicle length was observed in PK31 (22.70 cm) compared to PK37 (21.50 cm). No particular trend was found with increasing salinity levels on the panicle length of the two

genotypes. The longest (24.17 cm) panicle length was found in PK31 at 6 dSm^{-1}, and the smallest (20.67 cm) was found in PK37 at the control condition (Table 4). Though Abdullah *et al.* (2001), Aslam *et al.* (2003), and many other scientists reported a reduction in panicle length with increasing salinity levels, Khatun *et al.* (1995) found no significant variation in panicle length of rice against salt stress.

Effect of salinity on the number of grains (NGPP) and sterile grains panicle^{-1}

At both TS and PI stages, salinity significantly reduced the number of grains $panicle^{-1}$ in both genotypes. At the TS, PK37 showed a higher mean NGPP than PK31. Grain number declined sharply with increasing salinity, with a greater reduction in PK31. The highest NGPP (122.67) was recorded in PK31 under control conditions, while the lowest (0.00) occurred at 10 dSm^{-1}. At higher salinity, NSPP values in PK37 at 10 dSm^{-1} were comparable to PK31 at 8 dSm^{-1} (Table 5). Whereas, at the PI stage, the NGPP decreased gradually for both genotypes, and again, the decline was more prominent in PK31. The highest (126.67) NGPP was observed in PK31 at the control condition, and the lowest (95.33) was observed in the same at 10 dSm^{-1} (Table 5). Rad *et al.* (2011) found a significant effect of salinity on the grain yield of rice. Similar results were observed by Hakim *et al*. (2014), Mahmood *et al.* (2009), and Zeng *et al.* (2000).

Sterile grains $panicle^{-1}$ were higher in PK31 than PK37 at the TS. Both lines showed increasing sterility with rising salinity,

Table 5. Effect of salinity on number of fertile and sterile grains of the genotypes of rice

Rice lines	Salinity (dSm^{-1})	Fertile grains (no. $panicle^{-1}$)		Sterile grains (no. $panicle^{-1}$)	
		TS	PI	TS	PI
PK31	0	122.67 a	126.67 a	12.81 d	9.22 e
	4	106.33 bc	121.33 b	27.50 b	10.77 de
	6	67.00 e	113.67 cd	35.05 a	16.78 c
	8	15.33 g	105.33 f	13.34 d	27.33 b
	10	0.00 h	95.33 g	0.00 e	53.55 a
PK37	0	113.67 ab	115.33 c	3.40 e	3.55 f
	4	102.33 c	112.33 cd	9.61 d	6.22 f
	6	88.00 d	110.33 de	13.49 d	12.22 d
	8	54.33 f	106.00 ef	21.54 c	16.33 c
	10	13.33 g	103.00 f	13.33 d	24.66 b
CV (%)		4.82	1.50	11.68	5.20

CV = Co-efficient of variance, Means bearing the same letter do not differ at 5% level of significance, TS = Tillering stage, PI = Panicle initiation.

while seed set panicle^{-1} (SSPP) declined and became zero in PK31 at 10 dSm^{-1} due to no grain formation. At the PI stage, PK31 also had slightly higher sterility than PK37. Sterile grain numbers increased with salinity in both lines, reaching the highest level at 10 dSm^{-1}. PK31 at 6 dSm^{-1} and PK37 at 8 dSm^{-1} showed similar sterility levels. Chowdhury *et al.* (1993) reported differences in the number of sterile grains panicle^{-1} due to varietal differences. Abdullah *et al.* (2001) noted that the sterility in rice under salt stress was not merely due to the reduction or inhibition of different biochemical constituents and physiological functions, but also due to the limitation of soluble carbohydrate translocation in primary and secondary grain.

Effect of salinity on percent sterile grains and grain yield

For plants treated during the TS as well as the PI stage, it was discovered that grain sterility increased in both genotypes with increasing salinity levels, and similar patterns were observed at both TS and PI stage (Table 6). The greatest (100%) sterile grain percentage for the TS was 10 dSm^{-1} for PK37. The rise in sterile grain was discovered to be greater in PK31 than in PK37 up to 8 dSm^{-1}. Similar trends were seen in the PI stage, although at this point in PK37, salinity had considerably less impact. When treated at the PI stage, PK37 reported substantially less sterile grain at 10 dSm^{-1} than PK31, which had the highest (56.19) percentage of sterile grains at 10 dSm^{-1}. High sterility of grains under salt stress was observed by Makihara *et al.* (1999), Grattan *et al.* (2002), and Aref and Rad (2012).

At the TS, both genotypes had a similar pattern: a decline in grain weight was seen as saline levels increased. Compared to PK31 (4.24 g), PK37 showed a heavier (8.27 g) grain weight. The lowest (0.00 g) grain weight was noted in both PK31 and PK37 at 10 dSm^{-1}, while the maximum (14.93 g) grain weight was noted in PK37 under control conditions. The drop in PK31 with rising salinity levels was greater than PK37 up to 8 dSm^{-1}. Similar trends in the genotypes were also seen in the PI stage. The grain weight that was found to be highest (14.93 g) in PK37 under control conditions was similar to the grain weight (14.17 g) that was found in PK37 at 4 dSm^{-1}. PK31 showed the lowest (7.13 g) grain weight, which was at the 10 dSm^{-1} level of salinity. Grain yield differences due to variety were reported by IRRI (1978), and Narale *et al.* (1969). Zheng *et al.* (2023) also concluded that salinity-tolerant varieties at the seedling stage do not always maintain grain yield at a sufficient level under salinity.

Effect of salinity on total dry matter and HI

Both genotypes at the TS and PI stages showed a reduction of total dry matter with increasing salinity levels, and the same trend was seen at both TS and PI stage (Figure 1). At the TS, the drop was more severe. When genotypes were taken into account, PK31 reported a more severe drop in TDM. The advanced line with the highest (35.02 g) TDM at the TS was

Table 6. Effect of salinity on percent sterile grains and grain weight of the genotypes

Rice lines	Salinity (dSm^{-1})	Sterile grain (%)		Grain weight (g $plant^{-1}$)	
		TS	PI	TS	PI
PK31	0	10.45f	7.28ef	12.09b	12.75ab
	4	25.91e	8.88de	6.74d	11.95abc
	6	52.54c	14.76c	2.08f	11.56abc
	8	86.82b	25.96b	0.31g	10.93abc
	10	0.00g	56.19a	0.00g	7.13c
PK37	0	3.01g	3.08g	14.93a	14.93a
	4	9.39f	5.53fg	12.50b	14.17ab
	6	15.32f	11.07d	10.43c	12.65abc
	8	39.52d	15.41c	3.48e	11.95abc
	10	100.00a	23.96b	0.00g	8.75bc
CV (%)		6.35	6.64	5.81	16.24

CV = Co-efficient of variance, Means bearing the same letter do not differ at 5% level of significance, TS = Tillering stage, PI = Panicle initiation.

PK37 under control conditions, whereas the advanced line with the lowest (4.76 g) TDM was PK31 at 10 dSm^{-1}, which was statistically equivalent to the TDM (5.43 g) of PK31 at 8 dSm^{-1}.

Similar results were obtained in the PI stage as well, but the decline in TDM was not as much as observed at the TS. The lowest (20.94 g) TDM was discovered in PK31 at 10 $dSm^{-1,}$ while the maximum (35.12 g) TDM was discovered in PK37 under control conditions. Ologundudu *et al.* (2014) reported a reduction in dry matter production in rice with increasing salinity levels. Islam *et al.* (1995) also reported a similar response. Both genotypes demonstrated a discernible decline in harvest index with increasing saline levels during the TS as well as the PI stage. The genotypes were very different, and PK37 (mean 28.25%) was discovered to be better than PK31 (mean 19.89%). At control conditions, PK37 had the highest (42.65%) harvest index, whereas both genotypes had the lowest (0.00%) at 10 dSm^{-1}. In comparison to PK31, PK37 had a much higher harvest index up to 8 dSm^{-1}.

Even though the harvest index decreased with rising saline levels and the HI of PK37 (mean 40.12) was greater than PK31 (37.83) at the PI stage, statistically, the genotypes differed in HI (Figure 1). A decline in harvest index with increasing salinity levels was reported by Aref (2013), Mondal *et al.* (2013), and Zeng and Shannon (2000).

Total dry matter (TDM) decreased significantly with increasing salinity in both

genotypes at the transplanting (TS) and panicle initiation (PI) stages (Figure 1), with a more pronounced reduction at TS. PK31 was more adversely affected than PK37. At TS, the highest TDM (35.02 g) was recorded in PK37 under control conditions, while the lowest (4.76 g) was observed in PK31 at 10 dSm^{-1}, which was statistically similar to that at 8 dSm^{-1} (5.43 g). A similar trend was observed at PI, although the reduction was less severe. The highest TDM (35.12 g) was recorded in PK37 under control conditions, whereas the lowest (20.94 g) was found in PK31 at 10 dSm^{-1}. Similar reductions in dry matter production under salinity stress were reported by Ologundudu *et al.* (2014) and Islam *et al.* (1995).

Harvest index (HI) also declined with increasing salinity at both growth stages. PK37 consistently maintained a higher HI than PK31, indicating better tolerance to salinity. At TS, PK37 had the highest HI (42.65%) under control conditions, while both lines showed no grain yield (HI = 0%) at 10 dSm^{-1}. At PI, HI remained significantly higher in PK37 (40.12%) than in PK31 (37.83%). These findings agree with previous reports showing that increasing salinity reduces harvest index in rice.

Effect of salinity on shoot, root, and grain Na^+ concentration

Accumulation of Na^+ in shoot and root increased with rising salinity at both TS and PI stages, while grain Na^+ increased only at the PI stage. At TS, PK31 showed higher shoot Na^+ (0.164%) than PK37 (0.156%), with the highest value (0.191%) recorded in PK31 at 10 dSm^{-1} and the lowest (0.106%) at control. A similar pattern was observed at the PI stage, where PK31 again had higher mean

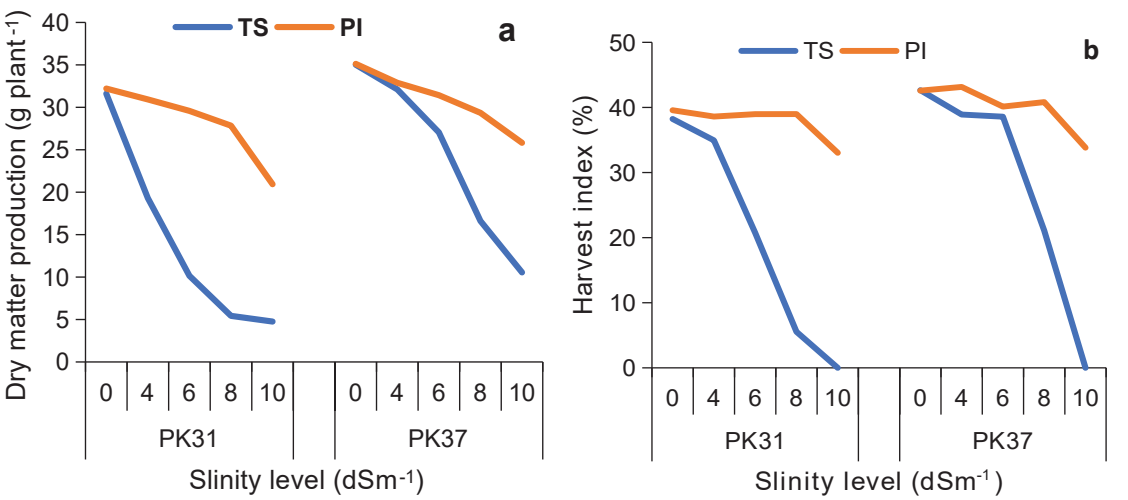

Fig. 1. Effect of salinity on total dry matter production (a) and harvest index (b) of the genotypes of rice. TS = Tillering stage, PI = Panicle initiation.

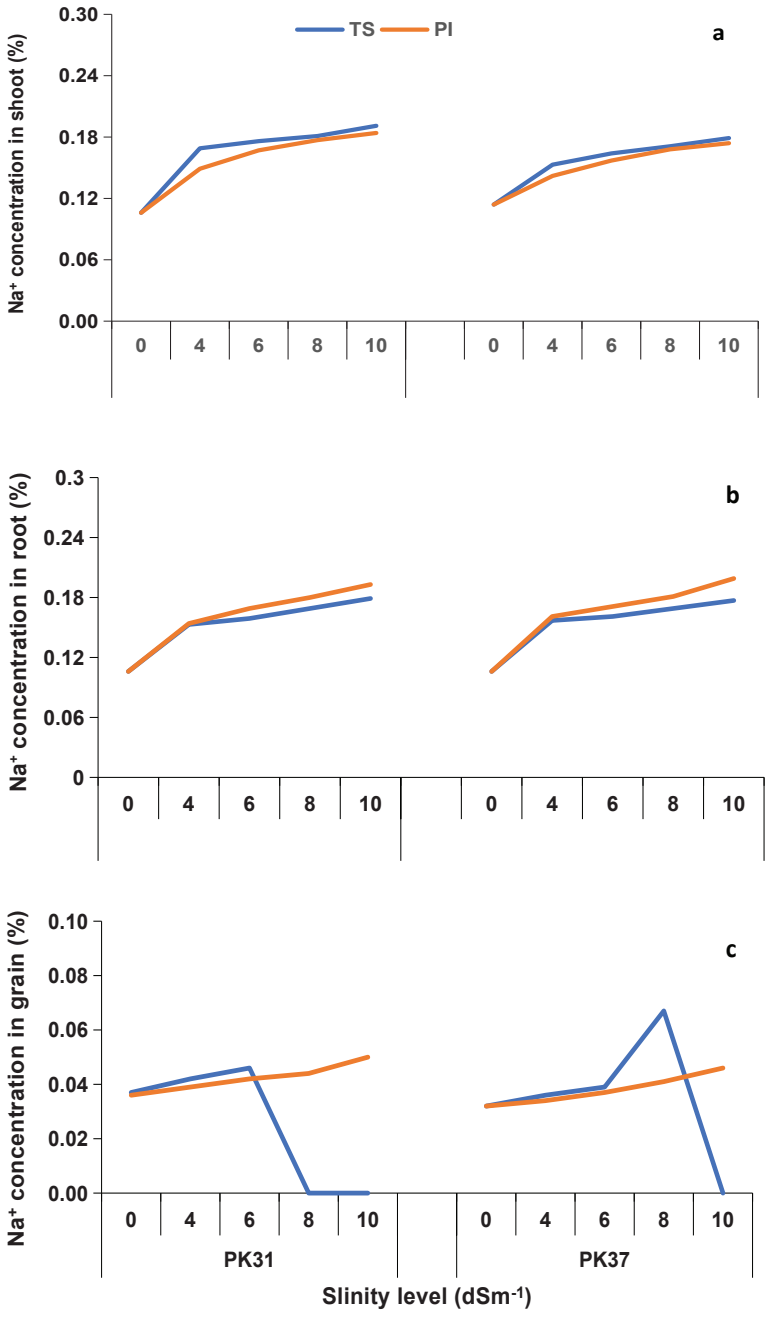

Fig. 2. Effect of salinity on Na^+ content in shoot (a), root (b) and grain (c) of the genotypes of rice. TS = Tillering stage, PI = Panicle initiation.

shoot Na^+ (0.157%) than PK37 (0.151%), with the same maximum (0.184%) and minimum (0.106%) values under 10 dSm^{-1} and control, respectively (Figure 2).

Root Na^+ concentration showed no significant difference between the two genotypes at either stage. However, the interaction between line and salinity treatment was significant. Na^+ levels increased in both lines with rising salinity at both TS and PI stages, and were statistically similar across treatments. Accumulation of Na^+ in the grain of the genotypes was also found to increase with increasing salinity levels at both stages.

Though the mean of PK31 (0.025%) was noted to be lower than that of PK37 (0.035%) at the TS, it is considered that PK31 didn`t yield enough filled grains at salinity levels above 6 dSm^{-1}. On the contrary, PK37 failed to produce filled grains at 10 dSm^{-1}. Na^+ concentration of PK31 up to 6 dSm^{-1} was higher than that of PK37. At the PI stage, the extent of increase in Na^+ concentration of the genotypes was comparatively lower than that found at the TS. PK31 (0.042%) accumulated more Na^+ ions in the grain compared to PK37 (0.038%). Hakim *et al.* (2014) also recorded a similar response in Na^+ ion concentration of the shoot and root of rice. Zhang *et al.* (2022) noted an increase in grain Na^+ content with increasing salinity stress (Figure 2).

Conclusions

In our research, we showed that the advanced line PK31 is more susceptible to salt stress than PK37, and the tillering stage had higher susceptibility to salt stress than the panicle initiation stage. Most physiological parameters like plant height, elongation rate, number of tillers per plant, effective tillers percentage, chlorophyll fluorescence (Fv/Fm ratio), shoot and root dry weight, grain weight per plant, grain sterility, harvest index, etc., have a negative relation with increasing salinity levels. Biochemical parameters were also affected by increasing salt concentrations, and proline content increased in both genotypes, but it was higher in tolerant PK37 than in PK31. Na^+ concentration increased in all shoots, roots, and grains of both genotypes, and again, the decline was more prominent in PK31 compared to PK37. No trend in MDA content with increasing salinity levels was found. Grain protein and amylose also revealed a strong negative correlation with salt stress. Growth and morphological parameters like plant height, percent effective tillers, grain sterility, chlorophyll fluorescence (Fv/Fm), and biochemical parameters like proline content, Na^+ content, grain protein, and amylose content can be used as an indicator of salinity tolerance in aromatic rice. Advanced line PK37 can be used in further study and has a great deal of promise to combat salt stress.

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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: MAB Mia (Md. Abdul Bast Mia). Methodology, Validation, and Data curation: MAB Mia and M Israr (Md. Israr). Visualization: M Israr. Original draft preparation, Reviewing, and Editing: MAB Mia, M Israr, MAA Mamun (Md. Abdullah Al Mamun) and S Pervin (Salma Pervin). All authors have read and agreed to the published version of the manuscript.

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