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MITIGATING ADVERSE EFFECTS OF SALT STRESS USING EXOGENOUS PROLINE IN RICE (*Oryza sativa*)

D. E. Jharna*, M. M. Hossain and F. Rabbi

Department of Biochemistry and Molecular Biology, Patuakhali Science and Technology University (PSTU), Dumki, Patuakhali. Bangladesh.

Abstract

Salt stress is certainly one of the most serious environmental factors limiting the productivity of rice plants, particularly in the coastal and salt-affected regions of Bangladesh. The present study was undertaken to investigate the effect of exogenous proline on the morphological, physiological, and biochemical responses of two rice (*Oryza sativa* L.) genotypes, namely BINA dhan23 and Sadamota under salt stress conditions (6 dS m^{-1}). The experiment was conducted in a net-house and laboratory of the Department of Biochemistry and Molecular Biology at Patuakhali Science and Technology University. The experimental treatments consisted of four combinations: T_1-control, T_2-proline spraying, T_3-salt stress and T_4-salt stress with proline spraying. A Randomized Complete Block Design (RCBD) with three replications was used. Data on plant parameters including plant height, tiller number, panicle and grain characteristics were recorded. The physiological and biochemical traits such as proline content, chlorophyll content, total sugar, and K^+/Na^+ ratios were analyzed in leaves. Results revealed that salinity significantly reduced yield-contributing traits such as panicle length, grain number, grain yield, and straw yield, BINA dhan23 produced substantially higher grain yield (21.8 g pot^{-1}) than Sadamota (5.58 g pot^{-1}), indicating its stronger reproductive resilience under salinity. The grain yield decreased under salt stress (10.6 g pot^{-1}) was partially recovered by proline spraying (11.4 g pot^{-1}). Similarly salt stress reduced straw yield (26.2 g pot^{-1}), while proline improved it under saline conditions (29.0 g pot^{-1}). Exogenous proline further enhanced leaf proline content (up to 114.9 mg 100 g^{-1} fresh leaf), total sugar (9.06 mg 100 g^{-1}), and chlorophyll (5.04 mg 100 g^{-1}), while improving the K^+/Na^+ ratio (0.78 vs. 0.69 in Sadamota). The findings suggest that the combined use of salt-tolerant genotypes and foliar proline treatment offers a promising approach for enhancing rice productivity in salinity-prone areas. Further field-level validation is recommended to confirm these effects under diverse agro-ecological conditions.

Keywords: Chlorophyll, K^+/Na^+ ratios, Proline, Salt stress.

Introduction

Salinity is one of the most critical abiotic stresses constraining rice (*Oryza sativa* L.) production worldwide, particularly in deltaic and coastal ecosystems where soil and water salinity are recurrent problems (Haque *et al*., 2008). Globally, over 1381 million

* Corresponding author: jharnabcm@pstu.ac.bd

hectares of land are affected by salinity, with an estimated 20% of irrigated agricultural lands suffering yield losses due to excess salt (FAO, 2024). In Bangladesh, about 1.05 million hectares of coastal land are salt-affected, predominantly during the dry season, severely reducing the productivity of rice-based cropping systems (Haque 2018; Khanam *et al.,* 2020). The adverse impacts of salinity include osmotic stress, ionic toxicity, nutrient imbalance, chlorophyll degradation, and disruption of physiological and metabolic processes (Sharmin *et al.,* 2013; Jharna *et al.,* 2001, Haque *et al.,* 2023abc, 2024a). These effects collectively inhibit plant growth, impair reproductive development, and ultimately reduce grain yield (Jharna *et al.,* 2017a; Akter *et al.,* 2020; Dutta *et al.,* 2025).

Rice, as a glycophyte, is highly sensitive to salt stress, particularly during early seedling and reproductive stages (Barman *et al.,* 2025). Salinity-induced yield loss can exceed 64% in susceptible cultivars (Zheng *et al.,* 2023). Thus, developing and deploying salt-tolerant rice varieties and complementary management practices is a priority for sustaining rice production in coastal regions. While genetic improvement for salinity tolerance remains a long-term goal, physiological interventions such as the exogenous application of proline have emerged as promising, immediate strategies for enhancing stress resilience (Farooq *et al*., 2023).

Proline, one of the mostly studied multifunctional amino acid, due to its roles in osmotic adjustment, stabilization of proteins and membranes, protection of photosynthetic pigments, and scavenging of reactive oxygen species (Jharna *et al.,* 2013, 2017b). Khanam *et al.* (2025) reported that proline content increases with increasing salinity stress; proline level is therefore act as an indicator of salt tolerance. Under salinity stress increasing proline accumulation effectively increases the osmotic pressure of the cell cytoplasm (Yan *et al.,* 2025). Although under saline conditions, plants naturally accumulate proline as part of their stress response; however, the endogenous levels are often insufficient to counteract severe stress which seeks additional supplementation of proline in plants (Verbruggen & Hermans, 2008). Recent studies have demonstrated that foliar application of proline can significantly improve plant water status, photosynthetic efficiency, ion homeostasis, and yield under salinity stress in cereals, including rice (Hayat *et al.,* 2012). However, the complex inter-relation of exogenous proline spraying with various rice genotypes under salinity stress is not well understood. The difference in genetic makeup among improved and traditional rice genotypes may respond differently to applied proline under salt stress, which needs thorough investigation. Despite growing evidence on the benefits of exogenous proline, its effectiveness varies with genotype, growth stage, and environmental conditions (Zhang *et al.,* 2025). Moreover, comparative assessments of high-yielding salt-tolerant varieties and traditional landraces under proline supplementation remain limited, particularly in the context of the South-Central coastal region of Bangladesh. This knowledge gap is critical, as genotype-specific responses can determine the practical applicability of proline-based interventions. The present study was therefore undertaken to evaluate the morphological, physiological, and biochemical responses of two contrasting rice genotypes BINA dhan23 (a high-yielding salt-tolerant variety) and Sadamota (a traditional landrace) to exogenous proline application under salinity conditions.

Methodology

Experimental site and duration

The experiment was conducted in the net-house and laboratory of the Department of Biochemistry and Molecular Biology, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh. The study was carried out during the Aman season 2024 under open sunlight. The natural temperature and humidity were maintained in the study site.

Experimental materials

Two contrasting rice (*Oryza sativa* L.) genotypes were selected for the experiment (BINA dhan23 and Sadamota). BINA dhan23 is a high-yielding salt-tolerant rice variety, developed by Bangladesh Institute of Nuclear Agriculture. The Sadamota is a traditional local landrace which is very much adapted in the coastal environment.

Experimental design and treatments

The experiment was laid out in a Randomized Complete Block Design (RCBD) with two factors and three replications. Factor 1 was variety (V_1 = BINA dhan23, V_2 = Sadamota), and Factor 2 was treatment, comprising four combinations: T_1: control (no salinity, no proline), T_2: proline spraying (75 mM), T_3: salt stress (6 dS m^{-1} NaCl) and T_4: salt stress + proline spraying (75 mM). A total of 24 pots were used, with each pot containing 8 kg of air-dried, sieved soil. Using 25-days old seedlings transplanting was done on 10 September 2024.

Fertilization

Basal fertilizers were applied at the rates of 100 mg N kg^{-1} soil, 25 mg P kg^{-1} soil, 40 mg K kg^{-1} soil, and 25 mg S kg^{-1} soil, using urea, triple superphosphate (TSP), muriate of potash (MOP), and gypsum, respectively. All fertilizers except urea were incorporated during final pot preparation, while urea was applied in two equal splits.

Imposition of salt stress and proline application

Salinity stress (6 dS m^{-1}) was imposed 20 days after transplanting by submerging the designated pots in a saline water tank. On the same day, 75 mM proline (Sigma-Aldrich, analytical grade) was sprayed as a foliar treatment, mixed with 0.1% Tween-20 as a surfactant. Second spraying of proline was done 35 days after transplanting during the morning hours to ensure optimum absorption.

Data collection on morphological parameters

At maturity all the plants were harvested at ground level. The BINA dhan23 was harvested on 16 November 2024 and Sadamota was on 7 December 2024. The parameters recorded were plant height (cm), number of tillers per pot, leaf length (cm) and width (cm), panicle length (cm), grains per panicle (no.), grain yield per pot (g) and straw yield per pot (g).

Physiological and biochemical parameters

Samples for physiological and biochemical analysis were collected 15 days after treatment application. Chlorophyll content was determined using the method of Coombs *et al.* (1985) by extracting fresh leaves with 80% acetone and measuring absorbance by spectrophotometer at 645 nm and 663 nm wavelength. The proline content was estimated by the method of Bates *et al.* (1973) using acid ninhydrin, with absorbance measured at 520 nm. Similarly, the total sugar content was determined following Dubois *et al.* (1956) using the anthrone method, with absorbance at 620 nm. The leaf Na^+ and K^+ content was measured with a flame photometer after wet digestion of dried leaf samples (Yoshida *et al.*, 1976). The K^+ /Na^+ ratio was calculated from the measured ion concentrations.

Statistical analysis

Data were statistically analyzed using the STAR (Statistical Tool for Agricultural Research) software developed by the International Rice Research Institute (IRRI). Analysis of variance (ANOVA) was performed, and treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level.

Result and Discussion

Effect of proline spraying and salt stress on growth parameters of rice genotypes

The single effect of genotype on plant height was highly significant ($p < 0.001$). Among the varieties, Sadamota showed the tallest plants (132 cm), which was 18% higher than BINA dhan23 (112 cm), reflecting genotypic variability in growth response under the given conditions (Table 1). The taller nature of traditional rice genotype Sadamota also reported by Sume *et al.* (2023). Likewise, the single effect of salinity and proline application was also highly significant ($p < 0.001$). The tallest plants (126 cm) were observed in proline spraying, closely followed by the control (125 cm). In contrast, plant height declined under salt stress, registering only 117 cm. However, the application of proline under saline conditions improved plant height to 120 cm, suggesting a partial mitigative effect of proline against salt-induced growth inhibition. The interaction between genotype and treatment was statistically significant ($p < 0.05$). Under control and only proline conditions, Sadamota maintained a consistent height of 135 cm, significantly higher than BINA dhan23 (115-116 cm; Table 2). Under salt stress, both genotypes showed reduced height, but Sadamota (126 cm) still outperformed BINA dhan23 (108 cm). Notably, under salt stress with proline application, plant height in Sadamota increased to 132 cm, while BINA dhan23 reached 109 cm, indicating that exogenous proline mitigated salt-induced reductions in plant growth for both genotypes.

Salinity inhibits plant growth by disrupting water balance, nutrient uptake, and cell elongation processes (Shila *et al.*, 2016). In this study, salt stress (6 dS m^{-1}) significantly reduced plant height in both rice genotypes, consistent with earlier reports that ionic toxicity and osmotic stress under saline conditions lead to stunted plant development (Sikder *et al.*, 2016; Kumar *et al.*, 2018). Exogenous application of proline showed a beneficial effect under both normal and saline conditions. The improved plant height in salt stress with proline spraying treatment suggests that proline supplementation helps mitigate the adverse effects of salt stress (Khanam *et al.*, 2025).

Table 1. Plant growth parameters of rice genotypes as influenced by proline spraying under salt stress condition

Treatment combinations	Plant height (cm) ©	Tiller pot-1 (no.)	Leaf width (cm)	Leaf length (cm) ©
Single effect of variety				
V1: BINA dhan23	112	14.6 B	1.49 A	37.4
V2: Sadamota	132	16.8 A	1.15 B	50.9
Significance level	***	***	***	***
SE (±)	0.61	0.416	0.416	0.77
Single effect of salinity and proline				
T1: Control	125	16.0 A	1.35 AB	46.5
T2: Proline spraying	126	16.8 A	1.37 A	43.5
T3: Salt stress (6 dS m^{-1})	117	13.7 B	1.26 C	40.7
T4: Salt stress+Proline spraying	120	16.2 A	1.29 BC	45.9
Significance level	***	***	**	***
SE (±)	0.86	0.589	0.589	1.09
Variety:treatments interaction				
Significance level	*	NS	NS	***
SE (±)	1.21	0.833	0.833	1.54
CV (%)	1.22	6.51	4.58	4.27

Similar capital letter in a column was not significantly different at 5% probability level

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Table 2. Interaction effect between variety and treatments on different plant parameters of rice

Treatments	Rice varieties	
	BINA dhan23	Sadamota
Plant height of rice (cm)		
T_1: Control	115 A b	135 A a
T_2: Proline spraying	116 A b	135 A a
T_3: Salt stress (6 dS m^{-1})	108 B b	126 C a
T_4: Salt stress+Proline spraying	109 B b	132 B a
Leaf length (cm) of rice		
T_1: Control	38.6 AB b	54.4 A a

Treatments	Rice varieties	
	BINA dhan23	Sadamota
T_2: Proline spraying	39.9 A b	47.1 B a
T_3: Salt stress (6 dS m^{-1})	34.8 C b	46.5 B a
T_4: Salt stress+Proline spraying	36.1 BC b	55.7 A a
Number of grain $panicle^{-1}$ of rice		
T_1: Control	98.73 A a	54.9 A b
T_2: Proline spraying	99.13 A a	55.3 A b
T_3: Salt stress (6 dS m^{-1})	94.80 A a	5.42 B b
T_4: Salt stress+Proline spraying	98.40 A a	6.08 B b

Similar capital letter in a column or similar small letter in a row was not significantly different at 5% probability level

The single effect of variety on tiller production was highly significant ($p < 0.001$). Sadamota produced a significantly higher number of tillers per pot (16.8) compared to BINA dhan23 (14.6) (Table 1). The single effect of salinity and proline treatment was also statistically significant ($p < 0.001$). Among the treatments, the highest number of tillers (16.8) was observed under only proline spraying, followed closely by salinity with proline spraying (16.2) and control (16.0). In contrast, the lowest tiller number (13.7) was recorded under salt stress, indicating a substantial reduction in tillering due to salinity. The application of proline under salt stress restored tiller number close to control levels. However, the interaction effect between variety and treatment was not significant, suggesting that the influence of treatment on tiller number was similar across both genotypes. In this study, salt stress significantly reduced tiller number per pot, likely due to osmotic and ionic toxicity that inhibit cell division and tiller initiation (Ahmed *et al.*, 2017; Sultana *et al.*, 2021; Kumar *et al.*, 2017). Application of exogenous proline significantly enhanced tiller production compared to salt stress alone, supporting its role as a protective osmolyte. The recovery of tiller number under salt stress with proline to levels comparable with the control suggests that foliar proline application can mitigate the inhibitory effects of salinity on vegetative growth.

A highly significant ($p < 0.001$) variation in leaf width and leaf length was observed between the genotypes (Table 1). BINA dhan23 recorded a significantly wider leaf (1.49 cm) than Sadamota (1.15 cm), while Sadamota showed markedly longer leaves (50.9 cm) compared to BINA dhan23 (37.4 cm), reflecting inherent varietal differences. The effect of salinity and proline treatments on those parameters were also significant ($p < 0.01$). The widest leaves were observed under treatment proline spraying (1.37 cm) and control (1.35 cm), while the narrowest leaves were under salt stress (1.26 cm). Leaf width slightly improved under salt stress with proline spraying (1.29 cm), suggesting that proline application partially mitigated the negative effect of salinity. Moreover, the longest leaves were found in control (46.5 cm) and salt stress with proline spraying (45.9 cm), followed by proline spraying (43.5 cm). The shortest leaves were recorded in salt stress (40.7 cm), confirming that salinity adversely affected leaf elongation. The application of proline under salt stress helped in restoring leaf length close to the control condition. The interaction between variety and treatment was also significant ($p < 0.001$), as detailed in Table 2. Under salt stress, leaf length reduced in both genotypes, with BINA dhan23 showing 34.8 cm and Sadamota 46.5 cm. However, the application of proline under salinity improved leaf length, especially in Sadamota, which recorded the longest leaves (55.7 cm), surpassing even the control. In contrast, BINA dhan23 remained comparatively shorter (36.1 cm) across all treatments. However, the application of proline (T_2 and T_4) significantly improved both parameters. Proline likely mitigates salinity effects by maintaining cell turgor, scavenging free radicals, and protecting cellular structures (Nguyen *et al.*, 2021).

Effect of proline spraying and salt stress on yield and yield components of rice genotypes

As shown in Table 3, a highly significant ($p < 0.001$) difference was observed in panicle length between the two rice genotypes. BINA dhan23 exhibited a longer panicle (24.7 cm) than Sadamota (20.4 cm). Salinity and proline treatments also had a significant ($p < 0.001$) effect on panicle length. The highest panicle length was recorded under T_2 (Proline spraying, 23.6 cm) and T_1 (Control, 23.2 cm), while the lowest was under T_3 (Salt stress, 21.5 cm). Application of proline under salt stress (T_4) increased panicle length to 21.9 cm. However, the interaction effect was not significant (NS), indicating consistent treatment effects across both genotypes. Table 3 further shows that grains per panicle were significantly influenced ($p < 0.001$) by variety, treatment, and their interaction BINA dhan23 produced significantly more grains per panicle (97.8) than Sadamota (30.4). Among treatments, the maximum grain count was recorded in T_2 (77.2) and T_1 (76.8). Salt stress drastically reduced grain number (T_3: 50.1), while proline application under stress (T4: 52.2) offered slight improvement. The interaction effect was highly significant (Table 2). Under control and only proline conditions, BINA dhan23 maintained a high grain count (~99 grains panicle^{-1}), while Sadamota showed moderate values (~55 grains panicle^{-1}). Under salt stress, grain production in Sadamota dropped sharply to only 5.42 grains, while BINA dhan23 still retained 94.8 grains. Similar trends continued under salinity and proline spraying; proline showing a protective effect in both varieties but particularly in BINA dhan23, which retained high productivity under stress.

Grain yield data (Table 3) revealed a highly significant varietal difference ($p < 0.001$). BINA dhan23 produced substantially higher grain yield (21.8 g pot^{-1}) than Sadamota (5.58 g pot^{-1}). Salinity and proline application also had a significant impact ($p < 0.01$). The highest yield was recorded in control (17.3 g pot^{-1}), followed by only proline (15.5 g pot^{-1}). Yield decreased under salt stress (10.6 g pot^{-1}) but was partially recovered by proline spraying (T_4: 11.4 g pot^{-1}). Straw yield (Table 3) further showed a significant ($p < 0.001$) difference between genotypes, with Sadamota outperforming BINA dhan23 (40.8 vs. 22.6 g pot^{-1}). Treatment effects were also significant ($p < 0.01$). The highest straw yield was observed in only proline spraying, 36.6 g pot^{-1} and control (35.1 g pot^{-1}). Salt stress reduced straw yield (T_3: 26.2 g), while proline improved it under saline conditions (T_4: 29.0 g pot^{-1}). The interaction effect was not significant for both grain and straw yield, indicating similar response patterns across genotypes.

Salinity adversely affected several yield-contributing traits such as panicle length, grain number, grain yield, and straw yield, which is consistent with previous findings (Haque *et al.*, 2024b, 2025ab). Salt stress likely caused reproductive failure, pollen sterility, and poor grain filling, contributing to yield reduction (Jodder *et al.*, 2016; Haque and Hoque 2023, Haque *et al.*, 2025c). BINA dhan23 consistently outperformed Sadamota in grain-related traits under all conditions, indicating its stronger reproductive resilience under salinity. Notably, grains per panicle and grain yield were drastically reduced in Sadamota under salt stress, reflecting its higher sensitivity. These observations are in line with reports suggesting that varietal tolerance plays a crucial role in maintaining reproductive output under saline environments (Chen *et al.*, 2024).

The application of proline significantly mitigated the negative effects of salt stress across all measured traits. Particularly under T_4 (salt stress + proline), BINA dhan23 maintained high grain number and yield, indicating that proline can enhance salt stress tolerance more effectively in stress-tolerant genotypes. In contrast, Sadamota, although superior in straw yield and vegetative vigor, performed poorly in reproductive traits under salt stress. This indicates that vegetative growth does not always correlate with reproductive success under salinity, highlighting the importance of stress-resilient reproductive physiology. The results emphasize that selection of tolerant varieties (like BINA dhan23) combined with foliar proline supplementation can significantly improve yield performance in saline conditions. Grain yield of rice was strongly dependent of the number of grain production per panicle (Fig. 1).

Table 3. Yield and yield components of rice genotypes as influenced by proline spraying under salt stress condition

Treatment combinations	Panicle length (cm)	Grain panicle^{-1} (no.)	Grain yield pot^{-1} (g)	Straw yield pot^{-1} (g)
Single effect of variety				
V1: BINA dhan23	24.7 A	97.8	21.8 A	22.6 B
V_2: Sadamota	20.4 B	30.4	5.58 B	40.8 A
Significance level	***	***	***	***
SE (±)	0.332	1.39	1.24	2.39
Single effect of salinity and proline				
T_1: Control	23.2 A	76.8	17.3 A	35.1 AB
T_2: Proline spraying	23.6 A	77.2	15.5 A	36.6 A
T_3: Salt stress (6 dS m^{-1})	21.5 B	50.1	10.6 B	26.2 C
T_4: Salt stress+Proline spraying	21.9 B	52.2	11.4 B	29.0 BC
Significance level	***	***	**	**
SE (±)	0.470	1.97	1.75	3.37
Variety: treatments interaction				

Treatment combinations	Panicle length (cm)	Grain panicle^{-1} (no.)	Grain yield pot^{-1} (g)	Straw yield pot^{-1} (g)
Significance level	NS	***	NS	NS
SE (±)	0.664	2.79	2.47	2.47
CV (%)	3.61	5.32	12.10	4.77

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According to STAR software, when interaction effect was significant, the lettering was given in interaction table, rather than single effects

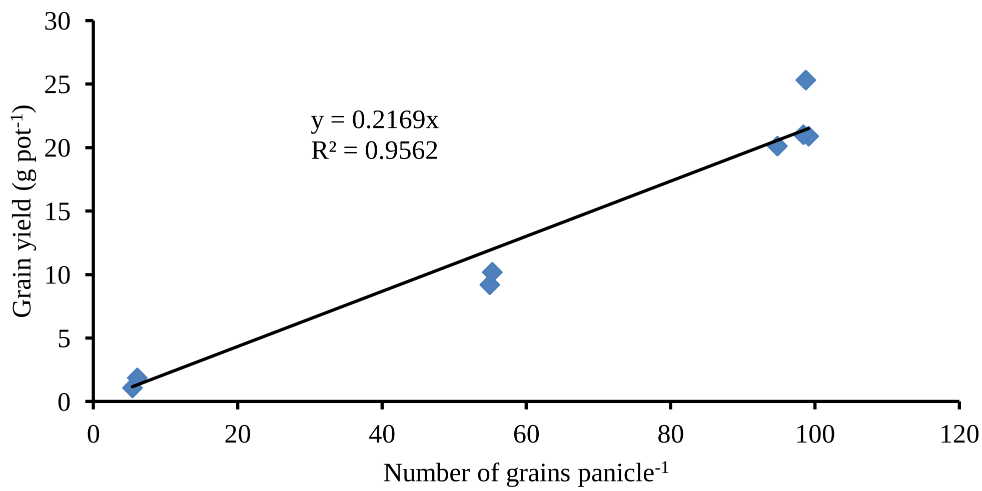

Fig. 1. Regression relation of number of grains panicle^{-1} with grain yield of rice

Effect of proline spraying and salt stress on proline accumulation in rice genotypes

Proline, a key osmoprotectant, accumulated significantly in rice leaves in response to genotypic variation and treatment conditions, as well as their interaction (Table 4). The proline content was significantly higher ($p<0.001$) in Sadamota (91.3 mg 100g^{-1}) compared to BINA dhan23 (61.9 mg 100g^{-1}). Among the treatments lowest proline level was found in control plants at 14.3 mg 100g^{-1}, representing basal metabolic levels. Salt stress alone increased proline to 105.3 mg 100g^{-1}, while proline spraying without stress raised it to only 72.1 mg 100g^{-1}, confirming the effectiveness of foliar proline application. The highest proline accumulation was observed in the combined salt stress and proline spraying treatment at 114.8 mg 100g^{-1}, indicating a synergistic effect where both endogenous synthesis (from salt stress) and exogenous application contribute to proline buildup. A highly significant ($p<0.001$) interaction between genotype and treatment was observed (Table 5). Notably in control conditions, Sadamota accumulated more proline (17.3 mg 100g^{-1}) than BINA dhan23 (11.2 mg 100g^{-1}). Under salt stress alone, both genotypes showed substantial increases, but Sadamota still maintained a slightly higher level (109.7 mg $100g^{-1}$) than BINA dhan23 (100.8 mg $100g^{-1}$). In proline spraying alone, Sadamota showed a dramatic spike in proline (126.3 mg $100g^{-1}$), far exceeding that of BINA dhan23 (17.9 mg $100g^{-1}$), indicating possible differences in foliar absorption or conversion efficiency. Interestingly, under combined salt stress + proline, BINA dhan23 (117.7 mg $100g^{-1}$) slightly exceeded Sadamota (111.9 mg $100g^{-1}$), though the difference was not statistically significant. Several studies also indicated that salt-tolerant rice cultivars accumulated higher proline than salt-sensitive rice under salinity stressed conditions (El-Banna and Mosa, 2024).

Table 4. Proline and total sugar content of rice genotypes as influenced by proline spraying under salt stress condition

Treatment combinations	Proline content mg $100g^{-1}$ fresh leaf ©	Total sugar content mg $100g^{-1}$ fresh leaf
Single effect of variety		
V_1: BINA dhan23	61.9	8.10
V_2: Sadamota	91.3	8.08
Significance level	***	NS
SE (±)	1.01	0.433
Single effect of salinity and proline		
T_1: Control	14.3	7.44 B
T_2: Proline spraying	72.1	7.10 B
T_3: Salt stress (6 ds m^{-1})	105.3	8.75 A
T_4: Salt stress + Proline spraying	114.9	9.06 A
Significance level	**	**
SE (±)	1.43	0.613
Variety:treatments interaction		
Significance level	***	NS
SE (±)	2.03	0.867
CV (%)	3.24	13.14

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Table 5. Interaction effect between variety and treatments on proline content (mg $100g^{-1}$ fresh leaf) of rice

Treatments	Rice varieties	
	BINA dhan23	Sadamota
T_1: Control	11.2 D b	17.3 Ca
T_2: Proline spraying	17.9 C b	126.3 Aa
T_3: Salt stress (6 dS m^{-1})	100.8 Bb	109.7 Ba
T_4: Salt stress+Proline spraying	117.7 Aa	111.9 Bb

Similar capital letter in a column or similar small letter in a row is not significantly different at 5% probability level

Effect of proline spraying and salt stress on total sugar content in rice genotypes

The total sugar content (mg $100g^{-1}$ fresh leaf) in rice plants was analyzed under varying treatments of salinity and proline application (Table 4). The results revealed that genotypic differences between BINA dhan23 and Sadamota were not statistically significant (NS), as both varieties recorded nearly identical total sugar levels (8.10 mg $100g^{-1}$ and 8.08 mg $100g^{-1}$, respectively). In contrast, the effect of salinity and proline treatments on total sugar content was statistically significant, indicating that stress and exogenous treatments played a major role in altering sugar metabolism. Among the treatments, the highest total sugar content was observed in salt stress combined with proline spraying at 9.06 mg $100g^{-1}$, followed by salt stress alone at 8.75 mg $100g^{-1}$. This increase in sugar under saline conditions could be attributed to osmotic adjustment and stress response mechanisms, where sugars act as compatible solutes to protect cellular structures and maintain turgor (Joseph *et al.*, 2018).

Chlorophyll content

Chlorophyll a content in rice leaves varied significantly ($p<0.01$) between the two genotypes studied (Table 6). Sadamota recorded significantly higher chlorophyll a (4.08 mg $100g^{-1}$) compared to BINA dhan23 (3.99 mg $100g^{-1}$), suggesting a genotypic tendency toward higher pigment concentration. However, the effect of salinity and proline treatments on chlorophyll a was not statistically significant, with salt stress slightly reduced chlorophyll a content, while proline spraying under salt stress marginally improved it, but not at a significant level. A notable increase in chlorophyll b was observed in the salt stress with proline spraying treatment (1.10 mg $100g^{-1}$), while salt treatment reduced it to 1.04 mg $100g^{-1}$).

Total chlorophyll content was also not significantly different between genotypes. The treatment effect was, however, highly significant ($p<0.001$). When salt was not imposed, the proline spraying had no significant effect on total chlorophyll content. However, under salt applied condition spraying had a significant effect to increase total chlorophyll content of rice, suggesting positive effect of proline on mitigating salinity stress in rice. This dramatic rise suggests that proline application under salt stress significantly enhanced chlorophyll synthesis or prevented its degradation, possibly due to proline’s role in membrane and pigment stabilization (Pranto *et al.*, 2025).

Table 6. Chlorophyll content of rice genotypes as influenced by proline spraying under salt stress condition

Treatment combinations	Chlorophyll a content (mg 100g^{-1} fresh leaf)	Chlorophyll b content (mg 100g^{-1} fresh leaf)	Total chlorophyll content (mg 100g^{-1} fresh leaf)
Single effect of variety			
V_1: BINA dhan23	4.08 B	1.16	5.24
V_2: Sadamota	3.99 A	1.14	5.13
Significance level	**	NS	NS
SE (±)	0.159	0.065	0.166
Single effect of salinity and proline			
T_1: Control	4.28	1.26 A	5.54 A
T_2: Proline spraying	4.24	1.20 A	5.44 A
T_3: Salt stress (6 dS m^{-1})	3.68	1.04 C	4.71 C
T_4: Salt stress+Proline spraying	3.94	1.10 B	5.04 B
Significance level	NS	***	***
SE (±)	0.225	0.09	0.235
Variety:treatments interaction			
Significance level	NS	NS	NS
SE (±)	0.318	0.13	0.332
CV (%)	9.55	6.34	6.21

Similar capital letter in a column was not significantly different at 5% probability level

Effect of proline spraying and salt stress on potassium (K+) and sodium (Na+) content in rice genotypes

The potassium content of rice plants was significantly influenced by rice genotype, salinity and proline treatments, and their interactions (Table 7). Among the genotypes, BINA dhan23 showed significantly higher K^+ (3.09%) compared to Sadamota (1.25%) under the tested conditions. Regarding treatment effects, the highest mean K^+ was recorded in the proline spraying (T_2) treatment (2.41%), followed by the control (T_1) (2.36%), while the lowest K^+ was observed under salt stress (T_3) (1.94%) and salt stress combined with proline spraying (T_4) (1.97%). The application of proline slightly improved the K^+ under salt stress compared to salt stress alone, although the effect was modest. The significant interaction emphasizing those genotypic responses varied across treatments (Table 8). In the control condition (T_1), BINA dhan23 had the highest K^+ (3.38%) compared to Sadamota (1.33%). Under salt stress (T_3), K^+ dropped substantially in both varieties, but the reduction was more severe in Sadamota. Interestingly, proline spraying under salt stress (T_4) led to a slight improvement in K^+ in BINA dhan23 (2.80%) compared to salt stress alone (2.75%), but the difference was not statistically significant as per letter annotations. These results are consistent with previous findings that potassium plays a key role in osmotic adjustment and salt tolerance, and that tolerant genotypes often maintain higher K^+ levels under salt stress (Haque 2020).

Table 7. Potassium and sodium content of rice genotypes as influenced by proline spraying under salt stress condition

Treatment combinations	K^+ content (%) ©	Na^+ content (%) ©
Single effect of variety		
V1: BINA dhan23	3.09	2.93
V_2: Sadamota	1.25	1.36
Significance level	***	***
SE (±)	0.059	0.053
Single effect of salinity and proline		
T_1: Control	2.36	1.69
T_2: Proline spraying	2.41	1.67
T_3: Salt stress (6 dS m^{-1})	1.94	2.63
T_4: Salt stress+Proline spraying	1.97	2.61
Significance level	***	***
SE (±)	0.084	0.075
Variety:treatments interaction		
Significance level	**	***
SE (±)	0.119	0.106
CV (%)	6.76	6.05

Similar capital letter in a column was not significantly different at 5% probability level

According to STAR software, when interaction effect was significant, the lettering was given in interaction table, rather than single effects

Table 8. Interaction effect between variety and treatments on K^+% of rice

Treatments	Rice varieties	
	BINA dhan23	Sadamota
	Potassium (%)	
T_1: Control	3.38 A a	1.33 A b
T_2: Proline spraying	3.44 A a	1.38 A b
T_3: Salt stress (6 dS m^{-1})	2.75 B a	1.14 A b
T_4: Salt stress+Proline spraying	2.80 B a	1.15 A b
	Sodium	
T_1: Control	2.31 B a	1.07 B b
T_2: Proline spraying	2.23 B a	1.10 B b
T_3: Salt stress (6 dS m^{-1})	3.60 A a	1.66 A b
T_4: Salt stress+Proline spraying	3.60 A a	1.63 A b

Similar capital letter in a column or similar small letter in a row is not significantly different at 5% probability level

The sodium content was also significantly affected by genotype, treatment, and their interaction (Table 7 & 8). BINA dhan23 exhibited a significantly higher Na^+ content (2.93%) than Sadamota (1.36%). Treatment-wise, salt stress and salt stress with proline significantly increased Na^+ (2.63% and 2.61%, respectively), compared to the control and proline alone (1.69% and 1.67%, respectively). Under salt stress (T_3), BINA dhan23 showed a dramatic increase in Na^+ (3.60%) compared to Sadamota (1.66%) (Table 8). Similar patterns were observed in T_4. The consistent pattern of lower Na^+ accumulation in Sadamota may be attributed to lower Na^+ uptake but at the cost of overall reduced growth and ion balance, as indicated by its lower K^+ values. This suggests that while BINA dhan23 accumulates more Na^+, it maintains a better K^+/Na^+ ratio, which may contribute to its higher salt tolerance. Exogenously sprayed proline on rice under salt stress reduces its sodium concentration by inhibiting Na^+ uptake and translocation, which helps to maintain a higher K^+/Na^+ ratio, that contributes to the overall improvement of growth and yield in salt-affected rice plants (Koc *et al.*, 2024).

The K^+/Na^+ ratio is a critical indicator of salt tolerance, as a higher ratio reflects a plant's ability to maintain potassium uptake while restricting sodium accumulation. Under control and proline spraying alone, both genotypes maintained relatively high K^+/Na^+ ratios, with BINA dhan23 consistently outperforming Sadamota (Fig. 2). Salt stress significantly reduced the K^+/Na^+ ratio in both genotypes. However, BINA dhan23 retained a higher ratio (0.76) than Sadamota (0.69), suggesting better ionic regulation under stress. Proline application under salt stress slightly improved the K^+/Na^+ ratio in both genotypes (0.78 and 0.71, respectively), indicating a mild mitigated effect. The graph clearly shows that BINA dhan23 maintains a higher K^+/Na^+ ratio across all treatments. Salt stress sharply reduces this ratio, but proline helps buffer the decline, more noticeably in BINA dhan23. This confirms that BINA dhan23 is more salt-tolerant, and proline can moderately support ionic balance under stress.

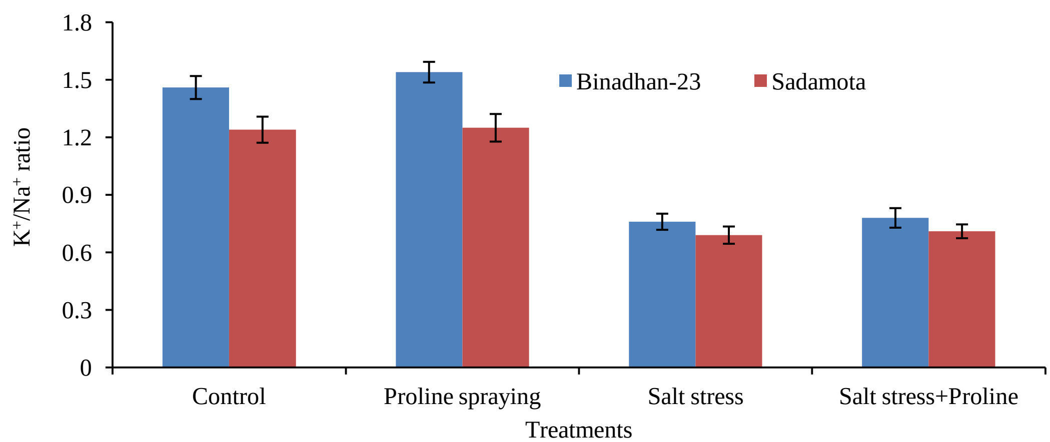

Fig. 2. K^+/Na^+ Ratio under different treatments of rice genotype Single and interaction effects were significant at 0.10% probability level

Conclusion

The findings of this study demonstrate that exogenous proline application can significantly mitigate the adverse effects of salt stress on rice growth and yield. Both BINA dhan23 and Sadamota responded positively to proline application under saline conditions, with improvements observed in key agronomic traits such as plant height, leaf development, grain number, and yield. Notably, proline application enhanced the accumulation of proline and helped maintain chlorophyll and ion balance, contributing to better stress tolerance. BINA dhan23 performed better in terms of grain yield and potassium retention, whereas Sadamota showed superior vegetative growth and proline accumulation under stress. These varietal differences suggest that proline's effectiveness may vary depending on genetic background. Overall, the results support the use of exogenous proline as a potential strategy to improve rice resilience in salt-affected areas. However, the main limitation of the current research; it was done in pot culture condition with only one induced salinity level (6 dS m^{-1}). The findings of the study are suggested to validate in field conditions under varying degree of salinity.

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Conflicts of interest

The authors declare no conflicts of interest regarding publication of this paper.

Authors' contribution

Study conception, research program formulation, materials supply, statistical analysis of data, tables and graph preparation, manuscript writing and editing, and research project funding was accomplished by Dilruba Easmin Jharna. Field activities, chemical analysis, data collection and manuscript writing were done by Md Mobarak Hossain. Fazla Rabbi helped in chemical analysis and data collection. All authors read and approved the final manuscript.

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