Original Research Article

Mitigation of salinity stress by application of plant growth promoting substances in rice

ABSTRACT

Aim: Salt stress adversely affects plant growth and development. Various mitigating strategies have been employed to enhance the adaptability of plants to salt stress. The present study was conducted with the objective of evaluating the recently developed CO55 rice variety's ability to withstand salt-induced stress during seedling growth. This evaluation included foliar spraying of plant growth promoting substances, along with the identification of effective plant growth-promoting substance that exhibit tolerance to salinity.
Study design: Completely randomized design.
Place and Duration of Study: Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore; March-April 2023.
Methodology: In the hydroponics experiment, the CO55 rice variety was subjected to foliar spraying of various plant growth promoting substances such as melatonin, salicylic acid, orthosilicic acid, and sodium selenate. Parameters like leaf drying score, osmotic potential, osmotic adjustment, sodium content, potassium content, Na^{+}/K^{+} ratio were assessed. One-way ANOVA were used to analyse the data.
Results: Specific pairwise differences between means were assessed at the 0.05 significance level using Fisher's least significant difference (LSD) test. Among the treatments applied, salicylic acid recorded the highest potassium content (3.94%), followed by the sodium selenate (3.59%).On the other hand, from the standard evaluation score, it was observed that CO55 rice variety seedlings was found to be tolerant when treated with salicylic acid, whereas it was susceptible under orthosilicic acid treatment.
Conclusion: Observations indicate that the treatment with salicylic acid at the concentration of 50µM and 100µM exhibited greater tolerance towards salinity during the seedling growth stages.

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Keywords: Rice, salinity stress, plant growth-promoting substances, salt stress mitigation, stress tolerance, CO55 variety.

1. INTRODUCTION

Rice (*Oryza sativa L.*) is a staple food crop that feeds more than half of the world's population, the demand for rice is expected to increase than any other crops [1]. It plays crucial role for ensuring food security for a global population [2]. The decline in rice yield coupled with the increasing population has serious concerns among rice-cultivating countries [3]. Salinity stress significantly affects global rice productivity [4]. It is an emerging threat to agriculture, causing declines in crop production [5]. Globally, high salinity impacts approximately one-third of irrigated agriculture and one-fifth of arable land. About 20% of agricultural areas and 50% of irrigated land are affected by high salt levels [6]. By 2050, over 50% of the world's arable land could be affected by salinization. Currently, more than 6% of arable land in worldwide is at risk due to excessive salinity [7]. Rice yield is negatively impacted when the electrical conductivity of irrigation water exceeds 32.8 mM NaCl [8].

Rice is sensitive to salt, particularly during its early growth stages, with the seedling stage being more vulnerable than tillering stage. Rice adopts two primary strategies to overcome salinity are ion exclusion and osmotic tolerance [8]. Ion exclusion depends on root pathways controlling Na^+ and Cl^- uptake, preventing excessive leaf accumulation. Sodium is expelled from xylem, and ions are pumped back into the soil. Osmotic tolerance enables the plant to withstand water scarcity associated with salt stress while maintaining leaf growth [9]. Scarcity of water triggers osmotic stress, leading to reduced nutrient transport efficiency, resulting in deficiencies, and leading to ionic toxicity [10]. The presence of excessive salinity in the soil poses a significant challenge to plant growth due to osmotic stress, ionic stress, and hormonal imbalances [11]. Ionic stress arises from an excessive accumulation of salt within the plant cells, while osmotic stress results from an oversupply of salt in the soil [12].The complexity of these stress factors gives rise to the intricate salt tolerance system in plants. Utilization of plant growth-promoting substances has shown promise in enhancing plant stress tolerance. Although it is not universally required by all plants it has a positive impact on plant growth and enhances their ability to cope with various biotic and abiotic challenges [13].

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Melatonin (MT) is a naturally occurring compound initially discovered in plants in 1995. It is recognized for its potential in mitigating salinity stress effects on crops, including rice [14]. As a growth promoter, melatonin regulates plant growth and mitigates impacts of both abiotic and biotic factors. These include salt stress, as well as drought, cold, high temperatures, darkness, and heat-induced leaf senescence. In the context of rice, applying melatonin externally has shown the ability to increase yields by 4-13%, and also it aids in enhancing the potassium-sodium ratio (K^+/Na^+), signifying improved potassium uptake and sodium exclusion [15].

Salicylic acid (SA), an endogenous growth regulator, is a key phenol compound discovered in 2003. Apart from its physiological regulation role [16], salicylic acid defends against biotic and abiotic stress like salt stress. It also aids germination under stress condition. During salt stress, salicylic acid lowers cellular Na^+ and Cl^- levels. Applying salicylic acid during vegetative and germination stages restores stress-free conditions. However salicylic acid application during reproductive stage do not boost morphological traits or yield [17].

Selenium (Se), a rare element dispersed in the earth's crust, is essential for humans, animals, and certain microorganisms. Genetic variations influencing selenium uptake and transport in plants significantly affect their absorption capacity. Exogenous selenium supplementation benefits crop growth under stress, promoting osmoprotectant synthesis [18] and activating detoxification processes [19].

Silicon (Si) constitutes around 27.8% - 32% of earth's crust, the second most abundant element after oxygen (46%). It is mainly in the form of quartz (SiO_2), abundant in sands. The plants can't use SiO_2 directly [20]. They absorb silicon as monosilicic acid (H_4SiO_4), known as orthosilicic acid (OSA). Silixol, a steady orthosilicic acid form, is a common silicon fertilizer enhancing crop growth [21]. It mitigates salinity stress impact, evident across crops like rice, barley, wheat, sorghum, cucumber, and tomato [22]. Moreover, silicon regulates phytohormone levels, aiding plants in tolerating salt-induced stress. This study aims to explore the effects of growth promoters like melatonin, salicylic acid, silicon, and selenium on rice growth in saline conditions during seedling growth.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The seeds were soaked in distilled water and then wrapped in cotton cloth for a day in a dark environment. The sprouted seeds were placed into a hydroponic system containing Yoshida nutrient solution. This system was set up on a thermacoal sheet covered with nylon mesh. The nutrient concentrations in the solution were listed in Table 1. The solution was changed every four days during the experiment. In this study, T_1 represented the treatment where no changes were applied, serving as the absolute control. T_2 involved the control group that was exposed to salt stress at a concentration of 100mM. T_3 and T_4 were treated 50µM and 100µM of melatonin. T_5 and T_6 were treated with different concentrations of salicylic acid viz., 50µM and 100µM, respectively. T_7 and T_8 were subjected to 0.15% and 0.25% of orthosilicic acid respectively. T_9 and T_{10} were treated with varying concentrations of sodium selenate which are 3ppm and 6ppm respectively. Salt stress at a concentration of 100mM NaCl was applied to seedlings (20-days old seedlings) in the Yoshida nutrient solution, as shown in Figure 1. Each treatment was replicated three times. The treatments were imposed as foliar sprays, facilitating absorption through the leaves of the plants.

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Table 1. Components of Yoshida nutrient solution [23]

Stock no	Reagent (AR grade)	Preparation g/L	Concentration of stock/L of nutrient solution (ml)
Stock I	NH_4NO_3	91.4	1.25
Stock II	$NaH_2PO_4.2H_2O$	35.6	12.5
Stock III	K_2SO_4	71.4	1.25
Stock IV	$CaCl_2.2H_2O$	117.35	1.25
Stock V	$MgSO_4.7H_2O$	324	1.25
Stock VI	$MnCl_2.2H_2O$	1.5	1.25
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.074	
	$ZnSO_4.7H_2O$	0.035	
	H_3BO_3	0.934	
	$CuSO_4.5H_2O$	0.031	
	$FeCl_3.6H_2O$	7.7	
	$C_6H_8O_7,H_2O$	11.9	

2.1. Preparation of the Yoshida nutrient solution for hydroponics

The Yoshida nutrient solution employed both macronutrients and micronutrients, as detailed in Table 1. Each macronutrient was dissolved separately and stored in an amber-colored bottle. Micronutrients were dissolved in 50ml of distilled water, mixed using a magnetic stirrer, and the volume was adjusted to 1000ml before being stored in an amber-colored bottle. The mixture was dissolved using distilled water with a pH of less than 5.5. For this experiment, a tray measuring 58 cm in length and 38.5 cm in width was used. The nutrient solution's pH was maintained between 4.5 and 5.3. It was monitored daily with a portable pH meter, and pH adjustments could be made using either HCl or NaOH.

2.2 Observations recorded

2.2.1. Visual Salt Injury Score (Standard evaluation system) (SES-IRRI)

Visual salt injury scores were assessed using the IRRI protocol [24]. The standard evaluation system with scores ranging from 1 to 9 was employed. Scores were assigned based on the levels of leaf drying observed upon the application of the treatments in the CO55 rice variety. Leaf drying commenced three days after the initiation of stress. The initial symptom was a white color appearing on the leaf tips, gradually spreading throughout the plants. Regular visual observations were conducted for the applied treatments and scored using the standard evaluation system. Symptoms and their corresponding scores are presented in Table 2 below.

Table 2. Standard evaluation system (SES-IIRI, 1997)

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

2.2.2. Osmotic potential (-Mpa) and Osmotic Adjustment (Mpa)

Fresh leaf samples were collected and immediately frozen using liquid nitrogen. Prior to collection, holes were made in the bottom of Eppendorf tubes. After freezing, the sample was centrifuged with an Eppendorf tube placed at the bottom. Sap from the leaf samples was collected for Osmolality measurement (mmol/kg) using a Vapour Pressure Osmometer (Vapro Model 5520; Wescor Inc., Logan, UT, USA). Osmotic potential was calculated using the appropriate formula..

$$\Psi s=-Crt$$

Where

C=Concentration
R=Universal gas constant (0.0832)
T=Temperature in Kelvin (310°K)
Osmotic potential in Mpa was calculated using formula

$$\text{Osmotic potential}=\frac{\text{Osmolality}\left(\frac{\text{mmol}}{\text{kg}}\right)(0.0832)(310)}{10000}$$

Osmotic adjustment was calculated by subtracting osmotic potential of stressed plant from control [25].

Osmotic Adjustment (Mpa) = Osmotic potential (control) - Osmotic potential (stress).

2.2.3. Sodium and Potassium content (%) and Na^+/K^+ ratio

Sodium and potassium estimated by the method suggested by [26]. Triacid mixture used to digest the sample contains Nitric acid: Sulphuric acid: Perchloric acid (9:2:1). Samples were dried at 50°C for 3 days, powdered, and 0.5 g was used for acid digestion. These samples were placed within 100ml conical flasks and treated with 15 ml of a triacid mixture. Digestion occurred in a chamber until the solution became colorless. The digested samples were cooled, diluted with distilled water, and filtered through Whatman number 1 filter paper, adjusting the volume to 100 ml. Diluted samples were neutralized using a 1:4 ammonium hydroxide solution. The content was subsequently analyzed using an Atomic Absorption Spectrometer (AAS).

$$\text{Sodium potassium } (Na^+/K^+) \text{ ratio} = \frac{\text{Sodium content (\%)}}{\text{Potassium content (\%)}}$$

2.3 Statistical Analysis

The design of the experiment was completely randomized design with three replications. Specific pairwise differences between means were evaluated at the 0.05 significance level using the Fisher's least significant difference (LSD) test.

3. RESULTS AND DISCUSSION

3.1. Visual Salt Injury Score (Standard evaluation system) (SES-IRRI)

The visual salt injury score developed by IRRI in 2013 helped to identify the salt tolerance capacity of CO55 rice seedlings under foliar spray with ten different treatments, as presented in table 3. Rice seedlings that were foliar-sprayed with 50µM and 100µM of salicylic acid, followed by 6ppm and 3ppm of sodium selenate, were found to be tolerant. Seedlings foliar-sprayed with 50µM and 100µM of melatonin exhibited moderate tolerance, while seedlings treated with 0.15% and 0.25% of orthosilicic acid showed similar susceptibility compared to the control. A lower score indicated a lesser visual impact under salt stress, whereas a higher score suggested significant salt injury. In this study, seedlings that were treated with 50µM and 100µM of salicylic acid scored 3 under 100mM salinity, indicating their tolerance at the 100mM salinity level. The effects of salinity (100mM) on plant growth-promoting substances such as melatonin, salicylic acid, orthosilicic acid, and sodium selenate were presented in figures 2 to 5.

Table 3. Effect of Plant growth promoting substances on standard evaluation system of rice seedlings under salt stress (100mM)

Treatments	SES Score
T_1: Absolute control	1
T_2: Control (salt stress)	7
T_3: 50µM of melatonin	5
T_4: 100µM of melatonin	5
T_5: 50µM of salicyclic acid	3
T_6: 100µM of salicyclic acid	3
T_7: 0.15% of orthosilicic acid	7
T_8: 0.25% of orthosilicic acid	7
T_9: 3ppm of sodium Selenate	3
T_{10}: 6ppm of sodium Selenate	3
Mean	4.4

Fig. 1. CO 55 (20 day old) rice seedlings that had been grown in the Yoshidha nutrient solution

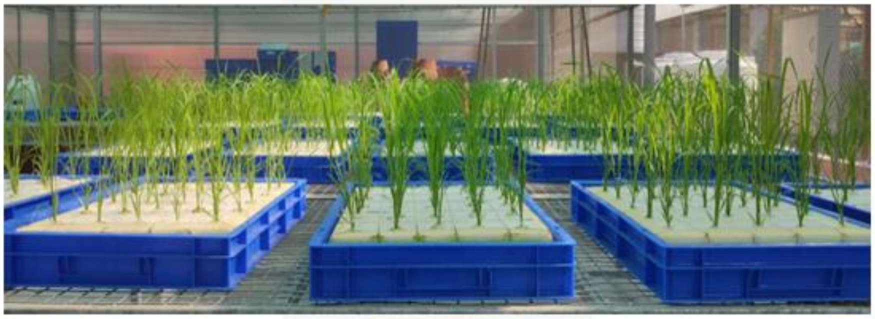

Fig. 2. Effect of salinity (100mM NaCl) and melatonin (50µM, 100µM) on rice seedlings

Fig. 3. Effect of salinity (100mM NaCl) and salicylic acid (50µM, 100µM) on rice seedlings

Fig. 4. Effect of salinity (100mM NaCl) and orthosilicic acid (0.15%, 0.25%) on CO55 rice seedlings

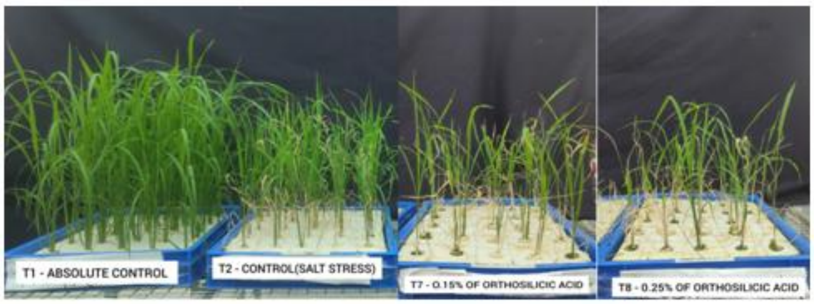

Fig. 5. Effect of salinity (100mM NaCl) and sodium selenate (3ppm, 6ppm) on CO55 rice seedlings

<u>3.2. Osmotic potential (-Mpa) and Osmotic Adjustment (Mpa)</u>

The effect of plant growth stimulating substances on osmotic potential of rice was given in the figure 6. A significant difference ($P<0.05$) in osmotic potential of rice was observed within the treatments. In general, the plants subjected to salt stress showed more negative osmotic potential values than the absolute control. Among the treatments, the plants applied with 50µM of salicylic acid (-2.38 Mpa) exhibited more negative osmotic potential which was found on par with 6ppm of sodium selenate (-2.36 Mpa) compared to the salt stress plants (-1.74 Mpa).

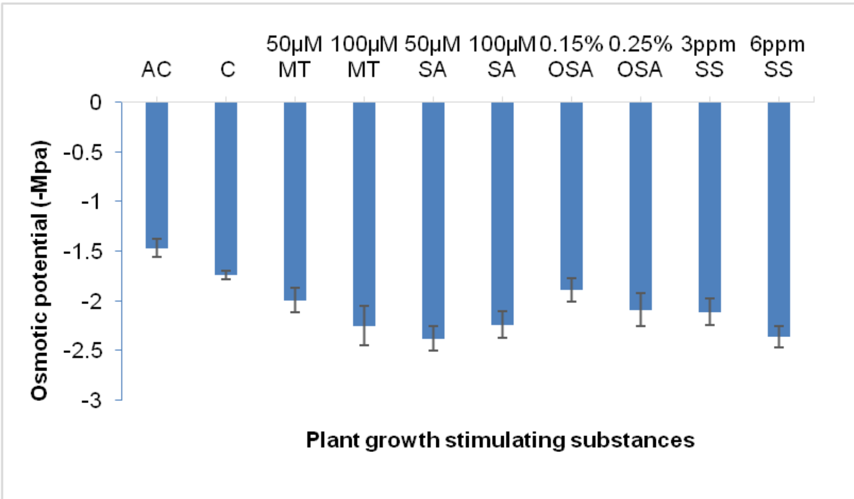

Fig.6. Effect of Plant growth promoting substances on osmotic potential (-Mpa) of rice seedlings under salt stress (100mM)

AC- Absolute Control, C=Control, MT=Melatonin, SA=Salicyclic acid, OSA=Orthosilicic acid, SS=Sodium Selenate.

Statistical analysis of plant growth promoting substances in rice plant revealed that mean data of osmotic adjustment were found significantly different ($P<0.05$) among the treatments (Table 4). Foliar application of 50µM of salicylic acid (0.91 Mpa) recorded increased osmotic adjustment that was found statistically on par with 6 ppm of sodium selenate (0.89 Mpa). However, the plant exposed to salt stress (0.27 Mpa) alone showed decreased osmotic adjustment. Osmotic potential influences the ability of the plants to withstand salt stress and helps to maintain cellular turgidity. In the present study, foliar application of 100 µM salicylic acid led to a reduction of 28.7% in osmotic potential when compared to plants subjected to salt stress. These findings are supported by [27], who reported that applying 100 µM salicylic acid to tomato plants alleviated salt stress (250 mM) by regulating osmotic potential. Osmotic adjustment helped the plants to maintain proper osmotic balance, ultimately protecting membranes from damage caused by salinity stress. Findings of [28], also stated that exogenous application of salicylic acid plays a role in maintaining osmotic balance and protecting the plasma membrane by modulating ion accumulation, including Na^+, K^+, and Ca^{2+}.

Table 4. Effect of Plant growth promoting substances on osmotic adjustment (Mpa) of rice seedlings under salt stress (100mM)

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Treatments	**Osmotic adjustment (Mpa)**
T_1: Absolute control	-
T_2: Control (salt stress)	0.42
T_3: 50µM of melatonin	0.52

T_4: 100µM of melatonin	0.78
T_5: 50µM of salicyclic acid	0.91
T_6: 100µM of salicyclic acid	0.77
T_7: 0.15% of orthosilicic acid	0.27
T_8: 0.25% of orthosilicic acid	0.62
T_9: 3ppm of sodium Selenate	0.64
T_{10}: 6ppm of sodium Selenate	0.89
Mean	0.65
SEd	0.14
CD(p<0.05)	0.28

SEd = Standard Error Difference; CD = Critical Difference

3.3. Sodium and Potassium content (%) and Na^+/K^+ ratio

A significant variation (P<0.05) were observed between the treatments for leaf sodium content and it was represented in figure 7. In leaf, an increased level of sodium content was noticed in control treatment (9.08%) followed by 0.15% of orthosilicic acid (8.67%) and decreased level of leaf sodium content was observed in 100µM of salicylic acid (1.64%). In rice among different treatments of plant growth promoting substances, a significant (P<0.05) highest leaf potassium content were observed in 100µM of salicylic acid (3.94%) followed by 6ppm of sodium selenate (3.59%). However significant (P<0.05) lowest leaf potassium content was recorded in 0.15% of orthosilicic acid (2.60%) which was statistically on par with control treatment (2.49%) and it was presented in figure 8.

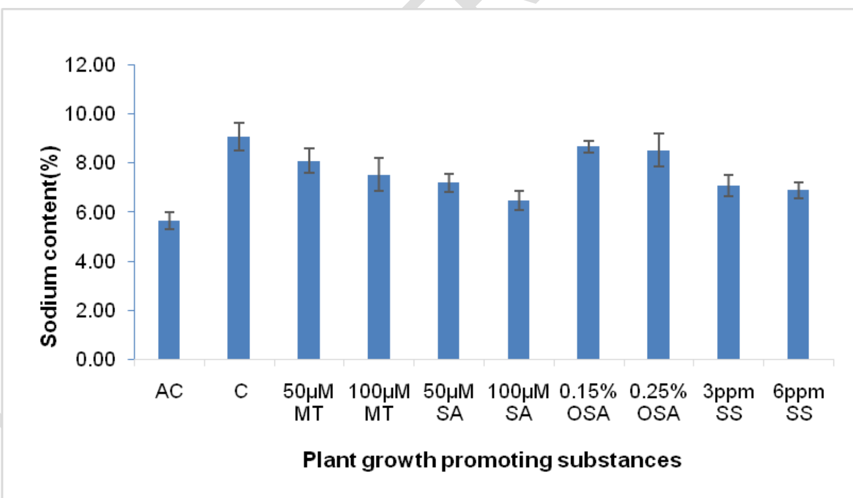

Fig. 7. Effect of Plant growth promoting substances on sodium content (%) of CO55 rice seedlings under salt stress (100mM)

AC- Absolute Control, C=Control, MT=Melatonin, SA=Salicyclic acid, OSA=Orthosilicic acid, SS=Sodium Selenate.

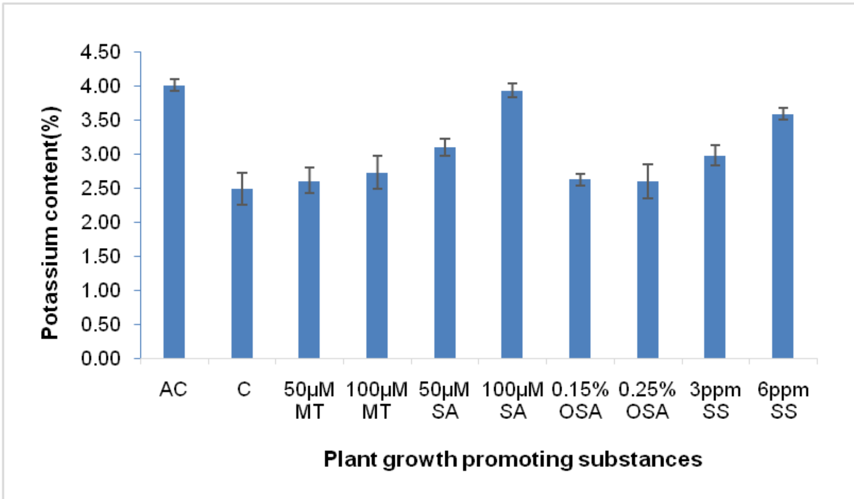

Fig. 8. Effect of Plant growth promoting substances on potassium content (%) of rice seedlings under salt stress (100mM)

AC- Absolute Control, C=Control, MT=Melatonin, SA=Salicyclic acid, OSA=Orthosilicic acid, SS=Sodium Selenate.

Sodium potassium ratio of rice leaf was presented in table 5. A significant difference (P<0.05) were found among the different treatments of plant growth promoting substances. The plants exposed to salt stress alone (3.65%) recorded increased sodium potassium ratio followed by 0.15% orthosilicic acid treated plants (3.30%). Whereas, the plant treated with 100µM of salicylic acid (1.64%) and 6ppm of sodium selenate (1.92%) recored decreased content of sodium potassium ratio in rice leaf. The sodium-potassium ratio undergoes significant changes in response to salt stress in plants, affecting their growth and survival [29]. In our study, we observed a notable reduction of 47.09% in leaf sodium potassium ratio in plants treated with 100 µM of salicylic acid compared to the control. Similar findings were reported by [27], found that applying 100 µM salicylic acid as a foliar spray on tomato plants effectively alleviated the reduction in sodium content caused by salt stress (250 mM), effectively regulating ion homeostasis. This reduction in excessive Na^+ accumulation is vital due to the competition between sodium (Na^+) and potassium (K^+) for essential metabolic processes, leading to alterations in the K^+/Na^+ ratio. Our results are confirmatory with [30], who observed that salicylic acid enhances H^+-ATPase activity in roots, prevents salt-induced potassium leakage from roots, and increases shoot potassium concentration during salt and oxidative stresses.

Table 5. Effect of Plant growth promoting substances on Na^+/K^+ Ratio (%) of rice seedlings under salt stress (100mM)

Comment [S9]: table do not cut

Treatments	Na^+/K^+ Ratio
T_1: Absolute control	1.41
T_2: Control (salt stress)	3.65
T_3: 50µM of melatonin	3.10

T_4: 100µM of melatonin	2.76
T_5: 50µM of salicyclic acid	2.32
T_6: 100µM of salicyclic acid	1.64
T_7: 0.15% of orthosilicic acid	3.30
T_8: 0.25% of orthosilicic acid	3.28
T_9: 3ppm of sodium Selenate	2.38
T_{10}: 6ppm of sodium Selenate	1.92
Mean	2.58
SEd	0.14
CD(p<0.05)	0.29

SEd = Standard Error Difference; CD = Critical Difference

4. CONCLUSION

In this hydroponic study, the foliar application of treatments containing various plant growth-promoting substances on CO55 rice variety seedlings under saline condition (100mM) was carried out. Among these treatments, foliar sprays containing 50 µM and 100 µM of salicylic acid, as well as 6 ppm and 3ppm of sodium selenate, exhibited remarkable effectiveness in alleviating salt-induced stress. This effectiveness was apparent through both visual assessments and physiological characteristics, as they achieved a lower Na^+/K^+ ratio and reduced osmotic potential. The study concluded that salt stress substantially altered physiological parameters. The exogenous applications involving 50 µM and 100 µM of salicylic acid, 6 ppm and 3 ppm of sodium selenate, successfully countered the impact of salinity stress. This resulted in notable enhancements in plant growth and developmental processes.

Comment [S10]: better complete with data from the result

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