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Mitigation of Salinity Stress by Application of Plant Growth Promoting Substances in Rice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 like melatonin, salicylic acid, silicon, and selenium, along with

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the identification of effective plant growth-promoting substances that exhibit tolerance to salinity. This study is required to develop salt-tolerant varieties capable of withstanding salinity stress during the seedling stage. The seedling stage is more susceptible to salinity, and also to increase the growth and yield of rice, thereby satisfying the country's agricultural needs.

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, and Na^+/K^+ ratio were assessed. One-way ANOVA was used to analyze 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%), and the lowest potassium content (2.60%) was found in orthosilicic acid. On the other hand, from the standard evaluation score, it was observed that CO55 rice variety seedlings were found to be tolerant when treated with salicylic acid, whereas it was susceptible under orthosilicic acid treatment.

Conclusion: Observations indicated that foliar application of salicylic acid at the concentrations of 50µM and 100µM exhibited higher tolerance towards salinity during the seedling growth stages.

Keywords: Rice; salinity stress; plant growth-promoting substances; Na^+/K^+ ratio.

1. INTRODUCTION

Rice (*Oryza sativa L.*) is a staple food crop that feeds more than half of the world's population, and the demand for rice is expected to increase more than any other crop [1]. It plays a 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 the tillering stage. Rice adopts two primary strategies to overcome salinity are ion exclusion and osmotic tolerance [8]. Ion exclusion predominantly depends on specialized root transport systems that control the intake of sodium (Na^+) and chloride (Cl^-) to avoid their excessive accumulation in the leaves. This mechanism involves removing sodium from the xylem and actively returning ions to the soil through specific pumps and channels [9]. Sodium is expelled from the 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]. Salt stress, due to ion accumulation (Na^+ and K^+), causes ionic toxicity, osmotic stress, ion imbalances, and reduced water potential. This significantly impacts plant physiological, morphology, and biochemical processes, ultimately affecting plant development and metabolism [13]. Elevated soil sodium levels restrict the ability of plants to absorb water and take up essential nutrients [14]. 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 [15].

Melatonin (MT) is a naturally occurring compound initially discovered in plants in 1995. It is recognized for its potential to mitigate salinity stress effects on crops, including rice [16]. 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 [17].

Salicylic acid (SA), an endogenous growth regulator, is a key phenol compound discovered in 2003. Apart from its physiological regulation role [18], salicylic acid defends against biotic and abiotic stress like salt stress. It also aids germination under stress conditions. 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 the reproductive stage does not boost morphological traits or yield [19].

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 [20] and activating detoxification processes [21].

Silicon (Si) constitutes around 27.8% - 32% of the 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 [22]. 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 that enhances crop growth [23]. It mitigates salinity stress impact, evident across crops like rice, barley, wheat, sorghum, cucumber, and tomato [24]. 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. MATERIALS AND METHODS

This study was carried out during March-April 2023 in the glasshouse of the Department of Crop Physiology, Coimbatore. 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 in the 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 are listed in Table 1. The Yoshida 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 100 mM. T_3 and T_4 were treated with 50 µM and 100 µM of melatonin. T_5 and T_6 were treated with different concentrations of salicylic acid *viz.,* 50 µM and 100 µM, 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 100 mM NaCl was applied to seedlings (20-day-old seedlings) in the Yoshida nutrient solution, as shown in Fig. 1. Each treatment was replicated three times. The treatments were imposed as foliar sprays, facilitating absorption through the leaves of the plants [25].

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 50 ml of distilled water, and mixed using a magnetic stirrer, and the volume was adjusted to 1000 ml 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

Table 1. Components of Yoshida nutrient solution [26]

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	

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 Observations Recorded

2.2.1 Visual salt injury score (Standard evaluation system) (SES-IRRI)

Visual salt injury scores were assessed using the IRRI protocol [27]. 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.

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). The 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 the formula

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

Osmotic adjustment was calculated by subtracting the osmotic potential of the stressed plant from control [28].

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

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

Sodium and potassium are estimated by the method suggested by [29]. The 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 100 ml 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).

Sodium potassium (Na^+/K^+) 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 100 mM salinity level. The effects of salinity (100 mM) on plant growth-promoting substances such as melatonin, salicylic acid, orthosilicic acid, and sodium selenate are presented in Figs. 2 to 5.

3.2 Osmotic Potential (-Mpa) and Osmotic Adjustment (Mpa)

The effect of plant growth promoting substances on the osmotic potential of rice is given in Fig. 6. A significant difference ($P<0.05$) in the 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).

Statistical analysis of plant growth promoting substances in rice plants 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, thereby it protects the cell membrane from damage through regulation of osmotic adjustment. In the present study, foliar application of 100 µM salicylic acid led to a reduction of 28.7% osmotic potential when compared to plants subjected to salt stress. These findings are supported by [30], who reported that applying 100 µM salicylic acid to tomato plants alleviates the salt stress by regulating osmotic potential. Findings of [31], also stated that exogenous application of salicylic acid plays a role in maintaining osmotic balance and protects the plasma membrane by modulating ion accumulation, including Na^+, K^+, and Ca^{2+}.

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

A significant variation ($P<0.05$) was observed between the treatments for leaf sodium content and it was represented in Fig. 7. In the leaf, an increased level of sodium content was noticed in the control treatment (9.08%) followed by 0.15% of orthosilicic acid (8.67%), and a 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 was 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 Fig. 8.

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: 50 µM of melatonin	5
T_4: 100 µM of melatonin	5
T_5: 50 µM of salicylic acid	3
T_6: 100 µM of salicylic 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

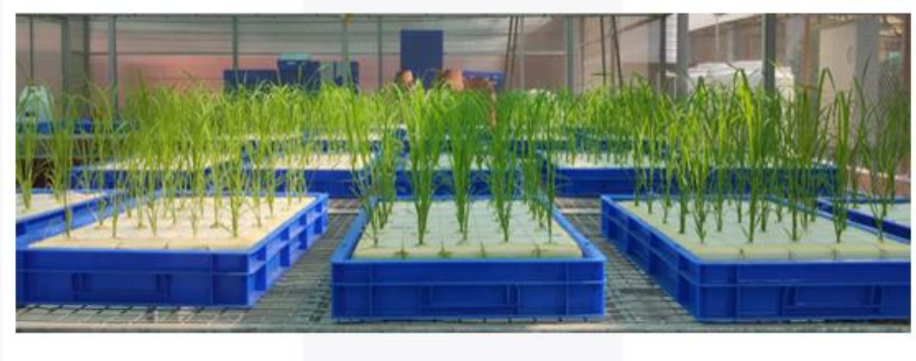

Fig. 1. Rice seedlings (20 days old) grown in the Yoshidha nutrient solution

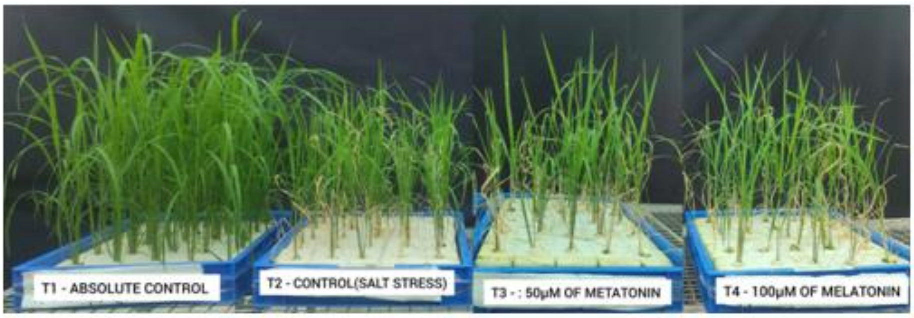

Fig. 2. Effect of melatonin (50µM, 100µM) on rice seedlings under salinity stress

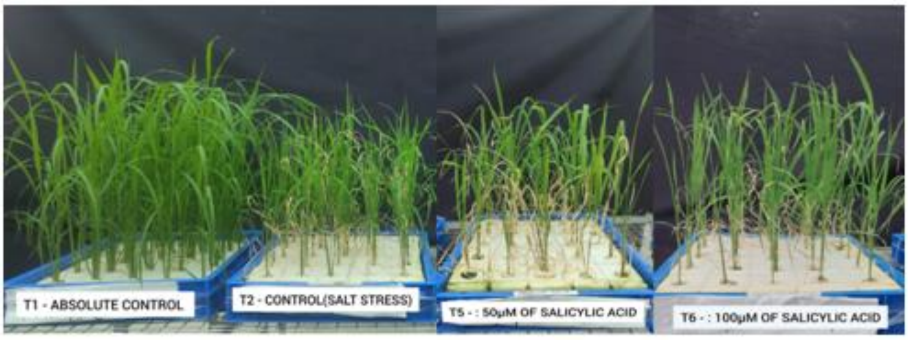

Fig. 3. Effect of salicylic acid (50µM, 100µM) on rice seedlings under salinity stress

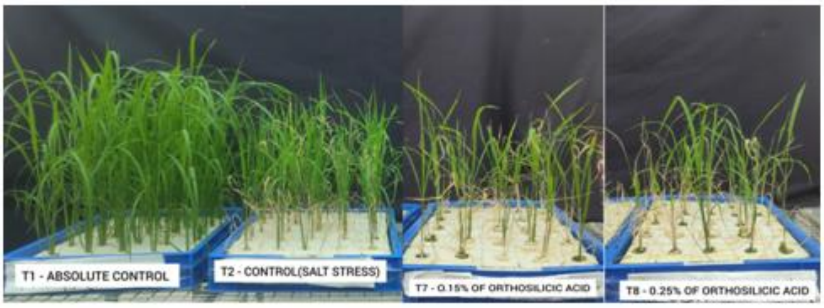

Fig. 4. Effect of orthosilicic acid (0.15%, 0.25%) on rice seedlings under salinity stress

Fig. 5. Effect of sodium selenate (3ppm, 6ppm) on rice seedlings under salinity stress

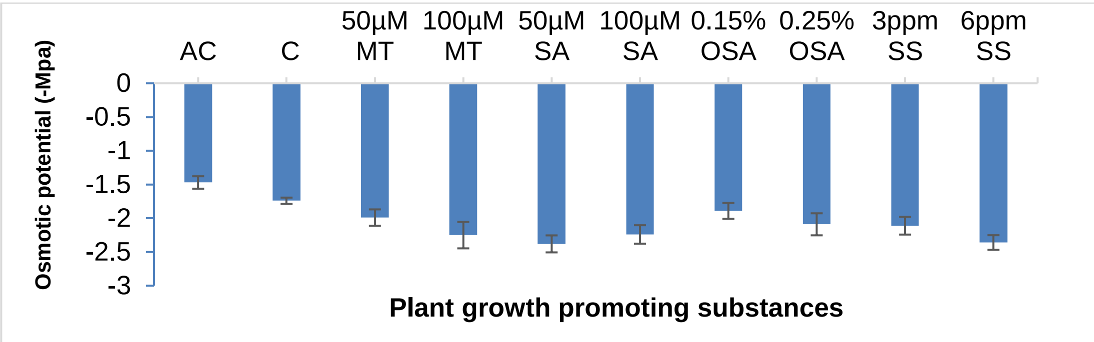

Fig. 6. Effect of Plant growth promoting substances on osmotic potential (-Mpa) of rice seedlings under salt stress (100mM)

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

Table 4. Effect of Plant growth promoting substances on osmotic adjustment (Mpa) and Na^+/K^+ ratio of rice seedlings under salt stress (100mM)

Treatments	Osmotic adjustment (Mpa)	Na^+/K^+ Ratio
T_1: Absolute control	-	1.41
T_2: Control (salt stress)	0.42	3.65
T_3: 50 µM of melatonin	0.52	3.10
T_4: 100 µM of melatonin	0.78	2.76
T_5: 50 µM of salicylic acid	0.91	2.32
T_6: 100 µM of salicylic acid	0.77	1.64
T_7: 0.15% of orthosilicic acid	0.27	3.30
T_8: 0.25% of orthosilicic acid	0.62	3.28
T_9: 3ppm of sodium Selenate	0.64	2.38
T_{10}: 6ppm of sodium Selenate	0.89	1.92
Mean	0.65	2.58
SEd	0.14	0.14
CD(p<0.05)	0.28	0.29

SEd = Standard Error Difference; CD = Critical Difference

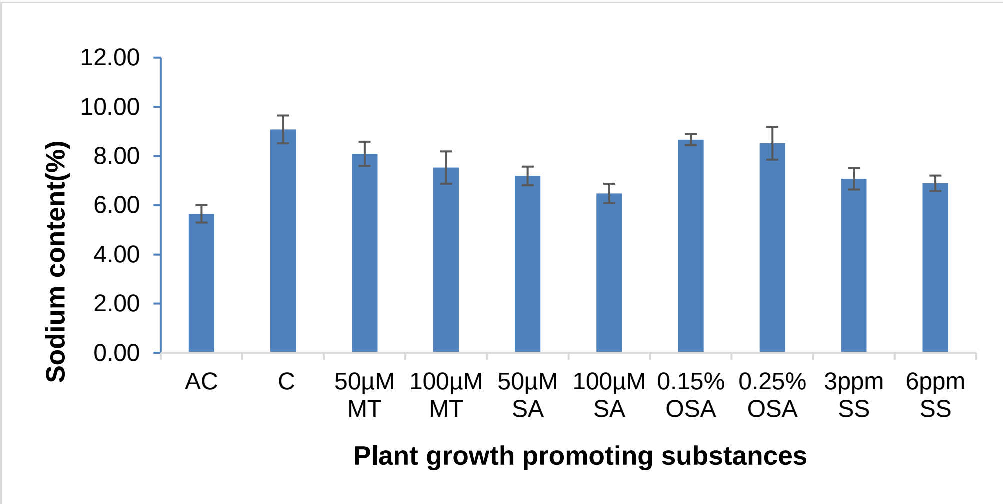

Fig. 7. Effect of Plant growth promoting substances on sodium content (%) of CO55 rice seedlings under salt stress (100mM)

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

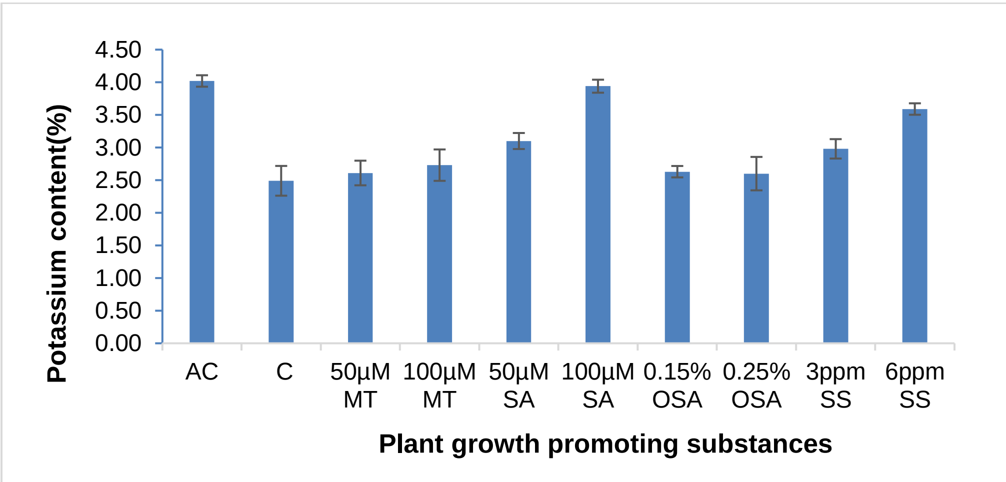

Fig. 8. Effect of Plant growth promoting substances on potassium content (%) of rice seedlings under salt stress (100mM)

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

The sodium potassium ratio of rice leaf is presented in Table 4. A significant difference ($P<0.05$) was found among the different treatments of plant growth promoting substances. The plants exposed to salt stress alone (3.65%) recorded an 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%) recorded decreased content of sodium potassium ratio in rice leaf. The sodium-potassium ratio undergoes significant changes in response to salt stress that affects plant growth and survival [32]. In this study, a notable reduction of 47.09% in leaf sodium potassium ratio was observed in the plants in plants treated with 100 µM of salicylic acid compared to the control plants. Similar findings were reported by [30], under salt stress that foliar application of 100 µM salicylic acid effectively reduced the accumulation of sodium content in the leaf of the tomato plant. This reduction of Na^+ accumulation is due to the competition between sodium (Na^+) and potassium (K^+) ions which is essential for various metabolic processes. Our results are confirmatory with [33], 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.

4. CONCLUSION

In this hydroponic study, the foliar application of treatments containing various plant growth-promoting substances on CO55 rice variety seedlings under saline conditions (100 mM) was carried out. Among these treatments, foliar spray containing 50 µM and 100 µM of salicylic acid, as well as 6 ppm and 3 ppm 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 with a value of 1.64% and osmotic potential was reduced by 28.7% when compared with salt-stressed plants. 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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