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COMMUNICATION

Exogenous Tyrosine Priming Enhances Salt Stress Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

Salt stress is a key abiotic stressor that contributes to reduced global rice production, particularly in salt-sensitive varieties such as Oryza sativa. This study aimed to assess the possibility of exogenous tyrosine (Tyr) seed priming in mitigating salt stress in rice seedlings (IR64 cultivar). The seeds were subjected to four treatment groups: control (water), 25 mM NaCl (salt stress), 5 mg l^{-1} tyrosine, and 5 mg l^{-1} tyrosine + 25 mM NaCl. Key morpho-physiological and biochemical parameters were measured after 14 days. Salt stress significantly inhibited seedling growth, decreased photosynthetic pigments, and elevated malondialdehyde (MDA) levels, indicating oxidative stress. In contrast, tyrosine-primed seedlings under salt stress exhibited notable improvements in seedling length (54.89%), fresh weight (58.88%), and dry weight (50%) relative to salt-stressed plants alone. Photosynthetic pigment levels, particularly total chlorophyll, improved by 55.88%, suggesting preserved chloroplast function. Moreover, tyrosine priming significantly decreased MDA content and increased superoxide dismutase (SOD) activity by 2.03-fold, indicating enhanced antioxidative

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defense. These findings support the role of tyrosine as a stress-mitigating priming agent, likely due to its function as a precursor for secondary metabolites and its involvement in redox signalling pathways. This study provides novel insights into tyrosine-mediated stress amelioration and suggests its applicability as a sustainable strategy to enhance salt tolerance in rice. Further field-scale studies are warranted to validate these findings under agronomic conditions.

Keywords: Tyrosine; Salt Stress Tolerance; Oryza Sativa; Photosynthetic Pigments; Antioxidant Defense

1. Introduction

Salt stress is one of the most critical abiotic factors limiting agricultural productivity worldwide, particularly in rice-growing regions. Globally, over 833 million hectares of land are salt-affected, posing a significant threat to rice (*Oryza sativa* L.), a staple food crop for more than half of the world's population. Rice is especially vulnerable to salinity during its early growth stages due to its inherent salt sensitivity [1]. High concentrations of sodium chloride (NaCl) in the soil induce osmotic stress, ion toxicity, nutrient imbalance, and excessive accumulation of reactive oxygen species (ROS). These effects collectively impair photosynthesis, damage cell membranes, and ultimately reduce crop yield [2]. In response to salinity-induced damage, plants activate a range of physiological and biochemical mechanisms, including osmolyte accumulation, antioxidant enzyme activation, ion homeostasis, and expression of stress-responsive genes [3]. However, these natural defense mechanisms are often insufficient in salt-sensitive cultivars like rice [4].

Seed priming, a pre-sowing treatment involving the application of bioactive agents, has gained recognition as a low-cost, environmentally friendly technique to enhance stress resilience in crops. It prepares seeds to better withstand adverse environmental conditions during germination and early growth [5]. Among various priming agents, amino acids have emerged as particularly effective due to their multifaceted roles in plant metabolism, growth regulation, and stress tolerance [6]. Tyrosine (Tyr), an aromatic amino acid synthesized via the shikimate pathway, not only serves as a building block for proteins but also functions as a precursor for a variety of bioactive compounds, including tocopherols, lignin, alkaloids, and phenolics. These compounds play pivotal roles in antioxidative defense and stress mitigation [7]. Exogenous tyrosine application has been shown to enhance antioxidant enzyme activity, stabilize membrane structures, and improve chlorophyll retention in crops such as maize [8,9], spinach [10], rice [11], and mung bean [12] under different abiotic stresses. Furthermore, tyrosine contributes to metabolic reprogramming by participating in redox regulation and acting as a precursor for signalling molecules involved in systemic acquired resistance and stress perception [13]. Despite increasing recognition of tyrosine's multifaceted roles in plant development and stress response, there is a notable lack of information regarding its potential application as a seed priming agent, particularly in rice under salinity stress. This research addresses that critical knowledge gap. To our knowledge, it is the first comprehensive investigation into the effectiveness of exogenous tyrosine priming in enhancing salt stress tolerance in *Oryza sativa*. By evaluating a range of morpho-physiological and biochemical parameters, this study provides new insights into how tyrosine modulates stress responses at multiple levels. The findings hold significant implications for agricultural development, particularly in the context of climate change and increasing soil salinization. Developing affordable, eco-friendly strategies like tyrosine priming not only improves crop performance under stress but also promotes sustainable rice production in marginal and salt-affected lands. This work contributes to the broader goal of ensuring global food security by enhancing the resilience and productivity of a staple crop that feeds more than half of the world's population.

2. Materials and Methods

2.1. Seed Materials and Treatment Procedure

The Indian rice seed cultivar (IR64) was obtained from the Indian Council of Agricultural Research (ICAR) - The Indian Agricultural Research Institute (IARI), New Delhi, India and the seeds were cultivated in the experimental field of Arignar Anna College (Arts and Science), Krishnagiri – 635 115, Tamil Nadu, India. For each time, 15 seeds were incubated on 2 layers of filter papers in petri-plates (9 cm in diameter). The room temperature was maintained at 27±2 °C. The plates were then treated with Tyr and NaCl, both alone and in combination, to determine priming efficiency. The groups listed here are:

1) C: Control seedlings received water;
2) 25 - SS: rice seedlings were treated with 25 mM NaCl alone;
3) 5 - Tyr: rice seedlings were treated with 5 mg l^{-1} tyrosine alone;
4) 5 - Tyr + 25 - SS; rice seedlings were treated with 5 mg l^{-1} tyrosine along with 25 mM NaCl.

The treatments were continued until the 14th day of seedling germination. After the end of treatments, the seedlings of different groups were subjected to various analyses.

2.2. Morphological Analysis

Seedling length was measured across different treatment groups. Fresh weight was recorded initially, followed by drying the seedlings in an oven at 50 °C for 24 hours. Once a constant weight was attained, the dry weight was documented.

2.3. Physiological Analysis

2.3.1. Determination of Photosynthetic Pigments

Fresh leaves from the rice seedlings were collected and homogenised with 80% acetone for analysing the photosynthetic pigments namely chlorophyll a, chlorophyll b, and total chlorophyll. Following detection at 470, 645, and 663 nm, the pigment concentrations in the supernatant were computed using the following formulas:

Chlorophyll a (mg g^{-1} FW) = $[((12.7) \times (A663)) - ((2.69) \times (A645)) \div 1000 \times W] \times V$

Chlorophyll b (mg g^{-1} FW) = $[((22.9) \times (A645)) - ((4.68) \times (A663)) \div 1000 \times W] \times V$

Total chlorophyll (mg g^{-1} FW) = $[((20.2) \times (A645)) + ((8.02) \times (A663)) \div 1000 \times W] \times V$

Where, A-absorption; W- weight of the sample; V- final volume of the extract, and FW- fresh weight [14].

2.4. Biochemical Characterization

2.4.1. Oxidative Marker Analysis: Lipid Peroxidation (LPO)

In this assay, 200 µl of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 8.1% aqueous thiobarbituric acid were combined in a reaction tube. To this mixture, 200 µl of plant extract was added, which had been obtained by homogenizing 250 mg of dried samples in 10 mM phosphate buffer (pH 7.0), followed by centrifugation at 17,000 rpm for 15 minutes at 4 °C. The reaction mixture was then incubated in a water bath for 60 minutes. After cooling to room temperature, 5 ml of a butanol: pyridine mixture (15:1, v/v) was added. The upper organic phase was separated, and the absorbance of the pink chromogen was measured at 532 nm. Tetramethoxypropane was used as the external standard, and lipid peroxidation was quantified as malondialdehyde (MDA) content, expressed in mg MDA/g dry weight (DW).

2.4.2. Enzyme Extract Preparation

In an ice bath, around 1 g of materials were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolidine (PVP). The enzyme activity was determined by centrifuging the resulting mixture for 20 minutes at 4 °C and 15,000 rpm, followed by collecting and storing the supernatant at −80 °C.

2.4.3. Superoxide Dismutase (SOD) Activity

Approximately 1000 mg of samples were ground in a 50 mM Tris-HCl/10 mM EDTA buffer solution (pH 8.5). Incubate 200 µl of extracted material in 7.2 mM pyrogallol at 25°C for 10 minutes. The reaction was halted using 100 µl of 1 N HCl, and the absorbance was measured at 420 nm. The percentage of SOD activity was calculated using the following formula:

SOD activity (%) = $[1-(A-B) \div C] \times 100$.

where A represents extracted samples with pyrogallol, B represents extracted samples without pyrogallol, and C represents the control/buffer alone with pyrogallol [3].

2.5. Statistical Analysis

All studies, including morphology (n = 15 × 3), physiology, and biochemical analysis, were conducted in triplicate (n = 5 × 3). The data were presented as mean values ± SE. Duncan's multiple range test (DMRT) was performed to compare statistical differences at a significance threshold of $P < 0.05$, using SPSS software version 20.

3. Results

3.1. Morphological Analysis

The 25 – SS plants exhibited a 48.32% reduction in seedling length compared to the control plants. However, the 5 – Tyr treatment resulted in a 43.9% increase in seedling length relative to the control. Similarly, the 5 – Tyr + 25 – SS plants showed a 54.89% improvement in seedling length compared to the 25 – SS treatment. Seedling fresh weight was reduced by 40.39% in 25 – SS plants relative to the control. In contrast, the 5 – Tyr treatment significantly enhanced fresh weight by 86.75% compared to the control. Likewise, the 5 – Tyr + 25 – SS plants exhibited a 58.88% increase in fresh weight compared to the 25 – SS group. Additionally, seedling dry weight decreased by 70.37% in the 25 – SS plants when compared to the control. Conversely, the 5 – Tyr + 25 – SS treatment led to a 50% increase in dry weight relative to the 25 – SS plants. Based on the above results, it can be determined that the exogenous priming of tyrosine was found to enhance seedling length, fresh, and dry weight under salinity stress conditions (**Table 1** and **Figure 1**).

Figure 1. Effect of priming with tyrosine on rice seedling morphology under salt stress conditions (after 15 days of treatment). (a) C: Control seedlings received water; (b) 25 – SS: rice seedings were treated with 25 mM NaCl alone; (c) 5 – Tyr: rice seedings were treated with 5 mg l^{-1} tyrosine alone; (d) 5 - Tyr + 25 – SS; rice seedings were treated with 5 mg l^{-1} tyrosine along with 25 mM NaCl. Scale bar is 1 cm.

Table 1. Morphological analysis of rice seeds was primed with exogenous supplementation of tyrosine.

Treatment groups	Seedling Length (cm)	Seedling Fresh Weight (g)	Seedling Dry Weight(g)
C	11.07 ± 0.03^{b}	03.02 ± 0.82^{b}	0.27 ± 0.01^{a}
25 - SS	05.72 ± 1.71^{d}	01.80 ± 0.03^{d}	0.08 ± 0.03^{c}

Table 1. *Cont.*

Treatment groups	Seedling Length (cm)	Seedling Fresh Weight (g)	Seedling Dry Weight(g)
5 - Tyr	15.93 ± 0.33^{a}	05.64 ± 1.01^{a}	0.23 ± 0.43^{a}
5 - Tyr + 25 – SS	08.86 ± 0.13^{c}	02.86 ± 0.01^{c}	0.12 ± 0.07^{b}

C: Control seedlings received water; 25 – SS: rice seedlings were treated with 25 mM NaCl alone; 5 – Tyr: rice seedlings were treated with 5 mg l^{-1} tyrosine alone; 5 - Tyr + 25 – SS; rice seedlings were treated with 5 mg l^{-1} tyrosine along with 25 mM NaCl.

Data represents mean values ± SE of three independent experiments (n = 15 x 3). Different letters next to numbers in the same column indicate significant differences between treatments ($P < 0.05$) as determined by DMRT.

Note: The data was recorded on the 15th day.

3.2. Photosynthetic Pigments

Chlorophyll a content was significantly decreased by 75.28% in the 25 – SS plants compared to the control. However, the 5 – Tyr + 25 – SS treatment enhanced chlorophyll a content by 59.09% compared to the 25 – SS group. Similarly, chlorophyll b content in 5 – Tyr + 25 – SS plants increased by 50% relative to the 25 – SS plants. In contrast, chlorophyll b was markedly reduced by 53.84% in 25 – SS plants when compared to the control. The total chlorophyll content was significantly higher in the 5 – Tyr + 25 – SS plants, showing a 55.88% increase compared to the 25 – SS group. On the other hand, 25 – SS plants exhibited a 70.43% reduction in total chlorophyll compared to the control. These findings indicate that tyrosine application under salt stress conditions helps maintain higher levels of photosynthetic pigments in rice seedlings (**Figure 2**).

Bars represent the mean values ± SE of three independent experiments ($n = 5 \times 3$). Means followed by different letters are significantly different according to Duncan's multiple range test at the 5% level.

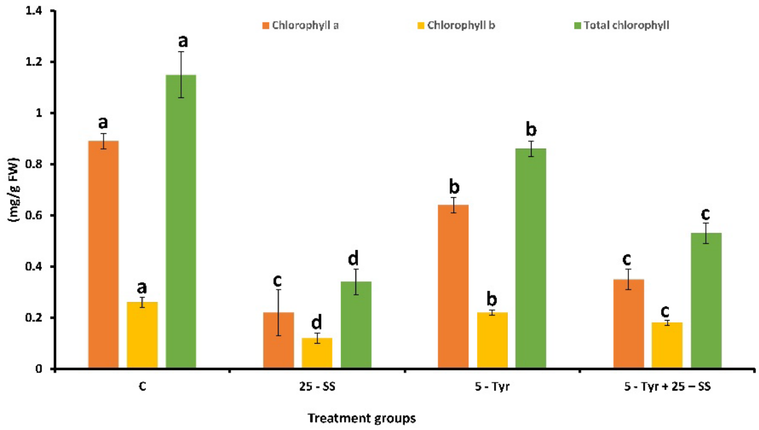


Figure 2. Influence of tyrosine on photosynthetic pigments in salt stressed rice seedlings.

3.3. Biochemical Characterization

Salinity stress significantly increased malondialdehyde (MDA) content by 2.46-fold in 25 – SS plants compared to the control, indicating elevated lipid peroxidation. In contrast, 5 – Tyr + 25 – SS plants exhibited a marked reduction in MDA content (24.65), even lower than the control (**Figure 3a**), suggesting reduced oxidative membrane damage. To mitigate the damaging properties of salinity stress and the accumulation of ROS, plants enhance their antioxidant defense mechanisms and elevate the synthesis of protective metabolites [3]. In the present study, SOD activity was significantly enhanced by 72.94% in 5 – Tyr plants relative to the control. Furthermore, 5 – Tyr + 25 – SS plants displayed a 2.03-fold enhanced in SOD activity compared to the control. In contrast, 25 – SS plants exhibited a 45.33% decrease in SOD activity compared to the control (**Figure 3b**). These results clearly indicate that exogenous priming with tyrosine not only alleviates membrane damage under salt stress but also significantly enhances the activity of enzymatic antioxidants, thereby contributing to improved oxidative stress tolerance in rice seedlings.

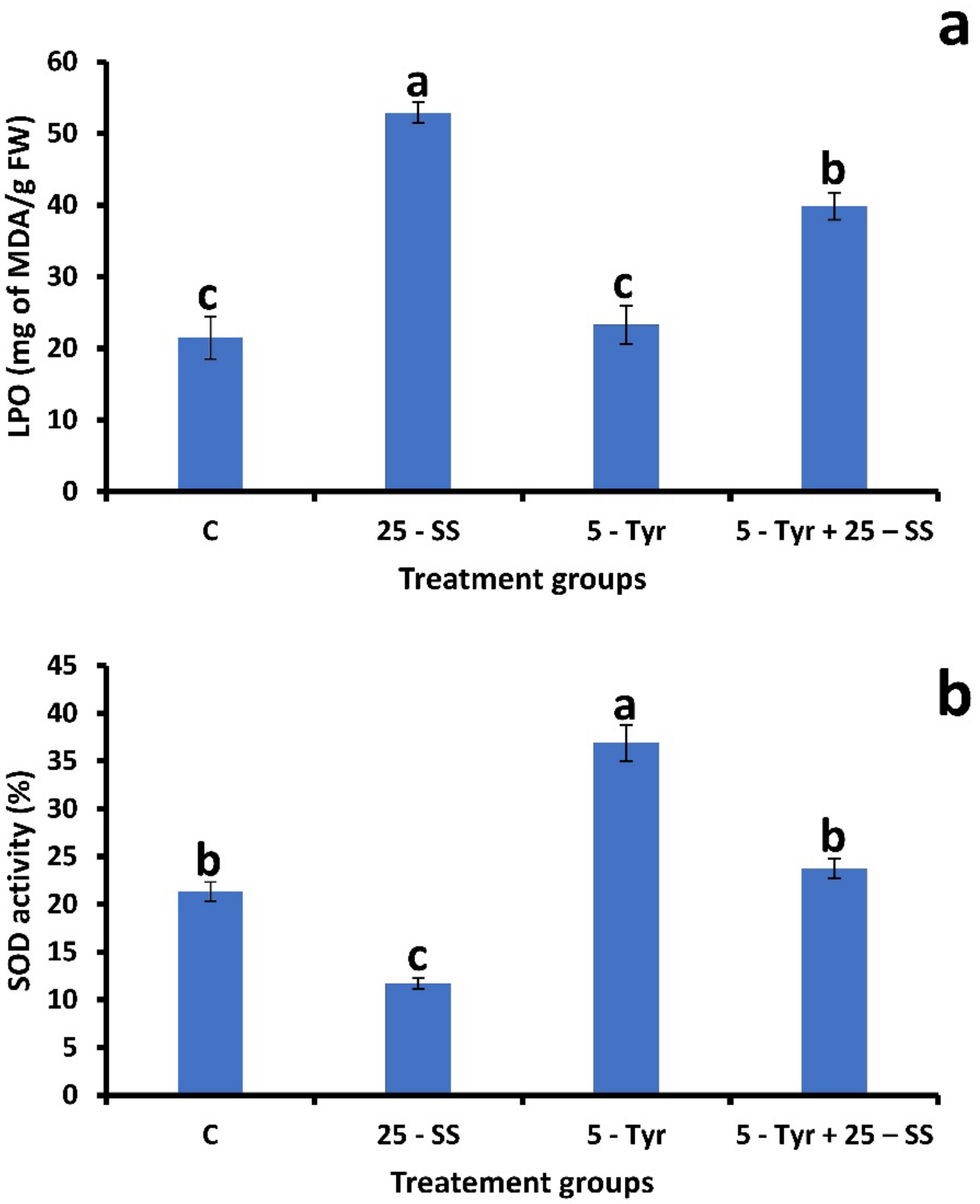
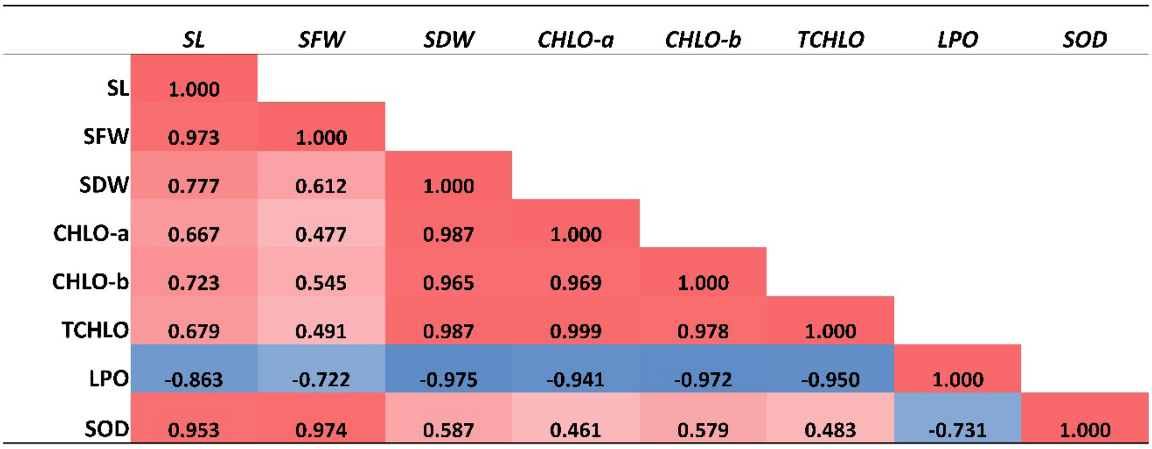


Figure 3. Analysis of (a) lipid peroxidation (LPO), and (b) superoxide dismutase (SOD) activity in the rice primed with tyrosine. Data represents mean values ± Standard error (n = 5×3). Values with different letters within columns are significantly different according to DMRT at 5% level.

3.4. Correlation (r2) Analysis

The associations between the various factors were examined using correlation (r^2) studies, as shown in **Figure 4**. The data revealed strong positive associations between seedling fresh and dry weight and seedling length. Furthermore, chlorophyll a, chlorophyll b, and total chlorophyll showed a favourable relationship with seedling fresh and dry weight. In contrast, there were negative relationships between lipid peroxidation and seedling length, fresh weight, dry weight, chlorophyll a, chlorophyll b, total chlorophyll, and superoxide dismutase.



	SL	SFW	SDW	CHLO-a	CHLO-b	TCHLO	LPO	SOD
SL	1.000							
SFW	0.973	1.000						
SDW	0.777	0.612	1.000					
CHLO-a	0.667	0.477	0.987	1.000				
CHLO-b	0.723	0.545	0.965	0.969	1.000			
TCHLO	0.679	0.491	0.987	0.999	0.978	1.000		
LPO	-0.863	-0.722	-0.975	-0.941	-0.972	-0.950	1.000	
SOD	0.953	0.974	0.587	0.461	0.579	0.483	-0.731	1.000

Figure 4. Correlation coefficient values (r2) among different parameters of rice treated with tyrosine under salt stress.

SL: Seedling length; SFW: Seedling fresh weight; SDW: Seedling dry weight; CHLO-a: Chlorophyll a; CHLO-b: Chlorophyll b; TCHLO: Total chlorophyll; LPO: Lipid Peroxidation; SOD: Superoxide dismutase.

4. Discussion

The present study demonstrates that tyrosine priming increases salinity stress tolerance in rice by improving growth parameters and modulating antioxidant responses. Salinity stress produced a significant decrease in seedling length, fresh and dry weight in rice, which is consistent with previous reports indicating that salinity induces osmotic stress, hampers water uptake, and disrupts cellular expansion and division [15]. However, priming with tyrosine (5 mM) resulted in a remarkable improvement in seedling length, fresh and dry weight in rice growth parameters even under saline conditions. This aligns with previous results like tyrosine enhanced root length, shoot length, number of leaves, leaves area, leaves dry weight, percentage of germination were enhanced in mung bean under cadmium stress [12]. Photosynthetic pigments were severely reduced in salt-stressed rice seedlings. This is consistent with previous research showing that salt stress destroys chlorophyll by increasing chlorophyllase activity and reactive oxygen species (ROS) production [16]. Notably, tyrosine priming significantly preserved chlorophyll content both in non-stressed and salt-stressed conditions, supporting its role in maintaining chloroplast integrity and photosynthetic efficiency. Tyrosine, being a precursor of important secondary metabolites and antioxidants, is known to participate in redox signalling and stress responses [17]. The increased MDA levels in 25 – SS plants highlight the extent of membrane lipid peroxidation under salt stress. However, the reduction in MDA content in tyrosine-treated plants indicates decreased membrane damage, likely due to improved ROS scavenging. Enhanced activity of SOD confirms the activation of the antioxidant defense system. Similar enhancements in antioxidant enzyme activities through amino acid priming have been observed in rice and wheat under abiotic stress conditions [18]. Overall, tyrosine priming appears to mediate stress tolerance by modulating the antioxidant machinery and preserving photosynthetic pigment stability, contributing to improved plant performance under salinity stress. These findings suggest that tyrosine could be a promising seed priming agent to enhance salt tolerance in rice cultivation.

The outcomes of this research study carry significant policy implications for climate-resilient and sustainable agriculture, mainly in regions affected by soil salinization. Soil salinity is a rising global concern, intensified by irrigation mismanagement, climate change, and increasing sea levels. Conventional approaches to fight salt stress such as breeding salt-tolerant cultivars or using chemical protectants are often time-consuming, expensive, or environmentally unsustainable. In contrast, amino acid-based seed priming, particularly with tyrosine, represents a low-cost, scalable, and eco-friendly agronomic intervention. Policymakers and agricultural extension services should consider promoting tyrosine-based seed priming as a part of integrated salt-stress management programs. Adoption of this practice could be encouraged through inclusion in national agricultural guidelines, farmer training modules, and sustainable rice intensification packages. Additionally, investment in public-private partnerships for amino acid formulation, seed treatment kits, and on-field demonstrations can enhance awareness and adoption among smallholder farmers. Integrating such innovative biostimulant strategies into agroecological policy frameworks can help improve productivity in salt-affected lands, contributing to food security, climate adaptation, and sustainable development goals (SDGs) particularly SDG 2 (*Zero Hunger*) and SDG 13 (*Climate Action*).

5. Conclusions

Our present work demonstrates that tyrosine priming increases salt stress tolerance in *Oryza sativa* by modifying critical morphophysiological and biochemical characteristics. Tyrosine-primed rice seedlings exhibited enhanced germination, seedling vigor, chlorophyll content, and antioxidant enzyme activities under salt stress conditions. Furthermore, decreases in malondialdehyde content indicate that the strengthened membrane integrity and elevation of cellular oxidative damage. These outcomes highlight the possible of tyrosine as a cost-effective and eco-friendly priming agent for enlightening rice productivity in salt environmental conditions. From a larger viewpoint, this research work supports the role of amino acid-based priming approaches in crop stress management, mostly under gradually changing climatic circumstances. Future research should discover the underlying molecular mechanisms of tyrosine-mediated stress mitigation, including its impact on stress-responsive gene expression and metabolic pathways. Field-level justification and valuation across diverse rice genotypes and agro-climatic zones are also essential to regulate the scalability and agronomic viability of this method. Moreover, contributing tyrosine priming with other agronomic practices and bio-stimulants could provide synergistic support, paving the way for sustainable and climate-resilient rice cultivation.

Author Contributions

K.S. conception and idea; K.S., S.D., and N.K. supervised the experiments; S.S. and R.P. performed the experiments; S.D., N.K. Wrote the manuscript under the guidance of K.S.; M.M., A.B.S.B., K.P., C.D.J.A., R.D., and R.M. preparation of the tables, and figures. Finally, the authors read and approved the manuscript.

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Conflicts of Interest

The authors declare no competing interests.

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