Jurnal Agroteknologi, Vol. 16 No. 1, Agustus 2025: 11–20
DOI: 10.24014/ja.v16vi1.36858
Available online at https://ejournal.uin-suska.ac.id/index.php/agroteknologi/
ISSN 2356-4091 (online), ISSN 2087-0620 (print)

EFFECTS OF SALINITY AND PYRITE ON THE GROWTH OF RICE SEEDLINGS *(Oryza sativa)*

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ABSTRACT

Rice is a key staple crop vulnerable to environmental stresses such as soil salinity and pyrite contamination, which can significantly inhibit seedling growth. These stressors disrupt physiological functions and worsen soil conditions, especially in degraded areas. This study aims to analyze the combined effects of salinity and pyrite on the morphological and physiological responses of rice seedlings under dual stress. The research was conducted at the UPT Seed Certification and Plant Seed Certification of Food Crops and Horticulture in Pekanbaru for two months. The experimental design used was a Completely Randomized Design (CRD) consisting of two factors: salinity stress (3 levels: 0 mM, 50 mM, 100 mM) and pyrite stress (3 levels: 0 mg, 200 mg, 400 mg), with four replications for each treatment. The parameters observed included plant height, leaf number, root length, stomatal count, plant necrosis, and leaf area. The results showed that salinity stress significantly reduced plant height and increased leaf necrosis, while the Mendol Pelalawan variety maintained relatively stable growth at moderate salinity (50 mM) through a possible hormetic response that promoted root elongation. Pyrite stress negatively affected leaf growth due to iron toxicity but tended to reduce salinity damage by improving soil chemical balance. No significant interaction effect was found, indicating that salinity and pyrite acted independently. Reduced stomatal numbers and increased necrosis under severe stress reflected the plant's adaptive strategy to conserve water, though at the cost of lower vegetative growth. These findings suggest the need to manage soil salinity and pyrite carefully to support rice productivity in tidal swamp areas.

Keywords: Environmental stress, Pyrite, Salinity, Seedling growth, Stress interaction.

INTRODUCTION

Rice (*Oryza sativa*) was one of the staple food crops in Indonesia, playing a strategic role in maintaining national food security. The low rice production in Riau Province, accounting for only 0.42% of the total national rice production in Indonesia, which reached 53,142,726.65 tons (BPS, 2025), was suspected to be related to the dominance of cultivation on suboptimal land. Rahmasari *et al*. (2020) explained that there were five categories of suboptimal land in Indonesia, namely acid dryland, dry climates, tidal swamps, peatlands, and lowland swamps. For example, Pelalawan Regency in Riau Province was one of the rice production canters, particularly in Kuala Kampar District, which had a rice field area of 5,922 hectares with a cultivation system in tidal swamp land (Laksamana & Kurniati, 2022). Tidal swamp land itself was a type of swamp land influenced by the rise and fall of sea levels along the coast and was generally flooded throughout the year. Masganti *et al*. (2022) revealed that the increasing seawater intrusion due to climate change had led to a decline in rice productivity, consequently reducing agricultural land quality. Since rice was known to be sensitive to salinity and other environmental stressors, understanding the growth-inhibiting factors in such ecosystems was deemed crucial.

Soil salinity was one of the major abiotic stresses that inhibited rice growth. Salinity affected both the morphology and physiology of rice plants. At the physiological level, high salinity caused osmotic stress, inhibited water uptake, and triggered ion imbalance, leading to stomatal closure and a decrease in the photosynthesis rate. As a result, rice experienced stunted growth, chlorosis, leaf necrosis, and a significant reduction in the number of tillers and grain weight (Rodríguez Coca 2023). Although rice had a certain level of tolerance to saline water, it was limited. According to Sopandie (2013), the salinity tolerance threshold for rice was 2.0 – 4.0 dS/m, with yields declining by 12% per dS/m starting at 3 dS/m. Based on a study where rice was treated with azolla and salinity stress, stress symptoms were observed at NaCl salinity levels of 4 dS/m or equivalent to 40 mM, indicated by decreased growth rate, number of tillers, dry weight of shoots and roots, stomatal density, chlorophyll content, flowering time, number of filled grains per panicle, and harvest index (Krismiratsih *et al.* 2020). This indicated that while rice could tolerate mild salinity, it could not adapt well to prolonged or high-salinity conditions.

In addition to salinity, the presence of pyrite (FeS_2) in tidal swamp soils also significantly affected soil quality and rice growth. Acid sulphate soils resulting from pyrite oxidation produced sulfuric acid, which drastically lowered soil pH. Maulidi *et al.* (2024) emphasized that pyrite was the primary source of soil acidity in tidal swamplands. The very low soil pH ($pH < 4$) dissolved heavy metals, particularly iron (Fe^{2+}) ions. Furthermore, during waterlogging, Fe^{3+} was reduced to soluble Fe^{2+}, which could be toxic to rice roots. As a result, tidal swamp lands with high pyrite content struggled to reach maximum productivity. Abdillah *et al.* (2024) even reported that the main causes of low rice yield in IR Zinc varieties grown on acid sulphate soils were high pyrite levels and high bulk density. It was therefore suspected that pyrite in tidal swamp soils reduced fertility through pH decline and metal toxicity that harmed rice growth. Heavy metals in pyrite inhibited seed germination and worsened rice plant development. Pyrite was commonly found in poorly drained soils, which further aggravated rice growth conditions, especially in degraded peatlands (Anwar & Masganti 2021).

Therefore, understanding how salinity and pyrite interacted was essential for developing strategies to manage degraded lands and enhance plant resilience to abiotic stress. This study consisted of an analysis of the combined effects of salinity and pyrite levels on the growth of rice seedlings. The simultaneous influence of these two abiotic stress factors provided insights into the morphological and physiological response mechanisms of plants under dual stress conditions.

MATERIALS AND METHODS

Time and Place

The research was conducted at the Seed Production and Certification Unit (UPT) for Food Crops and Horticulture, at Jalan Kaharudin Nasution No. 69, Pekanbaru. The research was conducted from August to October 2024.

Materials and Tools

The research used NaCl, aqua dest (distilled water), pyrite rock, label paper, Mendol Pelalawan rice seeds, nail polish, tape, litmus paper, paper and plastic. The research used materials including stationery, a ruler, germinator, thermometer, salinity meter, analytical balance, microscope, glass slides, oven, desiccator, petri dishes, and camera.

Research Method

The research was conducted experimentally using a Completely Randomized Design (CRD) with two factors. The first factor was salinity stress (C) which had three levels: control, 50 mM, and 100 mM and the second factor was pyrite stress (L) also with three levels: control, 200 mg, and 400 mg. Each treatment had 4 replications. Thus, a total of 36 experimental plots was used with each plot containing 25 plants and 10 plants were taken as samples from each plot, collecting a total of 900 plants.

The observed parameters included

- Plant height : Plant height was measured from the base of the stem to the highest growing point. Measurements were carried out weekly, starting from one week after planting until the third week after plantin.
- Number of leaves : The number of leaves was counted manually and recorded in the third week after planting.
- Leaf area : Leaf area was determined by cutting all the leaves from the plant and capturing their images using a digital camera with a measurement scale included. The images were then analyzed using ImageJ software to obtain the leaf area.
- Root length : Root length was measured from the base to the tip of the longest root of the rice plant. The measurement was conducted in the third week after planting.

- Number of stomata : Observations were carried out on healthy rice leaf samples, specifically on the middle part of the leaf. A thin, even layer of clear nail polish was applied to the lower surface of the leaf. After allowing it to dry for 10–15 minutes, the nail polish film was carefully peeled off and placed onto a microscope slide, then covered with a cover glass. The observations were conducted under a microscope. Images of the observations were captured using a digital microscope camera and analyzed using ImageJ software.
- Plant necrosis : Necrosis observations were conducted on the final day of observation by assessing the number and length of leaves exhibiting necrosis symptoms. The number of necrotic leaves was determined by counting the leaves showing necrosis on each rice plant. The length of leaf necrosis was measured from the tip of the leaf to the base of the affected area showing necrotic symptoms.

Data Analysis

The plant growth produced was analyzed to determine if there was an effect of salinity stress and pyrite stress. Data analysis in this study used analysis of variance (F test) by the SPSS application. For results of an F test that had a significant effect, it was progressively analyzed by Duncan's Multiple Range Test (DMRT) at 5% significance level.

RESULTS AND DISCUSSION

1. Plant Height (cm)

Plant height is an important parameter in plant growth studies, as it can be influenced by various abiotic factors, including salinity and pyrite. In this study, the effects of salinity stress and pyrite stress on plant height were observed over a period of three weeks. The analysis of variance results showed that the interaction between salinity stress and pyrite stress had a significant effect only in the second week on the plant height parameter of rice. The results of the post hoc test and the average plant height of rice over the three-week period are presented in Table 1.

Table 1. Effect of Salinity and Pyrite Stress on the Average Plant Height (cm) of Rice

Salinity Stress	Pyrite Stress			
	L_0 (control)	L_1 (200 mg)	L_2 (400 mg)	Mean C
		Week 1		
C_0 (control)	5.73 ± 0.22^{a}	4.87 ± 0.32^{a}	4.72 ± 0.50^{a}	5.11 C
C_1 (50 mM)	4.06 ± 0.23^{a}	3.86 ± 0.21^{a}	4.18 ± 0.38^{a}	4.03 B
C_2 (100 mM)	2.69 ± 0.22^{a}	2.71 ± 0.17^{a}	2.60 ± 0.10^{a}	2.67 A
Mean L	4.16 A	3.81 A	3.83 A	
		Week 2		
C_0 (control)	15.54 ± 0.14^{f}	13.14 ± 0.52^{de}	13.43 ± 0.36^{e}	14.04 C
C_1 (50 mM)	11.75 ± 0.79^{cd}	11.94 ± 0.40^{cd}	11.12 ± 0.61^{bc}	11.60 B
C_2 (100 mM)	8.87 ± 0.43^{a}	9.34 ± 0.14^{a}	9.74 ± 0.53^{ab}	9.31 A
Mean L	12.06 A	11.47 A	11.43 A	
		Week 3		
C_0 (control)	19.83 ± 0.40^{a}	17.56 ± 0.92^{a}	17.18 ± 0.53^{a}	18.19 B
C_1 (50 mM)	15.31 ± 0.97^{a}	16.77 ± 0.82^{a}	15.73 ± 1.25^{a}	15.94 A
C_2 (100 mM)	14.91 ± 0.45^{a}	14.98 ± 1.05^{a}	14.98 ± 0.80^{a}	14.96 A
Mean L	16.68 A	16.44 A	15.96 A	

Description: Values are presented as the mean±standard error (n=4). Numbers followed by lowercase letters in the same row and column indicate significant differences based on the DMRT test at the 5% significance level between combinations of salinity and pyrite treatments. Numbers followed by uppercase letters in the same row or column indicate significant differences based on the DMRT test at the 5% significance level between salinity treatments within the same pyrite level or pyrite treatments within the same salinity level. Numbers followed by different letters indicate statistically significant differences.

The results of this study showed that salinity stress clearly reduced the plant height of rice. In the first week, the 0 mM salinity treatment produced the tallest plants (5.11 cm), while the 100 mM salinity treatment reached only 2.67 cm. This pattern continued in the second and third weeks; the higher the salt concentration, the lower the plant height tended to be. These results were consistent with many previous studies which reported similar findings. For example, Székely *et al*., (2022) stated that salinity significantly inhibited rice growth, markedly reducing plant height ($p<0.001$). Liu *et al*., (2019) also reported that 100 mM NaCl stress caused up to a 42% decrease in plant height in salt-sensitive cultivars. Physiologically, this reduction in height was related to the osmotic effect and ionic toxicity of salt on the plants, which reduced cell division, cell elongation, and biomass accumulation (rice is highly sensitive to salinity).

The Mendol Pelalawan variety was reported to be salt-tolerant, so it tended to experience a milder reduction in height compared to sensitive varieties. The observed decrease in height in this variety was consistent with reports that salt-tolerant genotypes-maintained growth better than sensitive ones. Xu *et al.*, (2024) showed that the average reduction in height in salt-tolerant genotypes was only about 10.6%, whereas in sensitive ones it reached around 25%. Thus, although Mendol Pelalawan showed a reduction in height due to salinity, the relative decrease was smaller than what was reported for sensitive varieties. This explained why, even though the 100 mM salinity treatment still reduced height, the difference between the moderate (50 mM) and high (100 mM) treatments was no longer significant once the plants reached their tolerance threshold.

In the third week, it was observed that the difference in height between the 50 mM and 100 mM treatments was not significant. This result suggested that the growth inhibition effect had reached the variety's tolerance threshold. This meant that above a certain salt concentration (around 50–100 mM NaCl for salt-tolerant varieties), additional salt did not significantly decrease plant height further. This finding was consistent with the characteristics of salt-tolerant varieties, which only experienced limited reductions under high stress, major changes occurred up to moderate stress levels, then appeared to plateau (showing little difference between 50 and 100 mM). In other words, once salinity stress exceeded the plant's physiological capacity, the plant height could not decrease much further even if the salt concentration increased. This indicated that in the third week, the Mendol Pelalawan variety had already reached its growth tolerance limit to salinity stress, as suggested by the minimal difference between C_1 and C_2.

2. Number of Leaves

The number of leaves is an important parameter in assessing plant adaptation to environmental stress, as it is directly related to photosynthetic capacity and the plant's ability to produce. The analysis of variance showed that the interaction between salinity stress and pyrite stress did not have a significant effect on the number of leaves in rice plants. The post hoc test results and the average number of leaves of rice plants can be seen in Table 2.

Table 2. Effect of Salinity and Pyrite Stress on the Average Number of Leaves of Rice Plants

Salinity Stress	Pyrite Stress			
	L_0 (control)	L_1 (200 mg)	L_2 (400 mg)	Mean C
C_0 (control)	1.9 ± 0.05^a	1.8 ± 0.08^a	1.8 ± 0.06^a	1.86 A
C_1 (50 mM)	1.9 ± 0.06^a	2.0 ± 0.05^a	1.8 ± 0.06^a	1.87 A
C_2 (100 mM)	2.0 ± 0.03^a	2.2 ± 0.09^a	2.0 ± 0.09^a	2.06 B
Mean L	1.92 AB	2.00 B	1.86 A	

Description: Values are presented as the mean±standard error (n=4). Numbers followed by lowercase letters in the same row and column indicate significant differences based on the DMRT test at the 5% significance level between combinations of salinity and pyrite treatments. Numbers followed by uppercase letters in the same row or column indicate significant differences based on the DMRT test at the 5% significance level between salinity treatments within the same pyrite level or pyrite treatments within the same salinity level. Numbers followed by different letters indicate statistically significant differences.

The results of this study showed that the Mendol Pelalawan rice variety (salt-tolerant but less tolerant to pyrite) was able to maintain or even increase its leaf number under moderate salinity treatment. Under C_2 (NaCl 100 mM), the number of leaves increased (2.06 leaves) compared to the control (1.86 leaves) or 50 mM (1.88 leaves). This suggested that this variety had good salinity tolerance. In general, salinity caused osmotic stress and toxic Na^+ accumulation within plant cells, thereby inhibiting nutrient uptake and disturbing growth (Chen Y *et al.* 2022). Salt-tolerant varieties could overcome these negative effects through physiological adaptations, such as suppressing Na^+ uptake, maintaining a high K^+/Na^+ ratio, accumulating protective osmolytes like proline, and enhancing antioxidant enzyme activity (Nounjan & Theerakulpisut 2021). Mechanisms such as the activation of the SOS (Salt Overly Sensitive) pathway in the roots helped excrete Na^+ out of cells, preventing toxicity in aboveground tissues. Morphological adaptations also played a role, the development of a strong root system (greater biomass and root length) supported the uptake of water/salts and nutrients, thereby maintaining plant productivity. Chen Y *et al.* (2022) stated that root biomass, root length, and high root oxidation activity were positively correlated with rice yield under salinity stress. This combination of physiological and morphological strategies enabled Mendol Pelalawan to develop more leaves under 100 mM NaCl despite increasing salt pressure.

Anwar & Masganti (2021) stated that pyrite is a compound that readily undergoes oxidation during the dry season, leading to the formation of ferric iron (Fe^{3+}) and sulfuric acid. This process can lower soil pH and release iron in a form that is toxic to plants. Conversely, during the rainy season, when fields become flooded, reductive conditions are established, resulting in the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), which can also adversely affect rice plant growth. Such acid sulfate soil conditions are known to reduce soil fertility and rice productivity (Xiao *et al*. 2022). Under the 400 mg pyrite treatment (L2), the number of leaves decreased (1.86 leaves) compared to 200 mg (2.00 leaves), indicating that pyrite stress suppressed vegetative development. This reduction was consistent with sulfuric acid toxicity, which damaged the root system and disrupted nutrient uptake. Although the difference with the control (1.93 leaves) was not significant, the duration of the treatment or the variety's ability to maintain growth under moderate pyrite levels possibly explained this statistical similarity. In practice, pyritic soils tended to hamper rice growth due to extreme acidity and Al toxicity, so less tolerant varieties such as Mendol Pelalawan showed a decrease in leaf number at higher pyrite doses.

The interaction between salinity and pyrite stress did not show a synergistic effect on leaf number in this experiment. This indicated that the adaptation mechanisms for each stressor were relatively independent; tolerance to salinity (ionic/osmotic) did not automatically guarantee tolerance to acid/aluminum stress. In natural conditions (such as the tidal swamp areas of Mendol Island), plants often faced a combination of salt and pyrite stress, and their responses could be complex. Chen G *et al*. (2022) showed that plant responses to combined abiotic stress could be unique and not always predictable from single-stress responses. In other words, Mendol Pelalawan employed specific adaptation pathways for salinity, while pyrite stress required separate mechanisms. The fact that this variety was salt-tolerant but less tolerant to pyrite highlighted the importance of the variety's physiological traits genetic salt tolerance needed to be complemented by root adaptations and buffering systems to handle sulfuric acid conditions to grow optimally in complex environments. According to Taiz & Zeiger (2002), the interaction of different environmental stresses often resulted in complex plant responses, depending on the intensity and duration of the stress. Plants might prioritize resource allocation to maintain their main photosynthetic organs, such as leaves, to ensure the continuity of vital physiological processes.

3. Leaf Area (cm^2)

The analysis of variance (ANOVA) results showed that the interaction between salinity stress and pyrite stress did not significantly affect the leaf area parameter of the rice plants. The results of further testing and the average leaf area of the rice plants can be seen in Table 3.

Table 3 Effect of Salinity and Pyrite Stress on the Average Leaf Area (cm^2) of Rice Plants

Salinity Stress	Pyrite Stress			
	L_0 (control)	L_1 (200 mg)	L_2 (400 mg)	Mean C
C_0 (control)	1.022 ± 0.054^a	0.991 ± 0.110^a	$0,916 \pm 0.066^a$	0.976 A
C_1 (50 mM)	0.987 ± 0.015^a	0.958 ± 0.030^a	0.881 ± 0.038^a	0.942 A
C_2 (100 mM)	0.898 ± 0.073^a	0.888 ± 0.051^a	1.024 ± 0.055^a	0.937 A
Mean L	0.969 A	0.946 A	0.940 A	

Description: Values are presented as the mean±standard error (n=4). Numbers followed by lowercase letters in the same row and column indicate significant differences based on the DMRT test at the 5% significance level between combinations of salinity and pyrite treatments. Numbers followed by uppercase letters in the same row or column indicate significant differences based on the DMRT test at the 5% significance level between salinity treatments within the same pyrite level or pyrite treatments within the same salinity level. Numbers followed by different letters indicate statistically significant differences.

The local Mendol Pelalawan variety was developed on tidal swamp lands that often-contained pyrite compounds (acid sulfate soils), so this variety was known to be relatively salt-tolerant but sensitive to iron toxicity. The results of this study showed that pyrite (FeS_2) treatment at doses of 200–400 mg reduced the rice leaf area from 1.022 cm^2 (without pyrite) to 0.991 and 0.916 cm^2, respectively. This reduction was presumed to be caused by the toxic effect of pyrite, as pyrite oxidation released sulfuric acid (H_2SO_4) and dissolved iron, lowering the medium's pH and increasing the concentration of highly soluble Fe^{2+} ions (Yudianto 2016). As a result, excess Fe^{2+} within plant tissues triggered oxidative stress (Fenton reaction), which damaged leaf cells (manifested as chlorosis or rust-like striping) and reduced photosynthesis efficiency (Wairich *et al*. 2024). Physiologically and morphologically, this condition was reflected in growth inhibition of the leaves, namely a significant decrease in leaf number and leaf area.

While salinity stress generally also reduced leaf area, the findings of Jin *et al*. (2025) showed that salinity stress decreased leaf area through disruptions in turgor and photosynthesis (leaf curling, decreased SPAD/LAI). However, since the Mendol Pelalawan variety was salt-tolerant, the reduction in leaf area in this study was mainly attributed to pyrite toxicity. The combination of soil acidity and excess iron in the growing medium induced stomatal closure and reactive oxygen species (ROS) accumulation, which further inhibited leaf expansion (Wairich *et al.* 2024). Thus, although the interaction between salinity and pyrite was not statistically significant, these numerical results aligned with literature reports that excess Fe ions from pyrite hindered rice leaf growth.

4. Root Length (cm)

The results of the analysis of variance showed that the interaction between salinity stress and pyrite stress did not significantly affect the root length parameter of the rice plants. The results of further tests and the average root length of the rice plants can be seen in Table 4.

Table 4. Effect of Salt Stress and Pyrite Stress on the Average Root Length (cm) of Rice Plants

Salinity Stress	Pyrite Stress			
	L_0 (control)	L_1 (200 mg)	L_2 (400 mg)	Mean C
C_0 (control)	7.81±0.48^a	7.84±0.44^a	9.34±1.94^a	8.33 A
C_1 (50 mM)	10.65±0.59^a	9.73±0.37^a	10.66±0.45^a	10.35 B
C_2 (100 mM)	8.96±0.61^a	9.44±0.52^a	9.99±0.24^a	9.46 AB
Mean L	9.14 A	9.00 A	9.99 A	

Description: Values are presented as the mean±standard error (n=4). Numbers followed by lowercase letters in the same row and column indicate significant differences based on the DMRT test at the 5% significance level between combinations of salinity and pyrite treatments. Numbers followed by uppercase letters in the same row or column indicate significant differences based on the DMRT test at the 5% significance level between salinity treatments within the same pyrite level or pyrite treatments within the same salinity level. Numbers followed by different letters indicate statistically significant differences.

Based on the results of this study, the 50 mM NaCl salinity treatment (C_1) actually increased the average root length compared to the no-salt control (C_0), while the 100 mM concentration (C_2) did not extend the roots further, with root length changing from 8.33 → 10.35 → 9.46 cm. This was similar to the findings of Fan *et al.* (2024), who showed that the primary roots of rapeseed plants grew longer under moderate stress of 50 mM NaCl but were strongly inhibited at 150 mM NaCl. Likewise, Shelden & Munns (2023) reported that in cotton, moderate salinity (25–100 mM NaCl) actually stimulated primary root growth compared to the control without salt. In addition, the study by Hongqiao *et al.* (2021) on *Arabidopsis thaliana* showed that root fresh weight increased at 5 mM NaCl, whereas at concentrations ≥20 mM NaCl, the roots shrank.

According to Fan *et al.* (2024), this was due to the phenomenon of hormesis, where mild stress triggered a positive adaptive response. For example, NaCl at low concentrations could increase water use efficiency and the uptake of nutrients (such as C, S, and Zn) that supported root growth. However, when NaCl concentration became too high, the negative effects (metabolic disruption, ROS generation, nutrient deficiencies) dominated, preventing the roots from growing longer.

5. Stomatal Count

The analysis of variance results showed that the interaction between salinity stress and pyrite stress did not have a statistically significant effect on the stomatal count parameter of rice plants. The results of the further test and the average stomatal count of rice plants can be seen in Table 5.

Table 5. Effect of Salinity and Pyrite Stresses on the Average Stomatal Count of Rice Plants

Salinity Stress	Pyrite Stress			
	L_0 (control)	L_1 (200 mg)	L_2 (400 mg)	Mean C
C_0 (control)	43.08±1.20^a	38.92±3.25^a	34.00±1.22^a	38.667 A
C_1 (50 mM)	40.67±3.04^a	37.42±1.27^a	32.92±2.71^a	37.001 A
C_2 (100 mM)	38.42±2.25^a	37.58±3.78^a	43.00±2.73^a	39.667 A
Mean L	40.723 A	37.973 A	36.639 A	

Description: Values are presented as the mean±standard error (n=4). Numbers followed by lowercase letters in the same row and column indicate significant differences based on the DMRT test at the 5% significance level between combinations of salinity and pyrite treatments. Numbers followed by uppercase letters in the same row or column indicate significant differences based on the DMRT test at the 5% significance level between salinity treatments within the same pyrite level or pyrite treatments within the same salinity level. Numbers followed by different letters indicate statistically significant differences.

Rice plants typically reduced the number of stomata or closed their stomata as an adaptive response to water or salt stress to conserve leaf water. In this study, although the differences in stomatal number among treatments were not significant, the control plants (without stress) had the highest number of stomata, while the combination of 50 mM salinity stress and 400 mg pyrite (C_1L_2) showed the lowest stomatal number. This finding was supported by Yun *et al.* (2025), who reported that increasing salt concentrations triggered the accumulation of ABA hormone, which induced stomatal closure to prevent water loss. At the molecular level, increasing stress (salt or heavy metals) could genetically regulate the plant to suppress stomatal development. For example, the epigenetic regulator HDA704 in rice induced stomatal closure and reduced stomatal number, thereby slowing leaf water loss and increasing drought and salinity tolerance (Zhao *et al.* 2021). Heavy metal triggers, such as pyrite elements, were also suspected to raise ABA and ROS levels, which further caused stomatal closure and impaired photosynthetic function (Guo *et al.* 2023). Thus, although the changes in stomatal number in this study were relatively small, the tendency toward a decrease under combined severe stress reflected an adaptive water conservation mechanism in rice.

Stomatal closure in response to stress directly affected photosynthesis and transpiration processes. When stomata closed (either due to ABA increase from salinity or pyrite toxicity), CO_2 intake was limited, causing a sharp decline in the photosynthesis rate (Yun *et al.* 2025). This reduction in photosynthesis, along with the accumulation of toxic ions (Na^+, metal ions) in the leaves, led to decreased carbon assimilation and reduced plant growth. On the other hand, active stomatal closure reduced transpiration to limit water loss, but this also decreased leaf cooling and nutrient transport. Moreover, pyrite toxicity could damage chloroplasts through increased ROS levels (Guo *et al.* 2023), further reducing photosynthetic efficiency. Therefore, the combination of salinity and pyrite stress drove rice plants to perform physiological modifications of the stomata, such as closure and slight reduction in number, as a water conservation strategy this physiologically resulted in lower photosynthesis and transpiration rates.

6. Plant Necrosis

Necrosis in plants refers to the condition where leaf cells die, which can be caused by various stress factors such as salinity and pyrite. In this study, the parameters of leaf necrosis length and the number of necrotic leaves were used to assess the impact of stress on the health of rice plants. The analysis of variance showed that the interaction between salinity stress and pyrite stress significantly affected the necrosis length in rice plants, but did not have a significant effect on the number of leaves affected by necrosis. The results of further tests and the average necrosis in rice plants can be seen in Table 6.

Table 6. Effect of Salinity and Pyrite Stresses on the Average Necrosis in Rice Plants

Salinity Stress	Pyrite Stress			
	L_0 (control)	L_1 (200 mg)	L_2 (400 mg)	Mean C
	Leaf Necrosis Length			
C_0 (control)	0.000±0.000^{a}	0.219±0.610^{ab}	0.218±0.102^{ab}	0.145 A
C_1 (50 mM)	1.100±0.132^{c}	0.918±0.091^{bc}	0.604±0.071^{abc}	0.874 B
C_2 (100 mM)	3.580±0.356^{e}	2.105±0.469^{d}	2.711±0.255^{d}	2.799 C
Mean L	1.560 B	1.081 A	1.178 A	
	Number of Necrotic Leaves			
C_0 (control)	0.00±0.00^{a}	0.50±0.14^{a}	0.40±0.09^{a}	0.30 A
C_1 (50 mM)	1.33±0.08^{a}	1.35±0.13^{a}	1.23±0.11^{a}	1.30 B
C_2 (100 mM)	1.23±0.07^{a}	1.10±0.07^{a}	1.45±0.07^{a}	1.33 B
Mean L	0.81 A	1.10 B	1.03 B	

Description: Values are presented as the mean±standard error (n=4). Numbers followed by lowercase letters in the same row and column indicate significant differences based on the DMRT test at the 5% significance level between combinations of salinity and pyrite treatments. Numbers followed by uppercase letters in the same row or column indicate significant differences based on the DMRT test at the 5% significance level between salinity treatments within the same pyrite level or pyrite treatments within the same salinity level. Numbers followed by different letters indicate statistically significant differences.

Tiwari *et al.* (2022) stated that at the early seedling stage of rice (approximately 21 days old), plants were highly sensitive to salinity stress. The addition of salt (NaCl) disrupted water uptake and caused osmotic-ionic stress, which triggered the production of reactive oxygen species (ROS) and cellular membrane damage (Khan *et al.* 2023). This physiological phenomenon was presumed to result in reduced photosynthetic rates and pigment content, as well as to accelerate leaf necrosis and senescence. The results of this study reflected this, as the control without salt (C_0) showed no signs of leaf necrosis (necrosis length = 0.00 cm), whereas the 100 mM salinity treatment (C_2) produced the greatest leaf necrosis length (~3.580±0.356 cm). As soil salt levels increased, there was a clear increase in the number and length of necrotic leaves ($C_2 > C_1 > C_0$). This trend was consistent with the findings of Khan *et al.* (2023), who reported that high salinity drastically inhibited vegetative growth of rice seedlings and increased leaf damage. These results indicated that optimal conditions for seedling growth were achieved under the no-salt treatment (C_0L_0), while salt application significantly increased leaf damage.

The interaction data for salinity and pyrite stress showed a tendency for pyrite to mitigate stress symptoms. The C_2L_0 treatment (100 mM without pyrite) resulted in the longest leaf necrosis length (3.580±0.356 cm), while C_0L_0 (no salt/no pyrite) showed no necrosis, confirming that the combination of high salinity and no pyrite was the most severe stress condition. The addition of pyrite (L_1/L_2 treatments) was presumed to reduce necrosis length at high salinity levels compared to C_2L_0, although its effect depended on the dose. For the number of necrotic leaves, the salinity and pyrite interaction were not significant; however, single-factor analysis showed that high salinity significantly increased the number of necrotic leaves ($C_2 \approx C_1 >> C_0$), and the no-pyrite treatment (L_0) had the lowest number of necrotic leaves compared to the pyrite treatments (L_1/L_2). This indicated that pyrite tended to reduce the severity of leaf damage, although the absolute number of damaged leaves could slightly increase as better vegetative growth produced more leaves overall.

Physiologically, the ameliorative mechanism of pyrite could be explained as follows. Oxidized pyrite released H_2SO_4, which lowered soil pH and produced sulphate (SO_4^{2-}) that reacted with calcium to form gypsum. This process enhanced Ca^{2+} uptake and facilitated the leaching of sodium ions, thereby drastically reducing soil sodicity (Ahmed *et al.*, 2024). This local acidification helped remove excess Na^+ from the root zone. In addition, Fe^{2+} released from pyrite improved the plant's iron status, which was required for the photosynthetic electron transport chain and chlorophyll synthesis. Gao *et al.*, (2024) confirmed that Fe supplementation (e.g., through EDDHA-Fe application) under alkaline-saline stress improved photosynthesis and hormone/pigment metabolic pathways in rice, thereby increasing plant tolerance and photosynthetic efficiency.

The experimental data trend aligned with this mechanism: although the total number of necrotic leaves was slightly higher in the pyrite treatments (as plants grew better and developed more leaves), the relative proportion of damage decreased. Overall, the reduction in leaf necrosis length under pyrite treatments indicated that plants receiving pyrite were better able to withstand salinity stress. Ahmed *et al.*, (2024) highlighted the benefit of sulphur/iron amendments in reducing soil salt burdens and promoting plant growth in saline areas. In other words, the improvement in soil chemical properties and nutrient status due to pyrite synergistically enhanced the physiological resilience of rice seedlings against salinity stress, as reflected by the trend of lighter leaf necrosis under pyrite treatments.

CONCLUSION

Based on the research results, it can be concluded that salinity and pyrite stress significantly affect the morphology and physiology of rice seedling growth (*Oryza sativa*) in the seedling phase. Salinity stress, particularly at high concentrations (100 mM), affects plant height due to osmotic imbalance, the accumulation of Na^+ ions inhibiting enzyme activity, and cell damage caused by oxidative stress. Meanwhile, pyrite stress, especially at high doses (400 mg), worsens growth conditions through heavy metal toxicity and disruption of nutrient absorption. The interaction between salinity and pyrite shows less synergistic effects but is still harmful, with a decrease in growth parameters such as root length, stomatal number, and leaf area, as well as an increase in leaf necrosis. Although some parameters did not show significant statistical differences, the tendency of declining growth and plant health indicates that both stresses exacerbate the physiological condition of the plant. Overall, this study confirms that salinity and pyrite stress inhibit rice seedling growth through osmotic stress mechanisms and ion toxicity. The findings emphasize the importance of environmental management and the selection of rice varieties tolerant to salinity and heavy metals to minimize the negative impact on rice plant growth.

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