

## RESPON PERTUMBUHAN DAN PERKEMBANGAN TANAMAN PADI FASE PEMBIBITAN AKIBAT SALINITAS DAN PIRIT

### *GROWTH AND DEVELOPMENT RESPONSE OF RICE PLANTS DURING THE NURSERY STAGE TO SALINITY AND PYRITE STRESS*

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#### ARTICLE HISTORY:

Received: 5 May 2025

Peer Review: 15 June 2025

Accepted: 29 January 2026

#### KEYWORDS:

Environmental stress, plant physiology, synergistic effect

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Published by Department of  
Agrotechnology, Faculty of  
Agriculture, University of  
Lampung.

#### ABSTRACT

Salinity and heavy metal contamination, including pyrite, are environmental pressures that frequently limit plant growth. This study was designed to examine how salinity and pyrite, both individually and in combination, influence the early growth performance of rice seedlings. The experiment was arranged using a completely randomized design with two treatment factors. Salinity was applied at three concentrations: 0 mM (control), 50 mM, and 100 mM. Pyrite was also tested at three levels: 0 mg (control), 200 mg, and 400 mg. Each treatment combination was replicated four times, resulting in 36 experimental units. Every plot contained 25 plants, from which 10 plants were selected as samples for observation. In total, 900 plants were involved in the experiment. Growth observations included stem length, stem diameter, leaf length, chlorophyll content, root volume, fresh weight, dry weight, and leaf color. The results demonstrated that salinity imposed a stronger and more consistent negative effect on rice growth than pyrite. Increasing salinity concentrations led to marked reductions in stem length, leaf length, chlorophyll content, and total biomass accumulation. Although pyrite also suppressed growth, particularly by reducing stem length and fresh weight, its overall impact was less extensive than that of salinity. A significant interaction between salinity and pyrite was detected for fresh weight, indicating that the combined stresses altered plant growth responses beyond the effects observed under single-stress conditions. These findings suggest that salinity serves as the main limiting factor in the early growth phase of rice, while elevated levels of pyrite can aggravate growth suppression, especially under saline conditions.

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) was the main staple food crop that serves as a major source of carbohydrates for the majority of the world's population, particularly in Asia. In Indonesia, rice plays a crucial role in maintaining national food security. According to data from Statistics Indonesia of Riau Province (2024), the population of Riau Province is projected to reach 6.728 million people in 2024. With an average rice consumption of 1.33 kg per capita per week (Statistics Indonesia, 2025), the total rice demand in Riau Province is estimated to reach approximately 465,348 tons per year.

Although rice demand is very high, local rice production in Riau Province is still unable to meet the demand. Based on projections for 2024, the harvested area of rice in Riau Province is estimated to reach 57 thousand hectares, an increase of 5.09 thousand hectares or 9.80 percent compared to 2023, which recorded 51.91 thousand hectares. Rice production in 2024 is also predicted to increase to 225.84 thousand tons of dry milled rice (DMR), a rise of 19.86 thousand tons or 9.64 percent compared to 2023, which recorded 205.97 thousand tons. Meanwhile, the rice production for consumption in 2024 is estimated to reach 129.61 thousand tons, increasing by 11.40 thousand tons or 9.64 percent compared to 118.21 thousand tons in 2023 (Statistics Indonesia of Riau Province, 2024).

Despite the increase in local rice production, the figure is still far from sufficient to meet the rice demand in Riau Province. Riau Province rice deficit reached 335,738.48 tons per year after a total rice demand of 465,348.48 tons per year. This indicates that the majority of rice needs in the province had to be fulfilled through supply from other regions. This gap highlights the urgent need to continue efforts to increase local rice production, especially through the development of cultivation technologies that can adapt to suboptimal environmental conditions.

Low rice production yield in Riau Province is because most of the rice farmers there cultivated on land that is not suitable. According to Rahmasari *et al.*, (2020) five types of suboptimal land in Indonesia were acid dry land, dry climate land, tidal swamp land, peatland, and backswamp. Pelalawan Regency, located in Riau Province, is one of the rice production centers, particularly in Kuala Kampar District, which has a paddy field area of 5,922 hectares, where rice is cultivated was carried out on tidal swamp land (Laksamana & Kurniati, 2022). Tidal swamp are a type of wetland influence by the rise and fall of the sea level along the coast and is most often describe as a condition of continuous waterlogging through the year. Additionally, some parts of the swamp differed in their salinity degree, such as saline, brackish (slightly salty), and freshwater. Therefore, utilizing this particular land faced several challenges, that is managing water, presence of pyritic soil ( $\text{FeS}_2$ ), peat layer thickness, and risk of intrusion of sea water (Rahmasari *et al.*, 2020).

Climate change has increasingly challenged rice productivity, especially through the El Niño phenomenon that intensifies seawater intrusion in coastal regions. This process increases the frequency and severity of salinity events that interfere with rice growth and development. High salt concentrations induce osmotic stress, which prevents plant roots from absorbing water, resulting in nutrient deficiencies and dehydration (Seifikalhor *et al.*, 2019). Additionally, salinity causes ion toxicity from  $\text{Na}^+$  and  $\text{Cl}^-$ , which hinders plant growth (Isayenkov & Maathuis, 2019). Reactive Oxygen Species (ROS) are created when plants are exposed to too much salt, which sets off antioxidant defense mechanisms (Jan *et al.*, 2019). Damage to cellular structures is inevitable when ROS accumulation surpasses cellular detoxification capacity (Chen *et al.*, 2021). From the early seedling stage to the reproductive phase, when stress postpones panicle emergence and grain maturity, these physiological disruptions can impede rice development (Hassan *et al.*, 2023). Additionally, active germination is disrupted and ATP production is decreased by salinity during early development (Kadam *et al.*, 2017).

Apart from salinity, another significant obstacle to rice farming is pyrite ( $\text{FeS}_2$ ) in acid sulfate soils. By promoting chlorophyll synthesis and preventing ROS buildup in plants, pyrite can have a positive physiological impact (Tang *et al.*, 2023). However, pyrite oxidizes in dry, oxygen-rich environments to produce sulfuric acid, which drastically lowers the pH of soil (Anwar & Masganti, 2021). Toxic metal ions like  $\text{Fe}^{2+}$  and  $\text{Al}^{3+}$  are mobilized by this acidification, which decreases nutrient uptake and inhibits nitrogen fixation. Additionally, nutrient leaching from the root zone is encouraged by increased acidity. Rice plants and other aquatic life may be harmed by acidic runoff from oxidized pyrite that enters irrigation channels and agricultural water bodies.

Salinity and pyrite stress coexist in many rice-growing areas, resulting in complicated environmental limitations that lower productivity. Plant selection in target environments is challenging due to the variable nature of abiotic stress in field conditions (Yullianida *et al.*, 2014). To isolate particular stressors and control environmental variables, controlled selection is therefore required in greenhouses or labs. Although these stresses have been studied separately in earlier research, little is known about how pyrite and salinity interact to affect rice seedling growth. In order to support adaptive strategies for rice cultivation in multiple-stress environments, this study,

"Growth and Development Response of Rice Plants during the Nursery Stage to Salinity and Pyrite Stress" attempts to elucidate their individual and combined effects.

## 2. MATERIALS AND METHOD

### 2.1 Experimental Design

The Seed Breeding and Certification Unit for Food Crops and Horticulture, situated at Kaharudin Nasution Street No. 69, Pekanbaru, is where this study was carried out. The study was conducted from December 2024 to January 2025, a duration of one month.

The study's materials included pyrite stones, Mendol Pelalawan rice seeds, distilled water, and NaCl. Leaf color charts, germinators, analytical balances, pH meters, rulers, and petri dishes were among the instruments utilized.

A Completely Randomized Design (CRD) with two factors was used to conduct the experiment. The first factor was salinity stress, which came in three levels: control, 50 mM, and 100 mM. The second factor was pyrite stress, which came in three levels: control, 200 mg, and 400 mg. Four replications were used for each level. The experiment consisted of 36 plots, each containing 25 plants. From each plot, 10 plants were selected using random sampling to avoid bias and ensure equal selection probability. This procedure resulted in a total of 360 sampled plants.

The observations in this research included morphological and physiological characteristics of the plants, as follows: (a) Stem length; (b) Stem diameter; (c) Leaf color; (d) Leaf length; (e) Chlorophyll content; (f) Root volume; (g) Fresh weight; (h) Dry weight. The stem length (cm) of the plants was measured on day 25, by measuring from the base of the root to the tip of the stem where the first leaf is attached, using a ruler. The stem diameter (cm) was measured using a caliper on day 25. Leaf color was measured by matching the leaf with a leaf color chart on day 25, to observe morphological changes in the leaves.

Leaf length (cm) was measured from the tip to the base of the leaf using a ruler on the final day of observation. The total chlorophyll content of the leaves was measured on day 25 using a SPAD-502 chlorophyll meter. Root volume (cm<sup>3</sup>) was measured on day 25, by cutting the roots from the stem and cleaning them. The cleaned roots were placed into a graduated cylinder filled with water. The initial water volume was recorded before inserting the roots, and the final volume was recorded after inserting them. Root volume was calculated using the following formula:

$$\text{Root volume} = \text{Final volume} - \text{Initial volume} \quad (1)$$

Fresh plant weight (g) was measured on day 25. Fresh plant samples were weighed using an analytical balance to obtain the fresh weight. Dry plant weight (g) was measured by drying the plants in an oven at 70°C for 72 hours, then weighing them to determine the dry weight.

### 2.2 Data Analysis

To ascertain the impact of salinity and pyrite stress treatments, the physiological quality data of the seeds were examined. Analysis of variance (F-test) was used to analyze the data in this study using SPSS. Duncan's Multiple Range Test (DMRT) at the 5% significance level should be used to confirm F-test results that show a significant effect.

## 3. RESULT AND DISCUSSION

### 3.1 Effect of Various Stresses on the Growth and Development of Rice Plants

In addition to the potential presence of pyrite, tidal swamp areas also had the potential to experience high salinity (Pradiko *et al.*, 2017). Both salinity and pyrite can inhibit the growth and

development processes of rice plants. In this study, the combined effects of salinity and pyrite stress on rice seedling growth and development were evaluated based on various morphological and physiological parameters, as shown in Table 1.

Based on the analysis of variance presented in Table 1, salinity stress significantly affected several growth parameters, including stem length, stem diameter, leaf length, chlorophyll content, fresh weight, and dry weight of the plants. This suggests that salinity decreased plant vigor and biomass accumulation, most likely as a result of ionic and osmotic stress that hindered the uptake of nutrients and water. Stem length and fresh weight were greatly affected by pyrite stress alone, indicating that soil acidity and possible toxicity from pyrite oxidation may have disrupted plant growth and metabolism. Additionally, the substantial interaction between salinity and pyrite stress on plant fresh weight demonstrates that the two stressors worked in concert to intensify growth inhibition when compared to single stress conditions.

### 3.2 Stem Length

Stem length was one of the key morphological parameters observed in this study, as it directly affects plant structure and strength in supporting the leaf canopy and panicle. A stem that was sufficiently long and strong could help the rice plant remain upright, especially under wind pressure or when bearing grains. Based on the research results, the effect of various levels of salinity and pyrite stress on the average stem length of rice plants was shown in Table 2.

Table 1. Recapitulation of Analysis of Variance on the Effect of Salinity and Pyrite Stress on the Growth and Development of Rice Plants in the Seedling Stage.

| The Growth of Rice Plants | S  | P  | SxP | CV (%)             |
|---------------------------|----|----|-----|--------------------|
| Stem Length               | *  | *  | ns  | 9,13               |
| Stem Diameter             | *  | ns | ns  | 9,69               |
| Leaf Length               | *  | ns | ns  | 11,87              |
| Plant Chlorophyll         | *  | ns | ns  | 13,35 <sup>t</sup> |
| Root Volume               | ns | ns | ns  | -                  |
| Fresh Weight              | *  | *  | *   | 1,32               |
| Dry Weight                | *  | ns | ns  | 8,8                |

Note : (ns) no significant, (\*) significant at  $p < 0.05$ , S = salinity stress, P = pyrite stress. CV = Coefficient of Variation, <sup>t</sup> = data after square-root transformation.

Table 2. Effect of Salinity and Pyrite Stress on the Average Stem Length of Rice Plants.

| Salinity Stress          | Pyrite Stress            |                         |                         | Mean S |
|--------------------------|--------------------------|-------------------------|-------------------------|--------|
|                          | P <sub>0</sub> (control) | P <sub>1</sub> (200 mg) | P <sub>2</sub> (400 mg) |        |
| S <sub>0</sub> (control) | 7.49±0.31 <sup>a</sup>   | 6.57±0.37 <sup>a</sup>  | 6.88±0.16 <sup>a</sup>  | 6.98 C |
| S <sub>1</sub> (50 mM)   | 6.30±0.26 <sup>a</sup>   | 6.31±0.32 <sup>a</sup>  | 6.31±0.42 <sup>a</sup>  | 6.31 B |
| S <sub>2</sub> (100 mM)  | 6.33±0.23 <sup>a</sup>   | 5.32±0.21 <sup>a</sup>  | 5.68±0.24 <sup>a</sup>  | 5.78 A |
| Mean P                   | 6.71 B                   | 6.07 B                  | 6.29 AB                 |        |

Note: 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.

Table 2 showed that the single factor of salinity stress had a statistically significant effect on the stem length of rice plants. Plants grown without salinity stress (S<sub>0</sub>) had the highest average stem length of 6.98 cm, while those subjected to 100 mM salinity stress (S<sub>2</sub>) showed the lowest stem length, measuring 5.78 cm.

The decrease in stem length under salinity stress was primarily associated with physiological disruptions, such as a reduced ability of plants to absorb water due to the low osmotic potential of the soil. Ganapati et al. (2022) explained that when rice plants are exposed to saline soil, sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ) tend to build up inside the tissues, while important nutrients such as potassium ( $\text{K}^+$ ) and calcium ( $\text{Ca}^{2+}$ ) decline. This shift in ion balance places the plant under physiological strain. Excess  $\text{Na}^+$  and  $\text{Cl}^-$  can interfere with normal metabolic pathways, including the formation of growth hormones like auxin and gibberellin. Since these hormones regulate cell elongation, any disruption in their production can slow stem growth. A comparable trend appeared in the pyrite treatments. Plants grown without added pyrite (P0) produced the longest stems, with an average length of 6.71 cm. When 200 mg of pyrite was applied (P1), the average stem length decreased to 6.07 cm. This reduction suggests that pyrite, possibly through changes in soil chemical properties or metal availability, limited stem development. Together, these observations show that both salinity and pyrite can restrict stem elongation, although through somewhat different physiological pathways.

Pyrite is the rightful owner of sulfide that, when they are oxidized, they give up to the generate sulfuric acid ( $\text{H}_2\text{SO}_4$ ). The produced oxidation process consequently increased the solubility of the state of iron ( $\text{Fe}^{2+}$ ) and the gases ( $\text{H}_2\text{S}$ ), both of which are toxic to rice plants and reduce the availability of essential nutrients (Anwar & Masganti, 2021). The resulting soil acidity interfered with the uptake of nutrients that are necessary for stem development and elongation such as nitrogen, phosphorus, and potassium. Furthermore, the oxidative stress induced by pyrite exposure can trigger the overproduction of reactive oxygen species (ROS), leading to cellular membrane damage and inhibition of cell division and elongation in stem tissues.

The restricted stem growth also indicated that the plants had difficulty in keeping their structural integrity and functionality that are among the things necessary for water and nutrient transport. The shortened stems showed that the development of the vascular tissue is disturbed, and that is very important because the vascular tissue is the part that is responsible for the transport of water and nutrients from the roots to the shoots. In addition, the limited stem length was the most directly related to the reduction of the total biomass and thus the rice yield was affected.

Table 1. Effect of Salinity and Pyrite Stress on the Average Stem Diameter of Rice Plants.

| Salinity Stress          | Pyrite Stress            |                         |                         | Mean S |
|--------------------------|--------------------------|-------------------------|-------------------------|--------|
|                          | P <sub>0</sub> (control) | P <sub>1</sub> (200 mg) | P <sub>2</sub> (400 mg) |        |
| S <sub>0</sub> (control) | 0.68±0.05 <sup>a</sup>   | 0.71±0.06 <sup>a</sup>  | 0.63±0.04 <sup>a</sup>  | 0.67 A |
| S <sub>1</sub> (50 mM)   | 0.77±0.04 <sup>a</sup>   | 0.76±0.03 <sup>a</sup>  | 0.71±0.02 <sup>a</sup>  | 0.75 B |
| S <sub>2</sub> (100 mM)  | 0.78±0.03 <sup>a</sup>   | 0.70±0.04 <sup>a</sup>  | 0.79±0.02 <sup>a</sup>  | 0.76 B |
| Mean P                   | 0.74 A                   | 0.72 A                  | 0.71 A                  |        |

Note: 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.

### 3.3 Stem Diameter

Stem diameter is an important physiological parameter that reflects the plant's capacity for nutrient and water transport. This experiment analyzed the stem diameter of rice plants to find out

how the plants reacted to salinity stress, pyrite stress, and the combination of two stresses, consequently exposing the rice plants' physiological condition. A stem diameter at the best level could have a positive impact on the functioning of the vascular tissues (xylem and phloem) thus, allowing a more efficient transport of water and nutrients to all parts of the plant.

Table 3 revealed that salinity stress was the main factor affecting the stem diameter. The results of the experiment implied that salinity at 50 mM (S1) and 100 mM (S2) levels of salts in water result in a trend to increase stem diameter, which were 0.75 cm and 0.76 cm respectively, as compared to the non-salinity condition (S0), which was 0.67 cm. The increase in stem diameter with salinity stress probably represented the plant's adaptive response to environmental stress. It is known that a plant with a wider stem has more structural strength which has been proved to be a vital parameter for not only supporting the leaves, but also the panicles and other reproductive organs. Plants with sturdy stems were also more resistant to lodging (Syahputra, 2021).

Thicker stems allowed for more efficient transport of water, nutrients, and photosynthetic products, supporting both vegetative and reproductive growth. Plants with an exclusion mechanism stored salts in very low concentrations in the stem and shoots because they could retranslocate salts back to the root zone, while plants with an inclusion mechanism stored salts in higher concentrations in the stem and shoots. Ion transport played a critical role in plant adaptation to salinity, regardless of the specific tolerance mechanism (Sopandie, 2013).

When rice plants grow thicker stems under stress, it is usually not random. It is a structural response. A sturdier stem can help the plant cope with unfavorable conditions, including the presence of excess salt. Research by Zhang *et al.*, (2025) showed that changes inside the stem's vascular tissue—such as smaller xylem vessels and a higher number of vascular bundles—can limit the movement of sodium ( $\text{Na}^+$ ) through the plant. By restricting how far toxic ions travel, the plant reduces internal damage. At the same time, thicker stems often contain more cellulose, which strengthens the tissue. Stronger stems are better at supporting leaves and panicles and are less likely to collapse under wind or heavy rain (Sackey *et al.*, 2025).

Saline conditions also reduce the ability of roots to absorb water because high salt concentration increases osmotic pressure in the soil. To compensate, plants may invest more in reinforcing their transport system. This reinforcement often involves thicker cell walls due to additional lignin deposition. Lignin makes tissues more rigid, helping stems tolerate both mechanical pressure and ionic stress. Increased lignification under salt exposure is therefore considered part of a defensive adjustment (Zhang *et al.*, 2025).

However, the presence of excessive pyrite can shift this pattern. When pyrite releases soluble metals into the soil, these elements may disrupt nutrient uptake and interfere with normal cell processes. Under heavy metal stress, plants tend to slow down structural growth because more energy is directed toward maintaining basic physiological balance. As noted by Wang *et al.*, (2024) rice exposed to elevated metal concentrations often develops shorter and thinner stems due to impaired cell expansion and reduced vascular formation.

### 3.4 Leaf Length

Table 4 presented data on the effects of various levels of salinity and pyrite stress on the average leaf length of rice plants. Different levels of stress affected rice leaf length. Leaves played a vital role in the photosynthesis process, thus influencing yield when subjected to suboptimal environmental conditions.

Table 1 shows that the effect of salinity and pyrite stress on the potential leaf length of rice plants was not statistically significant, indicating that their interaction did not produce measurable differences in leaf growth. However, when analysed individually, salinity stress had a significant

effect on leaf length. Table 4 shows that the longest leaf length was found in the treatment without salinity stress (S<sub>0</sub>), measuring 11.21 cm, which was significantly different from S<sub>1</sub> (9.63 cm) and S<sub>2</sub> (9.18 cm). This indicates that conditions without stress allowed the plants to grow optimally. However, when analyzed individually, salinity stress had a significant effect on leaf length. This was caused by physiological disturbances due to the accumulation of salt ions.

Shorter leaves indicate that the plants reduce their surface area to minimize water loss through transpiration under stress conditions. Inhibited leaf growth also indicates that the plant's energy is more directed toward survival mechanisms rather than expanding vegetative organs. According to Lu & Fricke (2023), the root system uptake NaCl and transports it to the shoot continuously the transpiration stream which results in a very high concentration of NaCl in the leaf cells. This condition is no longer sustainable and the leaf cells' biochemical processes will be inhibited, especially photosynthesis, and in addition, a nutrient imbalance will ensue. Osmotic stress caused by salinity or drought leads to water deficiency in plants, thereby inhibiting the enzymatic activity required for cell division and leaf cell elongation. As a result, leaves grow shorter. The restriction of leaf length and area is an adaptive mechanism to reduce the transpiration surface area, thereby minimizing water loss. This response was observed by Ma *et al.*, (2020) who reported that under osmotic stress, cell turgor pressure decreases, and both leaf size and number become smaller. With a smaller leaf area, transpiration capacity is also reduced, helping the plant conserve water. However, reduced transpiration also decreases the leaf's evaporative cooling ability, which can affect the plant's temperature regulation and the transport of water and nutrients throughout the plant.

Table 2. Effect of Salinity and Pyrite Stress on the Average Leaf Length of Rice Plants.

| Salinity Stress          | Pyrite Stress             |                           |                           |         |
|--------------------------|---------------------------|---------------------------|---------------------------|---------|
|                          | P <sub>0</sub> (control)  | P <sub>1</sub> (200 mg)   | P <sub>2</sub> (400 mg)   | Mean S  |
| S <sub>0</sub> (control) | 12.34 ± 0.32 <sup>a</sup> | 10.99 ± 0.68 <sup>a</sup> | 10.29 ± 0.42 <sup>a</sup> | 11.21 B |
| S <sub>1</sub> (50 mM)   | 9.01 ± 0.77 <sup>a</sup>  | 10.46 ± 0.59 <sup>a</sup> | 9.42 ± 0.87 <sup>a</sup>  | 9.63 A  |
| S <sub>2</sub> (100 mM)  | 8.72 ± 0.59 <sup>a</sup>  | 9.08 ± 0.51 <sup>a</sup>  | 9.74 ± 0.37 <sup>a</sup>  | 9.18 A  |
| Mean P                   | 10.02 A                   | 10.18 A                   | 9.81 A                    |         |

Note: 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.

Table 3. Effect of Salinity and Pyrite Stress on the Average Chlorophyll Content of Rice Plants.

| Salinity Stress          | Pyrite Stress             |                           |                           |         |
|--------------------------|---------------------------|---------------------------|---------------------------|---------|
|                          | P <sub>0</sub> (control)  | P <sub>1</sub> (200 mg)   | P <sub>2</sub> (400 mg)   | Mean S  |
| S <sub>0</sub> (control) | 29.84 ± 0.19 <sup>a</sup> | 29.73 ± 0.41 <sup>a</sup> | 11.60 ± 0.25 <sup>a</sup> | 23.72 A |
| S <sub>1</sub> (50 mM)   | 25.75 ± 0.14 <sup>a</sup> | 40.53 ± 0.10 <sup>a</sup> | 28.70 ± 0.10 <sup>a</sup> | 31.66 B |
| S <sub>2</sub> (100 mM)  | 34.86 ± 0.12 <sup>a</sup> | 34.48 ± 0.20 <sup>a</sup> | 35.33 ± 0.28 <sup>a</sup> | 34.89 B |
| Mean P                   | 30.15 A                   | 34.91 A                   | 25.08 A                   |         |

Note: 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.

### 3.5 Plant Chlorophyll

In a plant, chlorophyll is the most important pigment in the Leaves, that performs the process of photosynthesis directly. Optimal chlorophyll content results in the efficient conversion of solar energy into chemical energy, which is the basis of plant growth and development. According to the

experiment, the chlorophyll content of rice plants under pyrite treatment or the interaction between salinity and pyrite had no significant influence, however, it was significantly affected by salinity stress alone.

Table 5 presents data on the effect of salinity stress on the average chlorophyll content of rice plants. Chlorophyll content serves as an important indicator for evaluating how salinity and pyrite stresses influence plant growth and the extent to which their interaction affects chlorophyll concentration in rice leaves.

Table 1 shows that the effects of salinity stress and pyrite on the chlorophyll content of rice plants were not significantly different. Table 5 showed that chlorophyll content increased with salinity stress at 50 mM (S1) to 100 mM (S2). The highest chlorophyll content was found at S2 (34.89), while the lowest was at S0 (23.72). The increase in chlorophyll content at 50 mM to 100 mM salinity stress indicated an adaptive response in rice plants. Salinity stress can stimulate an increase in chlorophyll production as a compensatory mechanism to enhance photosynthesis. Du *et al.*, (2025) stated that in rice under salinity stress, chlorophyll a and b contents increased significantly, which helps maintain photosynthetic activity and provides energy for the plant under stressful conditions. At the same time, plants exposed to high salinity also synthesize osmoprotectants (soluble compounds such as proline, glycine betaine, certain sugars, and polyamines) that accumulate in the chloroplasts to maintain the integrity of membranes and organelles. When rice plants face osmotic stress, they produce small organic compounds known as osmolytes. These molecules act like internal stabilizers, helping protect chloroplasts and cell membranes from damage. As long as this protection holds, chlorophyll can remain relatively stable. However, if salt exposure continues for a long period or becomes more intense, the plant's internal balance begins to weaken. Structural damage may follow, and chlorophyll levels gradually decline (Wang *et al.*, 2024).

Chlorophyll is directly linked to the plant's capacity to generate energy through photosynthesis. With sufficient pigment, the plant can maintain carbohydrate production, which supports height growth, tiller development, and grain formation. An increase in chlorophyll under moderate stress is often interpreted as a short-term adjustment strategy, allowing the plant to sustain metabolic activity despite less favorable conditions (Du *et al.*, 2025).

On the other hand, a noticeable drop in chlorophyll especially in treatments involving higher pyrite levels suggests internal physiological disruption. Soluble iron ( $\text{Fe}^{2+}$ ) released from pyrite can interfere with chlorophyll synthesis and damage chloroplast structure. Excess iron may also disturb the uptake and balance of other micronutrients such as manganese (Yuan *et al.*, 2024). Once these processes are affected, photosynthetic performance declines, reducing the plant's ability to grow and complete its life cycle under stress.

### 3.6 Root Volume

This constant root volume could indicate that the rice plants in this study, despite being subjected to salinity stress, did not experience a significant decline in root development. This could be attributed to several factors. As seen in Table 6, the effects of salinity stress and pyrites on the root volume of rice plants were presented.

The stable root volume observed in this study indicates that rice plants subjected to salinity and pyrite treatments did not experience significant suppression of root development. This condition may not only be attributed to the use of salinity-tolerant varieties and proper water management, but also to the regulation of soil chemical conditions that influence rhizosphere stability. Utami *et al.*, (2025) reported that continuous flooding increased  $\text{Fe}^{2+}$  solubility under strongly reducing conditions, which restricted root growth due to prolonged exposure to soluble iron and limited oxygen availability. High levels of  $\text{Fe}^{2+}$  in the soil can create problems for rice plants. When too much

of this soluble iron surrounds the roots, it can interfere with nutrient absorption and limit root elongation. However, previous findings showed that when paddy fields are not kept continuously submerged but instead allowed to dry for a period, part of the  $\text{Fe}^{2+}$  is converted into  $\text{Fe}^{3+}$ . This oxidized form is far less soluble and tends to settle as compounds such as iron hydroxide. Because of that shift, the intensity of iron toxicity during the next flooding phase becomes lower. Repeated wet-dry cycles also help prevent drastic pH fluctuations, which is especially important in Ultisol soils that are already chemically sensitive.

In this experiment, root volume remained relatively similar across treatments. This pattern suggests that the irrigation approach may have limited the accumulation of soluble iron near the roots, even though salinity stress was present. Salinity alone can disturb osmotic balance and affect root membrane function, yet stable soil conditions seem to have prevented additional stress from excess metals. The addition of pyrite did not visibly alter root size either. Its effects may have appeared in other aspects of plant performance, such as chlorophyll levels or fresh weight, rather than in structural

changes belowground. Overall, the steady root volume observed here supports earlier work by Utami *et al.*, (2025) which emphasized that alternating flooding and drying is an effective way to manage iron toxicity and maintain root function in difficult soil conditions.

Table 4. Effect of Salinity Stress and Pyrite on the Average Root Volume of Rice Plant.

| Salinity Stress          | Pyrite Stress            |                         |                         | Mean S |
|--------------------------|--------------------------|-------------------------|-------------------------|--------|
|                          | P <sub>0</sub> (control) | P <sub>1</sub> (200 mg) | P <sub>2</sub> (400 mg) |        |
| S <sub>0</sub> (control) | 0,1 <sup>a</sup>         | 0,1 <sup>a</sup>        | 0,1 <sup>a</sup>        | 0,1 A  |
| S <sub>1</sub> (50 mM)   | 0,1 <sup>a</sup>         | 0,1 <sup>a</sup>        | 0,1 <sup>a</sup>        | 0,1 A  |
| S <sub>2</sub> (100 mM)  | 0,1 <sup>a</sup>         | 0,1 <sup>a</sup>        | 0,1 <sup>a</sup>        | 0,1 A  |
| Mean P                   | 0,1 A                    | 0,1 A                   | 0,1 A                   |        |

Note: 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.

Table 5. Effect of Salinity Stress and Pyrite on the Average Fresh Weight of Rice Plants.

| Salinity Stress          | Pyrite Stress              |                            |                           | Mean S   |
|--------------------------|----------------------------|----------------------------|---------------------------|----------|
|                          | P <sub>0</sub> (control)   | P <sub>1</sub> (200 mg)    | P <sub>2</sub> (400 mg)   |          |
| S <sub>0</sub> (control) | 0.2664±0.016 <sup>b</sup>  | 0.1605±0.015 <sup>a</sup>  | 0.1572±0.006 <sup>a</sup> | 0.1947 A |
| S <sub>1</sub> (50 mM)   | 0.1946±0.009 <sup>ab</sup> | 0.2280±0.002 <sup>ab</sup> | 0.1874±0.005 <sup>a</sup> | 0.2033 A |
| S <sub>2</sub> (100 mM)  | 0.5524±0.037 <sup>d</sup>  | 0.5360±0.053 <sup>d</sup>  | 0.3780±0.029 <sup>c</sup> | 0.4888 B |
| Mean P                   | 0.3378 B                   | 0.3081 B                   | 0.2409 A                  |          |

Note: 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.

### 3.7 Fresh Weight and Dry Weight of Plant

Fresh weight is just the total weight of the plant, roots, stems, leaves, everything measured right after harvest, before it dries out. It's a snapshot of how much the plant has managed to grow by that point. In this study, measuring fresh weight was a way to see how rice plants reacted to salt and pyrite, and how these treatments affected their shape and overall health.

Table 7 illustrates the interaction between salinity and pyrite, providing a clearer understanding of how both factors jointly influence the growth and development of rice plants. Among all treatments, rice plants exposed to 100 mM salinity without pyrite (S2P0) produced the highest fresh weight. The addition of 200 mg pyrite (S2P1) under the same salinity level slightly reduced fresh weight, although the difference was not statistically significant. However, applying 400 mg pyrite (S2P2) at 100 mM salinity caused a sharp and significant decrease in fresh weight. Compared to the 50 mM (S1) and non-saline (S0) treatments, plants under 100 mM salinity without pyrite still maintained relatively higher fresh weight, suggesting that moderate salinity could be tolerated when pyrite levels were low. In contrast, excessive pyrite application appeared to induce toxicity that interfered with plant metabolism and limited growth.

Rice plants treated with S1P1 may show signs of tolerance to stress, as indicated by the increased chlorophyll content. It is known that the Mendol Pelalawan variety is resistant to salinity stress (Riau Agricultural Technology Assessment Institute, 2019). However, at the same time, this stress limits biomass accumulation, as seen in the lower fresh weight, because the plants must allocate energy and resources to survive rather than to grow plant organs.

Although S1P1 exhibited higher chlorophyll content, the lower fresh weight indicates that the plants are under stress, hindering physical growth despite increased chlorophyll production. High chlorophyll under stress may reflect the plant's effort to maintain photosynthesis, but this does not guarantee optimal photosynthesis efficiency, as salinity and pyrite stress disrupt other factors that affect overall plant growth, including water and nutrient transport, as well as energy production from photosynthesis. Du *et al.*, (2025) stated that the decrease in chlorophyll, particularly at high pyrite doses, indicates physiological disturbances in plants. Heavy metal stress (such as  $\text{Fe}^{2+}$  in pyrite) can reduce chlorophyll synthesis and damage the chloroplast structure, as well as interfere with the uptake of essential elements such as iron and manganese.

Dry weight refers to the plant's mass measured after the water content in the plant is removed through drying. Dry weight reflects the actual biomass produced by the plant, which represents its true growth and development, as it is not influenced by varying water content.

Table 6. Effect of Salinity Stress and Pyrite on the average Dry Weight of Rice Plants.

| Salinity Stress          | Pyrite Stress              |                           |                           |          |
|--------------------------|----------------------------|---------------------------|---------------------------|----------|
|                          | P <sub>0</sub> (control)   | P <sub>1</sub> (200 mg)   | P <sub>2</sub> (400 mg)   | Mean S   |
| S <sub>0</sub> (control) | 0.1289±0.002 <sup>a</sup>  | 0.1369±0.005 <sup>a</sup> | 0.1355±0.005 <sup>a</sup> | 0.1337 A |
| S <sub>1</sub> (50 mM)   | 0.1597±0.007 <sup>a</sup>  | 0.1689±0.003 <sup>a</sup> | 0.1565±0.003 <sup>a</sup> | 0.1617 B |
| S <sub>2</sub> (100 mM)  | 0.1568 ±0.014 <sup>a</sup> | 0.1668±0.008 <sup>a</sup> | 0.1696±0.005 <sup>a</sup> | 0.1644 B |
| Mean P                   | 0.1485 A                   | 0.1575 A                  | 0.1538 A                  |          |

Note: 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.

Although salinity stress typically inhibits plant growth, the results of this study revealed that, the increase in dry biomass at higher salinity treatments (S2) indicated the rice plants tolerance and adaptive responses, such as osmolyte accumulation and enhance root development. The application of pyrite to plants exposed to salinity might contribute to enhancing stress resistance and supporting better growth, even though there was a decrease in dry weight in some treatments. According to the Riau Agricultural Technology Assessment Institute (2019), the Mendol Pelalawan variety has been

reported to exhibit salinity tolerance, which could explain the plants' ability to maintain growth and accumulate biomass even under 50 mM and 100 mM salinity conditions. Varieties with salinity tolerance possess various physiological and morphological mechanisms that allow them to survive and grow well in saline conditions, which in turn supports the increase in biomass and optimal development of rice plants.

### 3.8 Leaf Color

The color of rice plant leaves is influenced by various physiological and environmental factors, including chlorophyll content, nutrient availability, and stressors such as salinity and pyrite. Darker or greener leaves indicate higher chlorophyll content, which functions more effectively in photosynthesis. In contrast, lighter or yellowing leaves suggest a reduction in chlorophyll, which may be associated with stress or disruptions in chlorophyll synthesis.

Leaf color can provide a quick visual clue about a plant's physiological condition. In rice, darker green leaves generally indicate a higher concentration of chlorophyll, the pigment responsible for capturing light energy during photosynthesis (Du *et al.*, 2025). When chlorophyll levels decline, leaves tend to lose their green intensity and may shift toward yellow or brown tones. This change often occurs when plants are exposed to stress, including high salinity or imbalanced nutrient supply.

Rahayu *et al.*, (2020) reported that increasing salt concentrations reduced leaf greenness in rice, as reflected by lower scores on the leaf color chart. Under saline conditions, the osmotic pressure of the soil solution rises, making it more difficult for roots to absorb water. In addition, excess sodium competes with essential nutrients such as nitrogen, magnesium, and iron key elements required for chlorophyll formation. As nutrient uptake becomes restricted, chlorophyll synthesis declines and visible yellowing may develop.

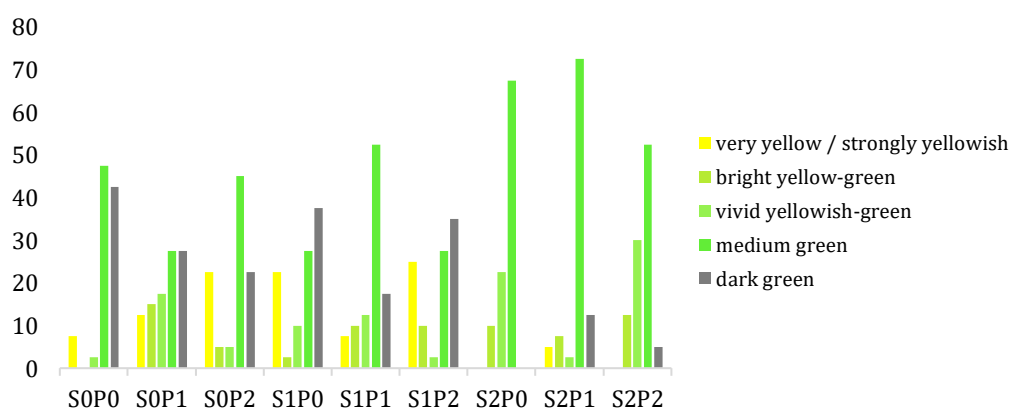


Figure 1. Leaf Color.

In the present experiment, plants grown without salinity or pyrite (S0P0) mostly showed medium to dark green leaves, consistent with a chlorophyll average of 23.72 under non-saline conditions. This suggests that photosynthetic capacity remained stable when water and nutrient uptake were not limited. Interestingly, in the S1P1 treatment (50 mM salinity combined with 200 mg pyrite), most leaves also remained medium green (52.5%) or dark green (17.5%), although some displayed lighter shades. The chlorophyll value increased to 40.53, which may indicate a temporary physiological adjustment to moderate stress.

Nevertheless, chlorophyll concentration does not automatically reflect actual photosynthetic performance. Salinity can cause partial stomatal closure, limiting CO<sub>2</sub> entry and slowing carbon

assimilation (Loudari *et al.*, 2022). Furthermore, the interaction between salinity and pyrite may influence soil pH and increase the availability of iron and manganese (Yuan *et al.*, 2024), which at excessive levels can disrupt chloroplast function. Therefore, even when leaves appear relatively green, underlying physiological constraints may still restrict plant growth.

#### 4. CONCLUSIONS

In this study, salt exposure clearly had the strongest influence on the early growth of rice seedlings. As the concentration of salinity increased, plants became shorter, stems were thinner, leaves were smaller, chlorophyll levels declined, and both fresh and dry weights decreased. These patterns show that excess salt disrupts normal growth processes and limits biomass formation from the early stages. The presence of pyrite also affected plant performance, although its impact was not as widespread. The main changes were seen in reduced plant height and lower fresh weight. At higher application levels, the negative effects became more evident. This response is closely related to changes in soil conditions, particularly increasing acidity and greater release of soluble metal ions, which can interfere with root activity and nutrient uptake. When both stress factors were applied together, the effect was more severe than when each was given separately. The reduction in fresh biomass was especially noticeable under high salinity combined with elevated pyrite. This indicates that rice seedlings respond differently under simultaneous stress, with growth suppression becoming more pronounced. Some adaptive tendencies were observed at moderate salinity, such as slightly thicker stems and improved chlorophyll content in certain varieties. These responses may reflect internal adjustment mechanisms that help plants cope with unfavorable conditions. Overall, salinity remains the dominant constraint on rice seedling growth, while pyrite can amplify its impact, particularly in soils where both stresses occur together.

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