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Evaluation of the Physiological Quality of Rice Seeds (*Oryza sativa*) under Salinity and Pyrite Stress Conditions

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Abstract. *Salinity and pyrite are abiotic stress factors that can affect the physiological quality of rice seeds. This study aimed to evaluate the effects of salinity and pyrite stress on the viability and vigor of rice seeds. The research was conducted using a completely randomized design (CRD) with two factors: salinity levels (0 mM, 50 mM, and 100 mM NaCl) and pyrite dosages (0 mg, 200 mg, and 400 mg). The observed parameters included germination, maximum growth potential, vigor index, growth speed, simultaneous growth, and growth rate. The results showed that salinity stress significantly affected the vigor index and growth speed, while pyrite stress only influenced the growth speed. The interaction between the two factors did not show a significant effect on all physiological quality parameters of the seeds. However, there was a tendency for a decline in germination percentage, maximum growth potential, vigor index, growth speed, and simultaneous growth. The combination of high salinity stress (100 mM) and high pyrite dosage (400 mg) caused more pronounced growth retardation, particularly after day 10. Although rice seeds were able to maintain tolerance at low to moderate stress levels, an increase in the intensity of stress from both factors could hinder water and nutrient absorption, thereby reducing overall growth performance. The findings of this study provide insights into the tolerance limits of rice seeds to salinity and pyrite stress, which can serve as a basis for managing suboptimal lands for more sustainable rice production.*

Keywords: *salinity stress; pyrite stress; rice seed; physiological quality.*

Type of the Paper: Regular Article.

1. Introduction

Rice is a strategic commodity that plays a crucial role in supporting food security in Riau Province. However, the production potential of rice in this region is facing challenges due to the dominance of swamp and peatlands, covering 55,822.02 hectares of established rice fields. These lands consist of 2,723.66 hectares of peat cultivation land, 11,098.58 hectares of tidal swamps, and 40,892.94 hectares of floodplain swamps, excluding rainfed or dry land that has the potential to be utilized as upland rice fields [1]. With a seed requirement of 25 kg per hectare, the total seed demand for tidal swamp areas alone reaches approximately 1,022 tons. This large demand highlights the urgency of providing high-quality seeds capable of adapting to extreme environmental conditions, such as salinity stress and the presence of pyrite layers.

Tidal swamp areas, which account for more than 70% of the total rice fields in Riau, often experience high salinity levels due to seawater intrusion, particularly in coastal areas such as

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Bengkalis, Pelalawan, Siak, and Indragiri Hilir Regencies. Rustiati et al. [2] stated that in coastal areas, soil salinity ranged from EC 2 dS/m to 18 dS/m during the dry season. This exceeds the physiological tolerance threshold of rice plants, which generally is only 3 dS/m. An increase in salinity above this threshold results in a 12% reduction in crop yield for every 1 dS/m increase above the critical limit. This is caused by an excess of Na+ and Cl- ions, leading to osmotic imbalance, nutrient absorption disruption, cell membrane damage, and a significant decline in seed germination and plant growth [3], impacting land productivity and the sustainability of farming in these areas.

Besides salinity, tidal swamp areas are also prone to pyrite (FeS_2) oxidation, a process that can drastically lower soil pH by as much as 2 to 3 units. Pyrite, a sulfur-based mineral, typically forms in waterlogged soils rich in organic material, where the presence of sulfur often comes from seawater intrusion [4]. When exposed to air, this compound generates sulfuric acid, which in turn increases the release of toxic ions like Fe^{3+} and Al^{3+}. These ions are harmful to plants, as they can restrict root development and interfere with vital enzymatic processes needed for growth [3]. In such challenging environments, having good seed physiological quality becomes crucial. Seeds that exhibit strong vigor, excellent germination performance, and better resistance to abiotic stress are more likely to thrive and grow optimally in swampy or marginal lands.

In many cases, farmers tend to choose seeds based only on their physical appearance, overlooking the more essential physiological traits that actually determine how well seeds can germinate and grow, especially under stressful conditions. In fact, certain superior varieties such as Inpara 8 have shown resilience against Fe toxicity, while Inpari 33 demonstrates tolerance to salinity during the early seedling stage [5]. However, these varieties were still not fully able to address the combination of salinity and pyrite stress. Therefore, evaluating the physiological quality of rice seeds under these dual stress conditions is highly relevant to support agricultural land diversification programs in Riau. This research is expected to provide scientific recommendations for farmers and policymakers regarding the use of quality and appropriate seeds to support sustainable food security.

2. Materials and Methods

2.1. Experimental Design

This research was conducted over a period of two months at the UPT Seed Production and Certification Center for Food Crops and Horticulture, located at Kaharudin Nasution Street No. 69, Pekanbaru.

The materials used in this research included NaCl, distilled water (aquades), pyrite stones, label paper, plain paper, Mendol Pelalawan rice seeds, and plastic. The equipment utilized consisted of stationery, a germinator, a ruler, a pH meter, and an analytical scale.

WThe research was carried out experimentally using a Completely Randomized Design (CRD) with two factors. The first factor was salinity stress, which had three levels: control, 50 mM, and 100 mM. The second factor was pyrite stress, also with three levels: control, 200 mg, and 400 mg. Each level had four replications, resulting in a total of 36 experimental units. Each replication contained 25 seeds, with 10 seeds sampled from each, making a total of 900 seeds used in the study.

The first solution prepared was the saline solution, made by dissolving NaCl according to the required dosage. Then, pyrite was weighed according to the treatment. After that, distilled water was added or mixed with the saline solution according to the treatment combinations.

The parameters observed during the study were germination percentage, maximum growth potential, vigor index, growth speed, simultaneous growth, and growth rate.

2.2. Data Analysis

The results of the physiological quality evaluation of the seeds were analyzed to determine whether there was an influence of salinity stress and pyrite stress. The data analysis in this study involved analysis of variance (F test) using the SPSS application and Excel for growth rate analysis. Results of the F test that showed a significant effect, which was further tested using Duncan’s Multiple Range Test (DMRT) at the 5% significance level.

3. Results and Discussion

Based on the data analysis results, it was observed that the interaction between salinity stress and pyrite did not give a significant effect on all variables of seed physiological quality. Meanwhile, the main effect of salinity stress showed a significantly different effect on the observed variables of vigor index and growth rate. The main effect of pyrite stress showed a significantly different effect on the growth rate variable.

3.1 Germination percentage (%)

The results of the variance analysis showed that the interaction between salinity stress and pyrite stress had no significant effect on the germination percentage parameter of rice plants. The results of further tests and the average germination percentage of rice plants are presented in Fig. 1.

Fig. 1 shows that the effects of salinity stress and pyrite stress on rice seed germination are not statistically significant. However, when observing the percentage of germination, the presence of salinity stress and pyrite stress both in single and combined treatments tends to reduce the germination rate of rice plants. In the control treatment (C_0L_0), the percentage of germination rate was 98%, which was lower than C_0L_1, reaching 99%. Additionally, C_2L_1 had a germination rate of 93%, which was lower compared to C_2L_2, reaching 95%. Salinity stress, caused by the accumulation of Na^+ and Cl^- ions in the soil, disrupted osmotic balance and reduced water absorption during seed imbibition [6]. These findings align with previous research by Chunthaburee et al. [7] which demonstrated that salinity stress reduced rice seed germination through a similar mechanism, where Na^+ inhibited the germination process. Salinity inhibited water uptake and delayed germination, although recovery was possible when physiological metabolism was restored [8]. Similarly, iron toxicity from pyrite (FeS_2) exposure induced oxidative stress, increased malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels, and impaired cellular metabolism, as previously reported by Zhang et al. [9]. These findings corroborated the observed reduction in germination rates under combined stresses.

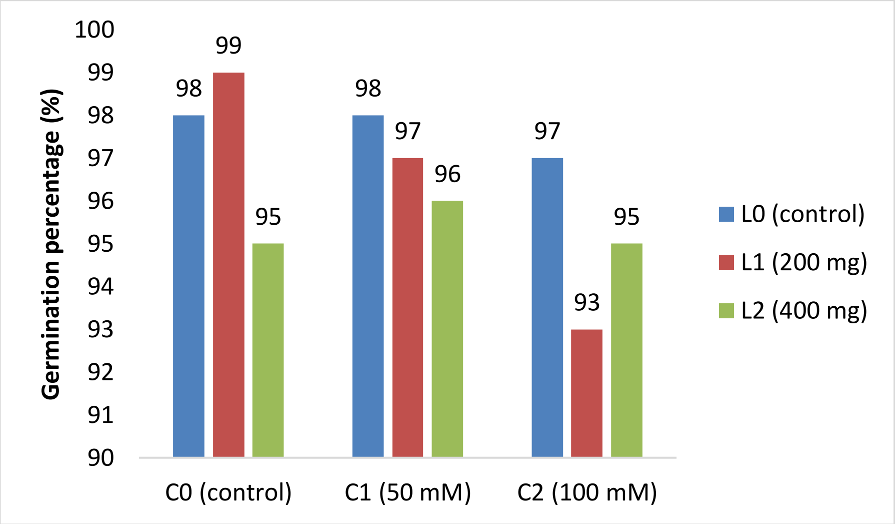

Fig. 1. The Effect of Salinity (C) and Pyrite Stress (L) on the Average Germination (%) of Rice Plants

Germination power reflects the ability of seeds to germinate and grow, which is crucial for determining initial growth potential. Based on Fig. 1, salinity stress and pyrite stress tend to reduce the germination rate of rice seeds, although this difference is not statistically significant. In the C_0L_0 treatment, the percentage of germination rate reached 98%, while in the C_0L_1 treatment, it reached 99%. Meanwhile, the C_2L_1 treatment resulted in a germination rate of 93%, which was lower than C_2L_2, reaching 95%. This reduction in germination was suspected to be related to the emergence of abnormal seeds caused by the influence of salinity and pyrite stress. Zhang et al. [9] stated that heavy metals such as Fe were shown to disrupt DNA repair mechanisms and induce oxidative damage, leading to delayed or impaired germination. Furthermore, high iron concentrations led to leaf bronzing scores (LBS) and reduced chlorophyll content, which likely contributed to metabolic disturbances during germination [10].

Munns and Tester [6] explain that salinity can cause excessive accumulation of Na^+ ions in plant tissues, disrupting cellular metabolism and inhibiting α-amylase activity, which is critical for breaking down starch into glucose. Similarly, Zhang et al. [9] reported that heavy metal toxicity, particularly from Fe, damaged cell membrane integrity, caused electrolyte leakage, and interfered with essential physiological processes such as photosynthesis and respiration. These disruptions likely impair energy availability for early seed growth, contributing to the observed reduction in germination rates under combined stresses. Consistently, a decrease in α-amylase activity under salinity correlates with lower germination rates, unless mitigated by bioactive compounds [8] .

In addition to salinity, heavy metals such as iron (Fe) in the form of pyrite (FeS_2) cause toxicity in plants by inducing oxidative stress and impairing antioxidant defense systems [9]. The accumulation of heavy metals interfered with nutrient uptake and transport, further exacerbating the negative impacts on seed viability. This might explain the reduction in germination rates observed in the combined treatments of salinity and pyrite stress, as seen in the C_2L_1 treatment (93%) compared to C_2L_2 (95%). This effect was previously reported [9], where iron toxicity led to thinner, shorter, and more fragile roots, directly impacting seedling establishment and growth. It was suspected that the impact of both stresses might have resulted in seeds with abnormalities, either in root or shoot formation, reducing germination rates and overall plant quality.

3.2 Maximum Growth Potential (%)

The results of the variance analysis presented show that the interaction between salinity stress and pyrite stress has no significant effect on the maximum growth potential parameter of rice plants. The results of further tests and the average maximum growth potential of rice plants can be seen in Fig. 2.

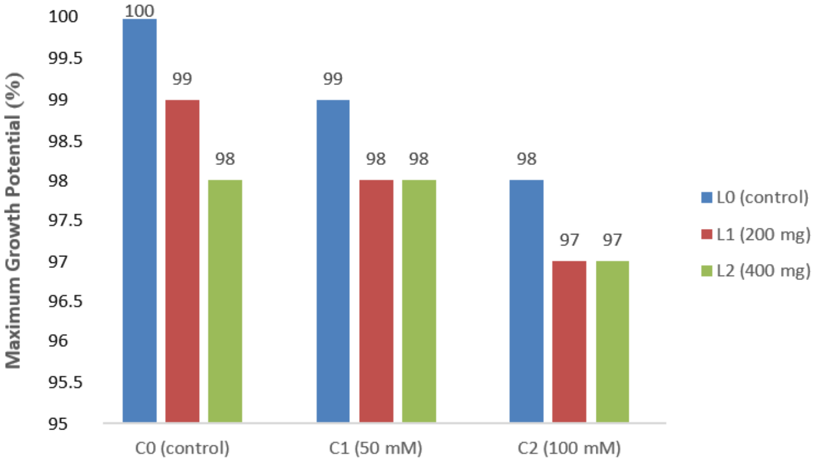

Fig. 2. The Effect of Salinity (C) and Pyrite Stress (L) on the Average Maximum Growth Potential (%) of Rice Plants

Fig. 2 shows that the effects of salinity stress and pyrite stress on the maximum growth potential of rice seeds are not statistically significant. However, when observing the presentation of maximum growth potential, the presence of salinity stress and pyrite stress both in single and combined treatments tends to reduce the maximum growth potential of rice plants. Rice plants that were not exposed to salinity stress and pyrite stress (C_0L_0) had the highest maximum growth potential, reaching 100%, while the interaction of salinity stress and pyrite stress with the lowest maximum growth potential was observed in C_2L_1 and C_2L_2, which reached only 97%.

The maximum growth potential indicates the maximal capacity of seeds under optimal conditions, providing insight into their genetic potential. Based on Fig. 2, the effects of salinity stress and pyrite stress on the maximum growth potential of rice seeds show no statistically significant results. Nevertheless, the reduction in growth potential from 100% (C_0L_0) to 97% (C_2L_1 and C_2L_2) highlights the cumulative impact of abiotic stresses. This aligns with findings demonstrating that salinity induces osmotic stress and ion toxicity, disrupting cellular redox balance and leading to oxidative damage, as noted in horticultural crops [11]. This aligns with previous findings by Aculey et al. [12], which demonstrated that seed quality characteristics, such as germination percentage and vigor, could be influenced by environmental factors, even if differences were not statistically significant. It was similarly emphasized that even under sublethal stress conditions, hormonal imbalances and altered antioxidant responses could quietly suppress seed metabolic activity, suggesting that visible growth limitations may emerge later from subtle physiological disruptions [13]. The slight decline in maximum growth potential under combined stresses suggests an early indication of stress-induced limitations on seed performance.

This phenomenon is consistent with the explanation by Nurjanah [14], who noted that increased osmotic pressure in the seed's surrounding environment hindered water absorption and disrupted the germination process. Similarly, heavy metal stress, particularly from pyrite (FeS_2), as explained by Anwar and Masganti [15], lowers soil pH through pyrite oxidation, reduces nutrient availability, and induces iron toxicity. These effects are further compounded by disruptions in potassium (K^+) and sodium (Na^+) homeostasis, critical for maintaining enzymatic activity and photosynthesis, as highlighted in horticultural studies [11]. These factors collectively impair root and shoot development, as also noted by Aculey et al. [12] who emphasized that seed vigor and growth potential are closely linked to the ability of seeds to during early growth stages. In line with this, hormonal regulators such as abscisic acid (ABA) and jasmonic acid (JA) become highly active during combined abiotic stress, fine-tuning antioxidant responses and influencing early seedling vigor through complex signaling cascades [13].

In the interaction between salinity stress and pyrite stress, as observed in treatments C_2L_1 and C_2L_2, the combined effects of both stresses were more detrimental. Salinity disrupted photosynthesis and water uptake, while pyrite-induced acidity exacerbated nutrient deficiencies. The dual stress triggered excessive reactive oxygen species (ROS) generation, leading to biomolecule degradation and impaired cellular functions, as shown in horticultural crops subjected to salinity stress [11]. This dual stress triggered reactive oxygen species (ROS), damaging cellular structures and activating complex stress signaling pathways. As explained by Khan et al., these ROS-mediated effects can be modulated by internal hormone signaling, where enhanced expression of stress-responsive genes plays a pivotal role in mitigating oxidative damage and preserving seed potential under unfavorable environments [13]**.** Although the reduction in maximum growth potential is not statistically significant, these findings underscore the mutual exacerbation of growth limitations caused by salinity and heavy metals. Therefore, the observed trends indicate the importance of selecting stress-tolerant rice varieties to mitigate the adverse impacts of combined abiotic stresses.

3.3 Vigor Index (%)

The results of the variance analysis presented show that the interaction between salinity stress and pyrite stress has no significant effect on the vigor index parameter of rice plants. However, the salinity factor shows a significant difference. The results of further tests and the average vigor index of rice plants can be seen in Fig. 3.

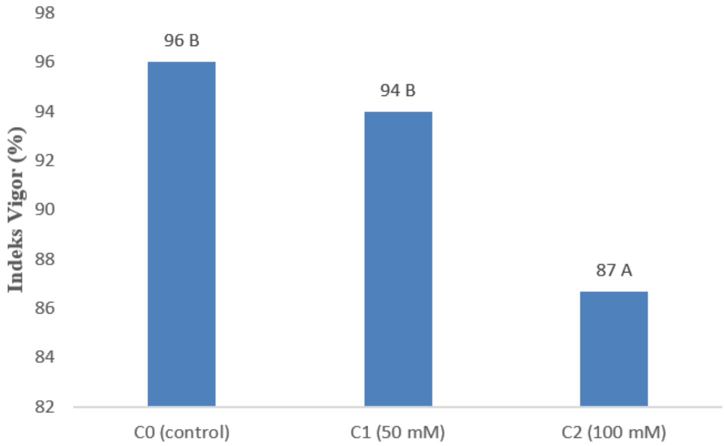

Fig. 3. The Effect of Salinity Stress (C) on the Average Vigor Index (%) of Rice Plants

Fig. 3 shows that the interaction between salinity stress and pyrite stress on the average vigor index of rice plants has a significant effect. However, when observed from single factors, salinity stress is suspected to influence the vigor index of rice plants. Statistically, the results showed that between C_0 (without salinity stress) with a vigor index of 96% and C_1 (50 mM salinity stress) with a vigor index of 94%, there was no significant difference. However, when the concentration of salinity stress increased to 100 mM (C_2), the vigor index dropped to 86.67%, which indicated a statistically significant difference. This suggests that increasing salinity could have a greater negative impact on the vigor of rice plants. This pattern is in line with findings from a large-scale evaluation of rice germplasm under salinity stress, where higher NaCl concentrations notably reduce seedling vigor index, particularly beyond the threshold of 120 mM, as reflected in reduced root length, shoot length, and fresh weight [16].

The vigor index was used to measure seed health and strength, relating to stress resistance. Based on the results in Fig. 3, the interaction between salinity stress and pyrite stress on the average vigor index of rice plants shows no significant statistical effect. However, when observed from single factors, salinity stress is suspected to influence the vigor index of rice plants. Statistically, there is no significant difference between treatment C_0 (without salinity stress) with a vigor index of 96% and treatment C_1 (50 mM salinity stress) with a vigor index of 94%. This decrease, although not significantly different, indicates an initial trend that could become more pronounced at higher concentrations. This aligns with findings showing that salinity reduces morpho-physiological traits like root and shoot dry weight, leaf area index, and chlorophyll content while increasing Na+-K+ ratios and total soluble sugars [17]. Genes such as OsRAV1 play a critical role in regulating seed vigor and salt stress resistance, as evidenced by higher germination rates in overexpression lines under salinity stress [18]. Similar reductions in vigor at early growth stages were also recorded under controlled salinity screening, where seedling vigor index was confirmed as the most responsive indicator to salt-induced injury in rice germplasm, strengthening its relevance as a physiological marker [16]. In agreement, Habib et al. [19] observed that early physiological responses, including reduced photosynthesis and protein synthesis, were triggered under combined abiotic stresses. These responses were more prominent in sensitive genotypes, reinforcing that vigor index can reflect underlying molecular disruptions, especially when oxidative and osmotic stress signals converge [19]. The slight reduction in vigor at 50 mM salinity likely reflects early stress responses, consistent with their findings that OsRAV1 expression is crucial for maintaining seed performance under adverse conditions [18].

Research has shown that plants exposed to high salinity experience a reduced ability to regulate osmosis, impacting their vigor [20]. In this study, the vigor index of rice plants decreases from 96% (C_0) to 94% (C_1) and 86.67% (C_2) as salinity increases. At lower salinity concentrations (50 mM), rice plants could still maintain a relatively high vigor index, despite a slight decrease. However, at higher concentrations (100 mM), the detrimental effects are evident, including reductions in photosynthesis, transpiration rates, and yield components, as noted in rice genotypes under salinity stress [17]. Gao et al. [18] reported that OsRAV1 enhanced initial germination rates under salt stress, suggesting its potential role in mitigating vigor loss at elevated salinity levels. Under combined salinity and thermal stress, sensitive rice cultivars show rapid physiological breakdowns, whereas tolerant types exhibit stable protein expression patterns and photosynthetic recovery. These contrasts mirror the observed variability in vigor under increasing salinity stress, underscoring the role of stress-specific protein regulation in maintaining early growth [19].

This decline in the vigor index is consistent with the effects of pyrite stress on rice plants as described by Anwar and Masganti [15]. Oxidized pyrite produces sulfuric acid, which lowers soil pH and affects the availability of essential nutrients for plant growth. In more acidic soils, rice plants might struggle to absorb critical nutrients such as phosphorus and potassium, which also reduce plant vigor. The oxidation of pyrite not only affects soil pH but also produces heavy metal toxicity resulting from pyrite reduction, which could affect plant enzymatic activity and inhibit growth. These effects parallel the findings of Singh et al. [21] who demonstrated that pyrites improve nutrient availability and mitigate sodicity stress by enhancing chlorophyll and sugar content at moderate levels, underscoring the dual role of pyrites as both a stressor and an ameliorating agent.These findings align with Gao et al. [18] which highlighted that abiotic stresses like salinity often act to impair seed vigor and seedling establishment, emphasizing the need for stress-tolerant varieties.

3.4 Growth Speed (Kct) (%/etmal)

The results of the variance analysis show that the interaction between salinity stress and pyrite stress has no significant effect on the growth rate parameter of rice plants. However, the individual factors of salinity stress and pyrite stress show significant results. The results of further tests and the average growth speed of rice plants could be seen in Fig. 4.

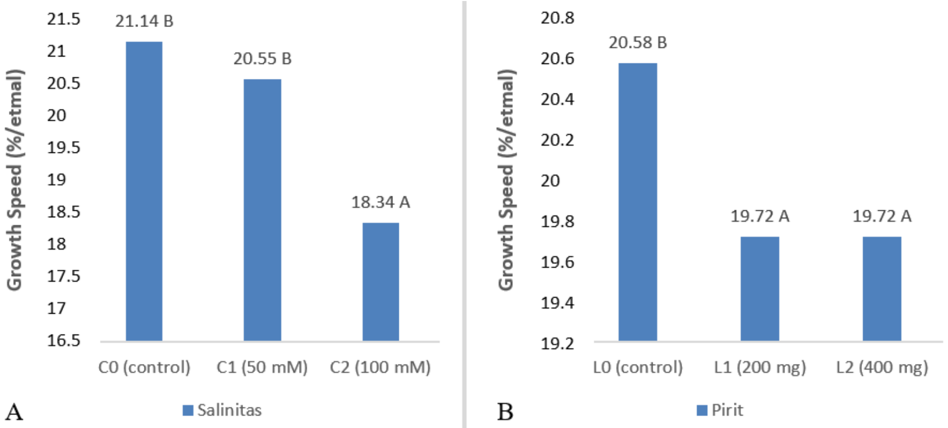

Fig. 4. A) The Effect of Salinity Stress on the Average Growth Speed (%/etmal) of Rice Plants, B) The Effect of Pyrite Stress on the Average Growth Speed (%/etmal) of Rice Plants

Fig. 4 shows that the single factor of salinity stress (C) has a significant effect on the growth speed of rice seeds, indicating that salinity levels significantly affect the optimal growth speed of rice plants. In the treatment without salinity (C_0), the average growth speed reached 21.14 %/etmal, which was not significantly different from the salinity treatment at 50 mM (C_1), with a growth speed of 20.55 %/etmal. However, when the concentration of salinity stress increased to 100 mM (C_2), the growth speed dropped to 18.34 %/etmal, indicating a statistically significant difference. These findings align with the results reported by Taratima et al. who found that increasing concentrations of NaCl negatively affected the growth of Luem Pua rice seedlings, reducing plant height, leaf number, and other essential growth parameters [22]. Similarly, Ata et al. [23] demonstrated that drought and salinity stress limited water uptake and slowed root elongation, emphasizing how osmotic stress disrupted plant development. Comparable trends were observed in rice cultivated on swampy soils with high iron and pyrite content, where growth performance varied depending on the genotype. Some varieties maintained better vigor despite the presence of FeS_2, suggesting they had genetically adapted to such harsh soil conditions [24]. Supporting this, previous studies showed that iron toxicity reduced shoot biomass and caused visible symptoms such as leaf bronzing. However, genotypic differences played a crucial role in determining tolerance, as seen in varieties like Taichung65 and INPARA2, which sustained growth under elevated iron conditions by restricting Fe accumulation in their shoot tissues [25]. In relation to stress responses during early development, environmental conditions such as salinity could influence enzymatic activity during germination, altering the physiological quality of rice seeds [26]. These shifts might affect early growth potential and metabolic balance, contributing to variations in vigor and seedling performance.

In addition to the salinity stress factor, the single factor of pyrite stress also showed a statistically significant effect on the growth rate of rice plants. In the treatment without pyrite stress (L_0), the average growth speed reached 20.58 %/etmal, which was significantly different from the treatments of pyrite stress at 200 mg (L_1) and 400 mg (L_2), with growth speed of 19.72 %/etmal, respectively. This indicates that increasing pyrite concentration in the soil could inhibit the growth rate of rice plants. The presence of heavy metals such as pyrite could lower soil pH and cause damage to plant roots, reducing the plant's capacity to absorb water and nutrients [27]. Ata et al. [23] reported that heavy metal toxicity, particularly from copper (Cu) and lead (Pb), significantly reduced protein content and enzyme activity in germinating seeds, further impairing physiological processes like chlorophyll synthesis and photosynthetic efficiency. Similar to findings in acid sulfate soils of Kalimantan, where high iron and low pH conditions limited rice growth during the dry season, the effect of pyrite stress is influenced not only by its concentration but also by seasonal soil conditions and varietal resilience [24]. Furthermore, the extent of Fe toxicity can vary depending on a plant's ability to manage Fe uptake and transport, with high FTRI (Fe Toxicity Response Index) genotypes showing both visual and physiological resilience under stress, suggesting that Fe handling efficiency is critical to sustaining growth under pyrite-influenced environments [25]. Nascimento et al. [26] also noted that seeds germinating under unfavorable abiotic conditions may experience reductions in key metabolic activities, including enzymatic breakdown of storage compounds, which could directly impact seedling vigor and growth capacity even before visible symptoms manifest.

The growth rate indicates the plant's growth rate, which is crucial for determining harvest time and productivity. As Fig. 4 indicates, the effect of the single factor of salinity stress on the growth rate of rice seeds shows a significant difference, indicating that salinity stress could affect plant growth rates. In the treatment without salinity stress (C_0), the growth speed of rice plants reached 21.14 %/etmal, which was not significantly different from the salinity treatment at 50 mM (C_1), with a growth speed of 20.55 %/etmal. Although the decrease in growth rate in C_1 was not significant, it indicated an initial trend that could become more pronounced at higher salinity concentrations. When the concentration of salinity stress increased to 100 mM (C_2), the growth speed decreased to 18.34 %/etmal, which showed a statistically significant difference. Increased salinity raises osmotic pressure in the soil, hindering the plant's ability to absorb water and nutrients necessary for its growth, leading to a reduced growth rate. Marschner [20] explained that plants had to expend more energy to regulate osmosis, diverting resources from growth to defense mechanisms. Some rice varieties have better tolerance mechanisms to salinity stress, allowing them to maintain higher vigor at certain salinity concentrations. This highlights the importance of selecting varieties that are proven to perform well in marginal environments, as demonstrated by Inpara 1 and Cilosari, which showed yield stability under pyrite-rich swamp soils [24].

In addition to salinity stress, the pyrite stress factor also has a significant effect on the growth speed of rice seeds. The research results show that the treatment without pyrite stress (L_0) had an average growth speed of 20.58 %/etmal, which was significantly different from the pyrite stress treatments at 200 mg (L_1) and 400 mg (L_2), with growth speed of 19.72 %/etmal, respectively.

Additionally, heavy metal exposure from pyrite can induce oxidative stress by generating excessive reactive oxygen species (ROS), which disrupt cell membranes and inhibit plant growth [28]. Taratima et al. [22] reported that high NaCl concentrations caused ion toxicity and oxidative stress, impairing cellular processes and reducing growth performance. These findings highlight the need for stress-tolerant rice varieties to mitigate the combined impacts of salinity and pyrite stress.

3.5 Simultaneous Growth (Kst) (%)

The results of the variance analysis show that the interaction between salinity stress and pyrite stress has no significant effect on the simultaneous growth parameter of rice plants. The results of further tests and the average simultaneous growth of rice plants are presented in Fig. 5.

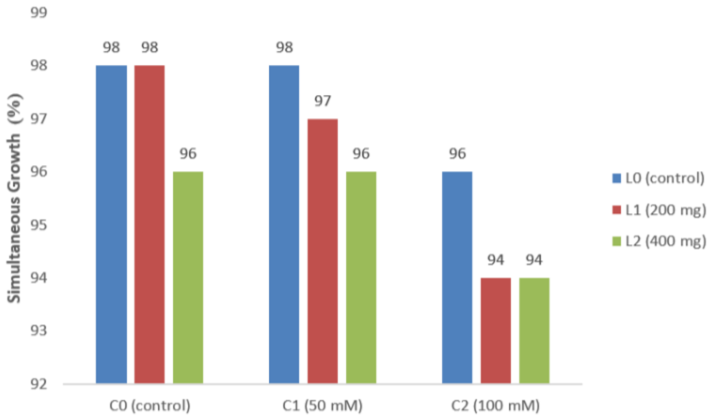

Fig. 5. The Effect of Salinity (C) and Pyrite Stress (L) on the Average Simultaneous Growth (%) of Rice Plants

Fig. 5 shows that the effects of salinity stress and pyrite stress on the simultaneous growth of rice seeds are not statistically significant. However, both stresses tend to reduce the percentage of simultaneous growth. In the control treatment (C_0L_0), the simultaneous growth rate reached 98%, similar to C_1L_0 and C_0L_1, each at 98%. In contrast, the lowest simultaneous growth rates were observed in C_2L_1 and C_2L_2 treatments, each at 94%.

The simultaneous growth describes the synchronization of growth, which is important for land management and achieving uniform yields. Based on Fig. 5, the effects of salinity stress and pyrite stress on the simultaneous growth of rice seeds show no statistically significant results, although observable differences could be noted. Simultaneous growth refers to the level of synchronization in the time seeds germinated and began their growth. Although not statistically significant, the decrease in simultaneous growth indicated that these stresses affected the growth potential of rice seeds, which could impact overall crop yield.

Rice plants that were not exposed to stress (C_0L_0) had the highest simultaneous growth rate, reaching 98%. A similar result was found in treatments C_1L_0 and C_0L_1, each showing a simultaneous growth rate of 98%. This indicates that both 50 mM salinity stress and 200 mg pyrite stress have no significant effect on the simultaneous growth of rice seeds. At these stress levels, rice plants were still able to maintain high simultaneous growth because the plants could cope with the stress impacts during the early stages of growth, consistent with previous research findings that low salinity stress or low-concentration heavy pyrite did not immediately reduce the ability of seeds to germinate or grow simultaneously. This is also suspected to be related to the use of rice seeds that were tolerant to these stresses.

However, when salinity stress increased , the simultaneous growth rate began to decline. In treatments C_2L_1 and C_2L_2, the simultaneous growth rate was recorded at 94%, lower than other treatments. This decline indicated that higher concentrations of salinity and pyrite stress could begin to affect the simultaneous growth of rice seeds. Krismiratsih et al. [29] stated that higher salinity stress led to a reduction in plant growth rates and health. Similarly, Khan et al. [30] reported that increasing salt concentrations significantly delayed seed germination and reduced root and shoot growth, likely due to ion toxicity and osmotic stress caused by high salinity.

High salinity stress increased soil osmotic pressure, which disrupted the ability of seeds to absorb water effectively during germination [6]. Delays or imbalances in germination may cause some seeds to exhibit stunted growth. Additionally, Mu et al. [31] highlighted that under salinity stress, the accumulation of reactive oxygen species (ROS) and oxidative damage further impair germination synchronization. Salt-stressed rice accumulated more Na^+ and produced more hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), damaging cellular structures and delaying seedling development. Rahman et al. [32] corroborated these findings, showing that oxidative stress under high salinity was shown to reduce α-amylase activity, impairing starch mobilization and energy availability for early seedling growth.

Heavy metals like iron (Fe) from pyrite also contribute to membrane damage and disrupt seed metabolism [3]. The combined stress of high salinity and pyrite exposure likely impairs seedling establishment by inducing both osmotic and oxidative stress, leading to the observed reduction in simultaneous growth, particularly in C_2L_1 and C_2L_2. This is consistent with Novianti et al. [10] who noted that iron toxicity led to thinner and weaker root systems, further compromising seedling uniformity. Rahman et al. [32] found that Fe toxicity induced lipid peroxidation and reduced photosynthetic efficiency, exacerbating growth asynchrony under combined stress conditions.

3.6 Growth rate (cm/day)

By observing the growth trend, the moment when stress had the most significant impact could be identified, as well as how efficiently plants adapted to each treatment. This can be seen in Fig. 6.

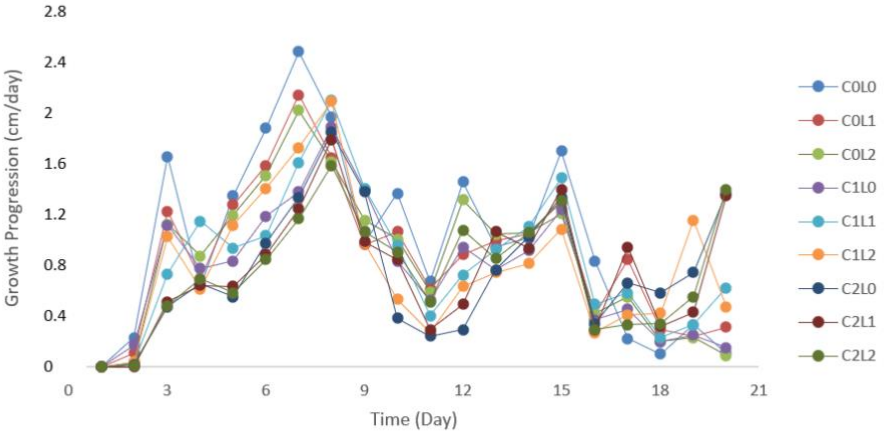

Fig. 6. Growth Rate of Rice Plants Under Salinity Stress (C) and Pyrite Stress (L)

The growth rate of plants is an important indicator for assessing physiological responses to environmental stress, such as salinity and pyrite. As Fig. 6 indicates, rice plants under the control treatment (C_0L_0) show the most stable growth with a daily rate reaching 2.47 cm on day 7. This growth reflects optimal environmental conditions without osmotic stress or heavy metal toxicity from pyrite, allowing maximal water and nutrient absorption. According to Taiz and Zeiger [33], water and nutrient availability are the main factors determining plant growth. However, after day 8, the growth rate decreased to 0.67 cm on day 11 before increasing again to 1.69 cm on day 15. This decline was likely due to internal resource limitations, as no external fertilizers were added, highlighting the dependency of rice plants on external nutrient inputs for sustained growth [34]. The findings align with the study by Singh et al. [21] which emphasized that nutrient deficiency caused by sodicity stress significantly reduced plant growth and physiological processes such as photosynthesis and sugar biosynthesis. Early-stage growth is often sensitive to changes in nutrient and water availability, where the absence of supplemental input can quickly limit cellular expansion and energy metabolism, even under non-stressful environmental conditions [35].

In contrast, the treatment with 100 mM salinity stress and 400 mg pyrite (C_2L_2) showed the lowest growth rate, only reaching 1.16 cm on day 7 and continuing to decline to 0.27 cm on day 16. This sharp decline indicated that salinity stress caused significant osmotic stress and inhibited water and nutrient absorption by the roots [3]. Sodium salinity increased external osmotic pressure as well as Na^+ ion toxicity in plant tissues, disrupting the ionic balance between Na^+ and K^+ [36]. Heavy metal toxicity, particularly from iron (Fe) in pyrite, further exacerbated root suppression and seedling growth inhibition, as reported by Girija et al. [34], who demonstrated that heavy metals like Hg, Co, Ni, and Cd severely reduced root and shoot lengths in rice seedlings. This type of combined stress ionic and metal-induced was also highlighted by Sarma et al. [35] as a key contributor to oxidative stress, where excess ROS accumulation damages cellular membranes and impairs both nutrient uptake and metabolic stability, thereby compounding growth limitations under dual-stress environments. Interestingly, despite experiencing high stress, the growth rate of treatment C_2L_2 slightly increased to 1.39 cm on day 20. This was suspected to be an adaptive response through salinity tolerance mechanisms such as osmotic adjustment, activation of antioxidant systems, and increased activity of protective enzymes [36]. Similar results were observed by Singh et al. [21] who noted that the application of pyrites improved nutrient availability and enhanced chlorophyll and sugar content under moderate sodicity stress (RSC levels of 2.5 meL^{-1}), suggesting a potential mitigating role of pyrites in reducing abiotic stress effects.

Overall, the combination of high salinity (100 mM) and high pyrite dosage (400 mg) significantly inhibited growth, especially after day 10, due to the accumulation of toxic ions like Na^+, which affected enzyme function in photosynthesis [6]. Chronic salinity stress was shown to inhibit the expression of genes involved in chlorophyll biosynthesis, ultimately affecting the plant's photosynthetic efficiency [36]. In a related study, Girija et al. [34] reported that exposure to heavy metals such as $CdCl_2$ significantly suppressed root and shoot development, with root length decreasing by as much as 73% compared to the control group. The combined presence of unmanaged salinity and pyrite stress had a pronounced negative impact on rice growth, highlighting the critical need for mitigation strategies such as seed priming or soil conditioning to improve plant tolerance. Previous findings demonstrated that Rhizobium inoculation, when applied alongside pyrite, enhanced plant resilience to sodicity stress by improving nutrient uptake and promoting sugar biosynthesis, emphasizing the value of integrated approaches in managing abiotic stressors [21]. Meanwhile, the non-stressed treatment (C_0L_0) allowed plants to achieve higher growth rates and maintain normal physiological development during their early stages, reinforcing the importance of minimizing abiotic pressures to ensure optimal crop performance [34].

4. Conclusions

The research demonstrates that the interaction between salinity and pyrite stresses does not significantly affect most physiological parameters of rice seeds. As single factors, salinity consistently reduces vigor index and growth speed, while pyrite mainly affects growth speed. Both stresses tend to lower germination percentage, maximum growth potential, and simultaneous growth, although these trends are generally not statistically significant. The highest daily growth rate occurred in the control (C_0L_0), approximately 2.47 cm on day 7, while the combined high-salinity and high-pyrite treatment (C_2L_2) showed the lowest rates (~1.16 cm on day 7, declining to 0.27 cm by day 16). Salinity reduces growth rate mainly via osmotic stress and Na^+ toxicity, while pyrite aggravates the effect through soil acidification and Fe toxicity. The dual stress caused the strongest inhibition particularly after day 10 although a modest adaptive recovery was observed toward the end of the experiment (small increase by day 20). The strongest inhibition occurred under the combined high-salinity and high-pyrite treatment.

Abbreviations

EC	Electrical Conductivity
dS/m	deciSiemens per meter
ROS	reactive oxygen species
FTRI	Fe Toxicity Response Index

Data availability statement

Data will be made available upon request.

CRediT authorship contribution statement

Hariy Laksamana conceptualization, methodology, includes data collection, data curation, writing, formal analysis, reference sourcing, editing, and administration. **Alhaviz** methodology flow, supervision, validation, and writing review. **Sri Utami** supervision, validation, and writing review.

Declaration of Competing Interest

The authors affirm that the publication of this article is free from any conflicts of interest.

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