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Physiological and morphological responses of three rice varieties under NaCl stress

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Abstract. Salt stress is a critical abiotic factor that adversely affects rice (*Oryza sativa* L.) growth and development, leading to significant yield reductions. This study investigates the physiological responses of three tolerant rice varieties—F478, INPARI35, and SIAK RAYA—under 100 mM NaCl stress. Several parameters were measured, including plant height, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight, and Chlorophylls were measured at intervals of 0, 3, and 6 days after salt exposure. Results indicate that salt stress significantly impairs growth parameters across all varieties, with varying degrees of tolerance observed. Notably, plant height and root length exhibited a marked decline under stress conditions. The correlation analysis revealed intricate interrelationships between growth traits under salt stress. The correlation matrix visualized these relationships, highlighting both positive and negative associations among traits. For instance, root length has almost negative correlation with all other parameters. Out of three rice varieties, Inpari35 demonstrated enhanced resilience, maintaining higher growth rates and biomass under stress conditions. Taken together, identifying salt-tolerant rice varieties will be crucial for mitigating the impacts of salinity on crop productivity and ensuring food security in saline-prone regions.

1 Introduction

Rice (*Oryza sativa* L.) is one of the main food crops from the Poaceae family worldwide, including in Indonesia. As a staple food crop, rice productivity is increasingly threatened by various abiotic stresses, such as salinity stress. Salinity stress negatively impacts plant growth, disrupts physiological processes, and causes decreased productivity. Rice is a glycophyte plant that is sensitive and vulnerable to salinity stress. Rice productivity can decrease by 10% at a salinity level of 3.5 dS/m and up to 50% at a salinity level of 7.2 dS/m [1]. This condition is further exacerbated by the increasing area of saline land caused by climate change that causes seawater intrusion, the use of saline irrigation water, and the excessive use of chemical fertilizers [2].

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Salinity stress can cause complex physiological disorders in rice plants such as decreased water absorption by roots due to low soil osmotic potential. In addition, high levels of toxic ions and the accumulation of toxic ions such as Na^+ and Cl^- in plant tissues cause nutrient imbalances due to competition with other essential ions such as K+, Ca2+, and Mg2+ [3]. In addition, salinity stress also reduces stomatal conductivity, thus limiting the ability of carbon fixation [4]. The combination of osmotic stress, ionic toxicity, nutrient deficiency, and oxidative stress has a direct impact on the overall growth of rice plants, including vegetative growth. This study was conducted to evaluate the morphological and physiological responses of three rice varieties (F478, Inpari 35, and Siak Raya) to salinity stress of 100 mM NaCl during the vegetative phase. The three rice varieties used in this study (INPARI 35, FI478, and Siak Raya) were selected based on their status as r developed superior varieties within Indonesian breeding programs. but, information regarding their physiological and morphological responses under salinity stress remains limited. This study is expected to provide insight into the level of resistance of the three tested rice varieties to salinity stress during the vegetative phase.

2 Research Method

Three rice varieties (*Oryza sativa* L.) selected in this study were F478, Inpari 35, and Siak Raya, obtained from the Indonesian Center for Rice Research (BBPadi) in Subang, West Java. These rice varieties were soaked in water for 24 hours to induce germination. The soaked rice seeds were then incubated at room temperature for 10 days by soaking them in distilled water to prepare them for planting in hydroponic media. The planting medium used in this study was hydroponic media. Seedlings were grown in a floating hydroponic system using ab mix nutrient solution, with aeration maintained continuously. This study used a factorial randomized block design (RBD) with two factors tested: (i) rice varieties and (ii) salinity treatment. The salinity stress treatment used sodium chloride (NaCl) added to the nutrient solution with a salinity concentration of 100 mM. Control plants were maintained in a normal nutrient solution without the addition of NaCl. Each treatment consisted of three replications. Salt stress was applied when the seedlings reach the early vegetative stage (14 days after planting) which is indicated by 3–4 leaves that have developed. Morphological and physiological parameters were measured on days 0, 3, and 6 after salt stress. The parameters measured included: plant height (cm), root length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), root dry weight (g), and chlorophyll content. Data were analyzed using Analysis of Variance (ANOVA) to determine the effect of treatment on the measured parameters. If treatment significantly affected the parameter changes, the Duncan Multiple Range Test (DMRT) was used at a 5% significance level. Correlation analysis was conducted to evaluate the interrelationship between growth and physiological traits under salinity stress.

3 Result and Discussions

3.1 Effect of Salinity Stress on Plant Height

There are no significant differences were observed on plant height at normal and stress condition. Under salinity stress, plant growth still increases, but the height is generally lower than under normal conditions. Siak Raya has the slowest growth under salt stress and Inpari 35 maintains better growth than other varieties (Fig. 1). Salinity stress causes a decrease in leaf length, leaf elongation rate, and leaf width. It also causes a decrease in leaf number, delayed leaf emergence, decreased leaf area, reduced leaf growth, and a higher rate of leaf senescence [5]. Salinity can lead to decreased plant productivity due to osmotic and toxic stress. Leaf growth is the earliest response of rice plants exposed to salinity stress. Salinity stress results in a decrease in CO2 assimilation rate, growth, and dry matter accumulation, thus inhibiting rice growth [6].

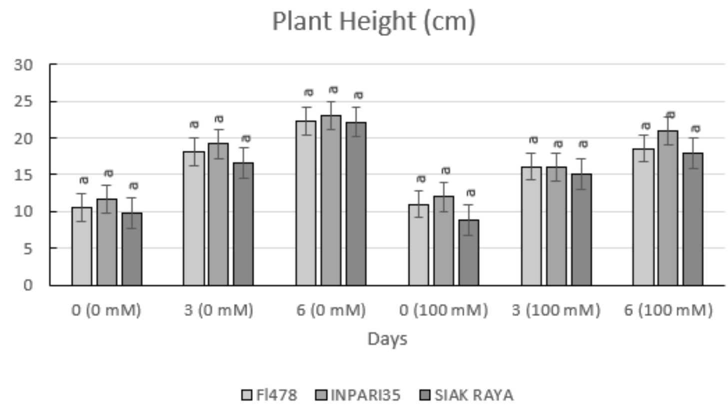

Fig. 1. Effect of Salinity Stress on Plant Height. Different letters indicate significant difference according to DMRT at $p<0.05$

3.2 Effect of Salinity Stress on Root Length

There are no significant differences were observed on root length at normal and stress condition. Under salinity stress conditions, Inpari 35 and Siak Raya showed slower root growth. F1478 was able to maintain or even slightly increase root growth indicating a physiological tolerance mechanism (Fig. 2). Roots are the most vulnerable part of the plant to salinity stress due to direct exposure. However, roots have a good adaptation with primary stopping meristematic activity at the root tip [6]. Salinity stress can cause ionic stress due to the absorption of Na+ and Cl− ions which can reduce the osmotic potential between the roots and the soil solution, which will reduce the availability of water infiltration, resulting in ion toxicity and reduced nutrient absorption [4]. The cessation of root meristematic activity, osmotic pressure that reduces the availability of water infiltration and inhibits nutrient absorption will have an impact on reducing root length under salinity stress conditions.

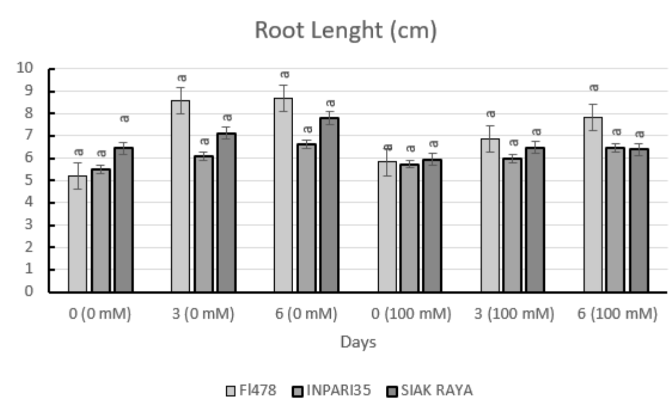

Fig. 2. Effect of Salinity Stress on Root Length. Different letters indicate significant difference according to DMRT at $p<0.05$

3.3 Effect of Salinity Stress on Shoot Fresh Weight

There are no significant differences were observed on shoot fresh weight at normal and stress condition. Although there was no significant difference, shoot fresh weight under salinity stress showed a decrease compared to normal treatment. Inpari 35 maintains better growth than other varieties (Fig. 3). Salinity causes a decrease in leaf length, leaf elongation rate, and leaf width due to changes in root water potential, resulting in poor leaf water supply and reduced turgor pressure. Salinity stress also causes a decrease in leaf number, delayed leaf emergence, decreased leaf area, reduced leaf growth, and a higher rate of leaf senescence. This is due to NaCl uptake, impaired ion homeostasis, decreased photosynthesis, and photoassimilate production. The conditions caused by salinity stress will result in a decrease in shoot height, shoot fresh weight, and shoot dry weight [5].

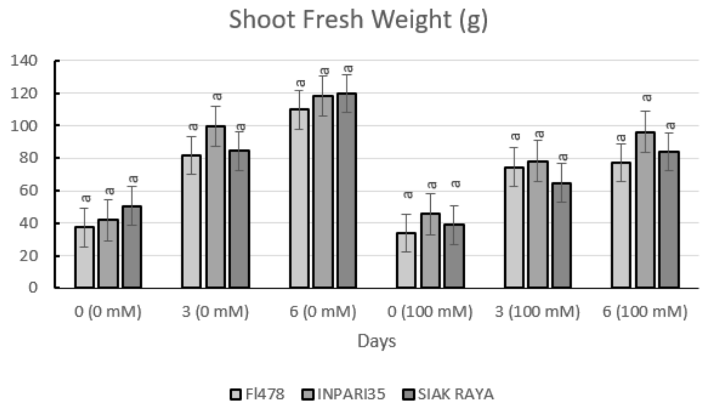

Fig. 3. Effect of Salinity Stress on Shoot Fresh Weight. Different letters indicate significant difference according to DMRT at $p<0.05$

3.4 Effect of Salinity Stress on Leaf Dry Weight

There are no significant differences were observed on leaf dry weight at normal and stress condition. Although there was no significant difference, Among the tested varieties, FI478 and Siak Raya tended to maintain higher leaf dry weight (Fig. 4). Salt stress triggers complex plant responses, including ionic imbalance, changes in osmolyte levels, accumulation of reactive oxygen species (ROS), and hormonal changes. Salt stress increases Na^+ concentrations in cells and decreases K^+ uptake. Salt stress also induces excessive ROS production, leading to oxidative stress that damages cell membranes, proteins, and DNA. Plants increase levels of enzymatic antioxidants (such as peroxidase and catalase) and nonenzymatic antioxidants (such as ascorbic acid and glutathione) to counteract excess ROS and prevent oxidative damage. Salt stress causes plants to close their stomata to reduce water transpiration, but also limits CO_2 uptake, resulting in reduced plant height, yellowing, wilting, and inhibited photosynthesis [7]. The impacts of salinity stress, which cause ionic imbalances, accumulation of reactive oxygen species (ROS), and inhibited photosynthesis, can lead to decreased leaf dry weight.

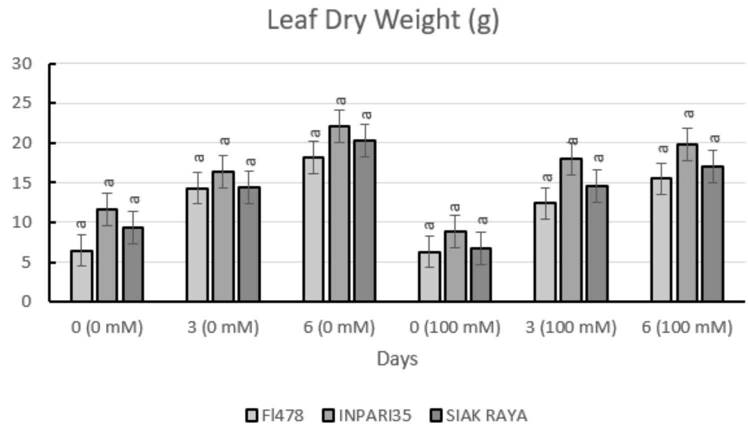

Fig. 4. Effect of Salinity Stress on Leaf Dry Weight. Different letters indicate significant difference according to DMRT at p<0.05

3.5 Effect of Salinity Stress on Root Fresh Weight

There are no significant differences were observed on root fresh weight at normal and stress condition. Under salinity stress, root fresh weight increase at day 3 and decrease at day 6. Among the tested varieties, Inpari 35 maintains better root fresh weight than other varieties (Fig. 5). Salinity stress affects the ability of rice plants to accumulate and produce biomass. This is because high salt concentrations in the soil have an osmotic effect and increase the soil's water retention capacity, thereby increasing the accumulation of specific ions in the plant. Salinity stress inhibits photosynthesis by reducing carboxylation in chloroplasts, thus disrupting the plant's ability to produce electron transport, reducing groups, and adenosine triphosphate (ATP) synthesis in the plant's thylakoids. Furthermore, there is a decrease in water potential within cells and the plant's inability to absorb water, which induces stomatal closure, thus limiting carbon dioxide (CO2) assimilation. This significantly reduces fresh and dry biomass of rice under salinity stress [8].

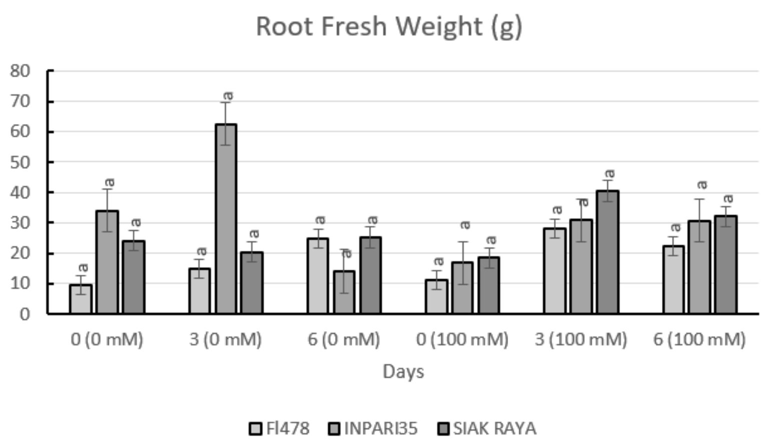

Fig. 5. Effect of Salinity Stress on Root Fresh Weight. Different letters indicate significant difference according to DMRT at p<0.05

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3.6 Effect of Salinity Stress on Root Dry Weight

There are no significant differences were observed on root dry weight at normal and stress condition. Under salinity stress, root growth continues to increase gradually, indicating an adaptive response of roots to the saline environment. Inpari35 maintains better root dry weight than other varieties (Fig. 6). Plants susceptible to salinity have large amounts of Na+ ions that accumulate in the rice plant, reaching toxic levels. However, salt-tolerant rice adapts by having a stronger Na+ exclusion capacity, which can cause Na+ efflux from the roots to the environment. Furthermore, a low Na+/K+ ratio due to lower Na+ accumulation can induce the Na+/K+ antiporter to regulate Na+ sequestration in the vacuole. Salt stress generates large amounts of reactive oxygen species (ROS), which reduce chlorophyll synthesis, damage nucleic acids and proteins, cause membrane lipid peroxidation, and alter enzyme activity. Salt-tolerant rice adapts by reducing ROS through an antioxidant enzyme system that increases the activity of CAT, SOD, and POD, and a non-enzymatic pathway that produces the osmotic regulator Pro, soluble proteins, and MDA. Higher levels of antioxidant enzymes and osmotic substances in salt-tolerant rice can protect the membrane system and maintain photosynthetic assimilates [10]. The adaptation factors possessed by these tolerant plants make the damage caused by salinity stress lower.

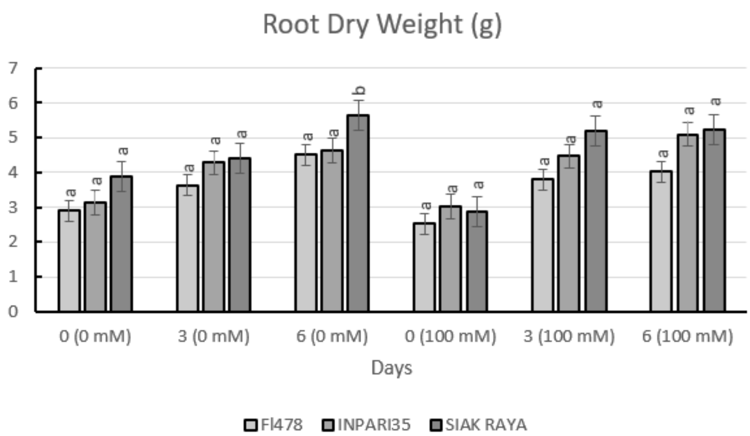

Fig. 6. Effect of Salinity Stress on Root Dry Weight. Different letters indicate significant difference according to DMRT at $p<0.05$

3.7 Effect of Salinity Stress on Chlorophyll Pigments

There are significant differences were observed on chlorophyll pigments at normal and stress condition. All varieties experienced a significant decrease in chlorophyll a, b and total levels (Fig. 7). Salinity stress significantly reduces fresh and dry biomass of rice. Rice growth is also reduced when exposed to salt stress. This is caused by a decrease in water potential in cells, which induces stomatal closure and limits carbon dioxide (CO2) assimilation. Salinity stress can induce premature leaf senescence and reduce rice leaf area. Salt stress decreases photosystem II (PS-II) activity due to the impact on chlorophyll synthesis and photosynthetic capacity in plants, which limits carbon assimilation, inhibits electron transfer, and dissociates thylakoid membranes. Nutrient imbalance caused by salinity stress inhibits the absorption of other essential nutrients. Nitrogen and magnesium, which are essential elements for chlorophyll, are disrupted in their absorption [8].

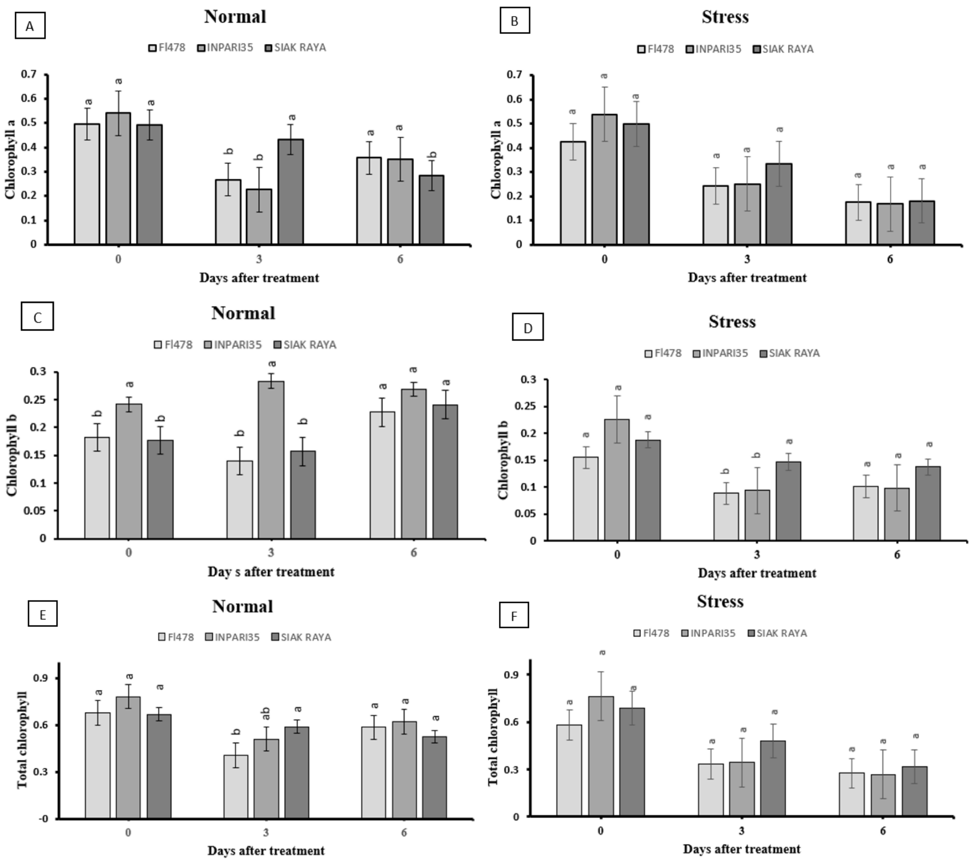

Fig. 7. Effect of Salinity Stress on Chlorophyll contents. (A-B) Chlorophyll a, (C-D) Chlorophyll b and (E-F) total Chlorophyll. Different letters indicate significant difference according to DMRT at $p<0.05$

All varieties maintained growth under salt treatment, as evidenced by the similar comparison between the control and salinity stress treatments. INPARI35 exhibited the lowest growth and biomass accumulation decline under stress, indicating its relatively superior salt tolerance compared to FI478 and Siak Raya. Salinity stress is generally caused by high concentrations of Na+ and Cl in the growing medium. Na+ and K+ enter cells using the same transporter series and compete with each other. K+ has a function for the catalytic activity of many important enzymes, K+ is also required for osmoregulation and protein synthesis, maintaining turgor pressure in cells and making photosynthetic activity optimal. Therefore, maintaining cellular Na+/K+ homeostasis is an important factor that determines the ability of plants to survive during salinity stress. The mechanism of rice plants that are tolerant to salinity stress in maintaining ion balance is by reducing Na+ ions in rice plants including increasing Na+ flow out of cells, limiting Na+ uptake, and Na+ compartmentalization in vacuoles. Salinity stress also causes osmotic stress and encourages tolerant plants to increase the accumulation of osmolyte compounds such as sugars, proline, glycine betaine, polyamines. These compounds play a role in osmotic adjustment under salinity stress by reducing cell osmotic pressure and stabilizing proteins and cellular structures of rice plants [9].

3.8 Correlation Analysis

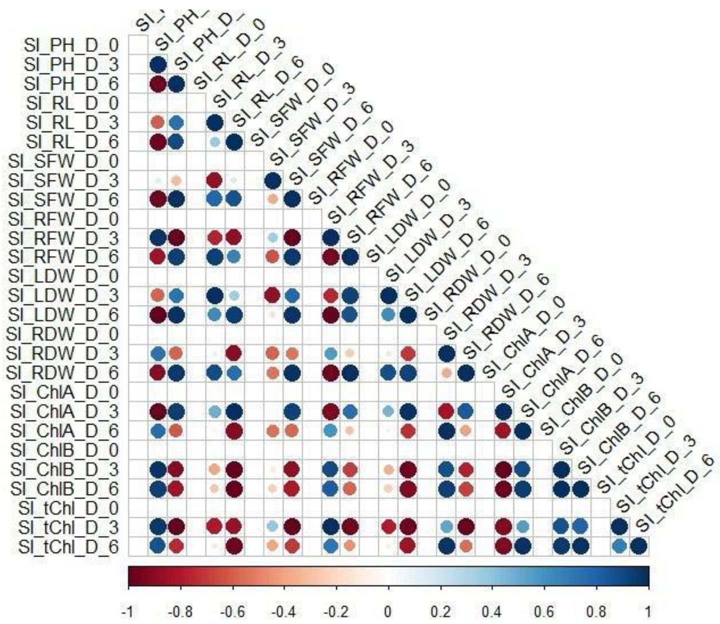

Fig. 8. correlation analysis for the salinity stress treatment

Based on the correlation analysis results for the salinity stress treatment, it was clear that plant growth at various observation times was strongly positively correlated. Several parameters, such as plant height, root length, shoot fresh weight, root fresh weight, leaf dry weight, and root dry weight on day 0, day 3, and day 6, showed a strong positive relationship. This is indicated by the deep blue color in the correlogram. This suggests an association where rice plants with larger initial size and biomass tend to be linked with better growth performance under salinity stress conditions. Positive relationships between growth components, such as between SFW and LDW or RL and RFW/RDW, also reflect an associated trend between shoots and roots. Increases in aboveground biomass (shoots) appear to be aligned with increases in root biomass possibly due to associated patterns of photosynthesis and water and nutrient uptake.

Furthermore, photosynthetic pigments (ChlA, ChlB, and total chlorophyll/tChl) showed a very strong positive correlation, indicating synchrony rather than direct causation in the biosynthetic pathway, as they are controlled by the same metabolic pathway. However, the direction of the relationship between pigments and growth changed after salt stress. On day 0 (before stress), high chlorophyll content was positively associated with growth, reflecting high photosynthetic capacity and greater biomass accumulation. After stress, this relationship tended to shift to a negative direction. This can be explained as a possible adaptive trade-off rather than a direct causal outcome [11].

Negative correlations over time, for example between PH_D6 and ChlB_D3, indicate an association with different adaptation strategies between plants. Some plants that experienced a more rapid decline in chlorophyll at the beginning of the stress may have been able to adapt through increased root growth or other physiological efficiency mechanisms, thus maintaining good growth in the later phase of the observation. Overall, these results indicate that positive correlations between growth parameters reflect a close link between plant vigor and biomass; photosynthetic pigments indicate biosynthetic synchrony, while the change in direction of the correlation between pigments and growth after salt stress indicates different physiological adaptation mechanisms between genotypes in response to salinity stress [12].

4 Conclusion

The results demonstrated that exposure to 100 mM NaCl induced mild reductions in growth and biomass accumulation among the tested rice varieties, but none exhibited severe growth inhibition. Overall, the Inpari 35, FI478 and Siak Raya varieties have good adaptation and resistance to salinity stress. Inpari 35 can be characterized as the most salt-tolerant genotype at the early vegetative stage, This shows that Inpari 35 has adaptive physiological mechanisms, such as the ability to maintain Na^{+}/K^{+} ion homeostasis and suppress damage caused by ROS.

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