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RESEARCH ARTICLE

Effects of salinity on growth traits, physiological parameters and chlorophyll in vegetative phase of Indonesian local rice (*Oryza sativa* L.) varieties

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Abstract

Rice (*Oryza sativa* L.) is one of the most important staple food crops for most of the world's population. However, rapid population growth and the conversion of agricultural land to other uses have increased the demand for higher rice production. One approach to increase rice yields is to expand the planting area on high salinity land using rice varieties with enhanced tolerance under salinity stress. This study aims to test the level of resistance of several rice varieties during the vegetative phase under salinity stress. The rice varieties tested in this study include Pamelen, Inpago 10, Gilirang and Inpari 30. The steps of this study include preparation and sowing of rice seeds, making planting media, planting hydroponically and treating salinity stress, harvesting, observing and measuring treatment results. A factorial randomized block design (RBD) was used, with two factors: variety and salinity level (0, 100 and 200 mM NaCl), with three replications. The results obtained from this study were that the administration of salinity stress caused the four rice varieties to experience morphological, physiological and chlorophyll pigment disorders. Based on the results of research and statistical analysis conducted, the Inpari 30 variety shows the highest tolerance ($p < 0.05$) across most traits.

Keywords: chlorophyll pigment; morphology; *Oryza sativa;* physiology; rice; salt stress

Introduction

Rice (*Oryza sativa* L.) is a staple food crop for most of the global population (1). In previous studies (2), Indonesia's rice production in 2022 reached 54.75 million tons, yielding 31.54 million tons of milled rice. However, the country's growing population has led to an increasing demand for rice as a staple food (3). Supply shortages resulted by the incline of rice consumption continues to rise without a corresponding increase in production, it may result in (4). This challenge is further exacerbated by land conversion and conflicts over land use due to urban expansion and housing demands, reducing the available agricultural land (5, 6). Therefore, strategies to enhance rice production are essential to meet future demand.

One approach to increasing rice production is the utilization of marginal lands to expand cultivation areas (7). Marginal lands have inherent limitations, such as low organic matter content, making them less optimal for agriculture (8). In Indonesia, an archipelagic country, high-salinity soils are among the most common types of marginal land (9). Research on salinity stress in plants is crucial, but the variability and heterogeneity of soil conditions pose challenges for field studies. Therefore, controlled environmental conditions are necessary for effective research (10). Hydroponic system provide an alternative by offering better control over environmental factors, optimizing nutrient supply, aeration and plant care (11).

Salinity is an abiotic stress caused by excessive salt accumulation in the soil or air, negatively impacting plant growth. High salt levels lead to physiological drought, reducing water uptake efficiency in plants (12). Salinity stress is a global issue, affecting up to 20 % of agricultural land and significantly reducing crop yields (13). In Indonesia, saline soils are primarily found in coastal and arid regions. In coastal areas, salinization occurs due to seawater intrusion, while in low-rainfall regions, high evaporation rates prevent the natural leaching of salt from the soil (14). Other contributing factors include saltwater irrigation, marine mudflows, salt rock erosion, fossil and mineral deposits, oil and gas mining waste and excessive use of chemical fertilizers (15).

Salinity stress is particularly detrimental during the early vegetative phase, as young plants are highly sensitive and vulnerable to damage (16). It inhibits growth and development, disrupts metabolism such as protein and lipid synthesis and affects cellular homeostasis (17). Excessive salt levels cause osmotic imbalances, reducing nutrient uptake and inducing the generation of reactive oxygen species (ROS) (18). These disruptions lead to reduced turgor pressure, cell dehydration and in severe cases, cell death. Increased sodium (Na^+) and chloride (Cl^-) ion uptake can be toxic, causing leaf chlorosis, curling and desiccation (12). Additionally, salinity stress reduces cellular energy, causes redox imbalances and inhibits photosynthesis, ultimately leading to abnormal plant growth (19).

To mitigate the effects of salinity, developing salt-tolerant rice varieties is crucial (20). Rice resistance has been studied using transcriptomic approaches (21, 22). Furthermore, efforts to improve resistance have been conducted using complementary testing approaches using rice genes inserted into *Arabidopsis* (23, 24). This study focused on evaluating the salt stress tolerance of several high-yielding rice varieties bred for resistance to biotic and abiotic stresses. The varieties selected for the study were based on their resistance to several stresses. Gilirang is resistant to brown planthoppers and bacterial leaf blight (25). Pamelen is resistant to bacterial leaf blight, moderately resistant to blast and tungro. Inpago 10 is resistant to drought and Ai toxicity at 60 ppm Ai 3+, resistant to blast race 033 and moderately resistant to blast race 133 and 073. Inpari 30 is moderately susceptible to brown planthopper biotypes 1 and 2, susceptible to biotype 3, moderately susceptible to bacterial leaf blight pathotype III and susceptible to pathotypes IV and VIII. Inpari 30 is resistant to soaking (26). These varieties are resistant to several stresses. However, there is no information yet regarding their response to salinity stress. Given the potential of saline lands for agriculture, further research is essential to develop salt-tolerant rice varieties (27). Therefore, this study aimed to assess the salinity tolerance of the rice varieties Pamelen, Inpago 10, Gilirang and Inpari 30 during the vegetative phase.

Materials and Methods

The rice varieties selected in this study were Pamelen, Inpago 10, Gilirang and Inpari 30 obtained from Rice Research Center (BBPadi) Subang, West Java. A total of 120 good rice seeds of each variety were collected and soaked in water for 24 hr to induce germination. The water-soaked rice seeds were then incubated at room temperature for 10 days by soaking them in distilled water for sowing in hydroponic system. The planting medium used in this research is hydroponic system. The hydroponic growing medium is made from water:nutrient A:nutrient B in a ratio of 990 mL:5 mL:5 mL. This dosage is based on the instructions for use on the AB mix hydroponics Surabaya packaging with nutrient A containing the macronutrients N, P, K, Ca, Mg and S and nutrient B containing the micronutrients Fe, Mn, Zn, B, Cu and Mo. This hydroponic growing medium is periodically stirred to prevent sedimentation of the nutrients and distribute them throughout the medium. The hydroponic planting medium uses plasterboard and cork as a support for the plants to grow and stay above the surface of the solution.

Rice was sown in six trays without applying salt stress as an initial adaptation to the hydroponic seedling medium. Salt stress treatment was carried out when the rice plants were 7 days after sowing (DAS) in 4 tanks with salinity concentrations of 0 mM, 100 mM and 200 mM.

The salt solution is prepared from 3 M NaCl by weighing 175.32 grams of NaCl and then dissolving the NaCl in 1 L of water. The 100 mM salt stress treatment used 333.3 mL of NaCl solution and the 200 mM salt stress treatment used 666.6 mL of NaCl. In more detail, the NaCl addition follows the following formula:

M1. V1 = M2. V2à3000 mM. V1 = 100 mM. 10,000 mLàV1 = 333.34 mL (Eqn. 1)

Information:

V1 = Volume before dilution; V2 = Volume after dilution; M1 = Concentration before dilution; M2 = Concentration after dilution

Rice plants are harvested twice, the first harvest is done when the rice plants are 7 days after sowing and is harvested as day 0 of stress and when the rice is 10 days after sowing as day 3 after stress. This is based on preliminary trial data showing that rice plants exhibited significant changes on the third day after stress. The parameters measured in this study include plant height (cm) calculated from the base of the crown to the growing point of the crown, plant root length (cm) measured from the base of the root to the root tip, shoot fresh weight and root fresh weight (mg) which were weighed directly after harvest using an analytical balance, shoot dry weight and root dry weight (mg) were weighed using an analytical balance after the shoot and roots were placed in the oven for 3 days. Water content was calculated using the shoot fresh weight and dry weight. Electrolyte leakage was obtained by measuring the electrical conductivity after the sample was harvested (EC1) and the electrical conductivity after the sample was placed in the autoclave (EC2). Electrolyte leakage was calculated using the following formula:

$$EL\,[\%] = (EC1/EC2) \times 10 \quad \text{(Eqn. 2)}$$

Chlorophyll was measured by 20 mg of fresh leaves obtained from each plant sample and added 1 mL of 80 % acetone in a 1.5 mL microtube, incubated in the dark for 24 hr at 4 °C. Chlorophyll was observed with a spectrophotometer by measuring the absorption of Chl a and Chl b at wavelengths of 646 nm and 663 nm. Chlorophyll concentration was calculated according to previous studies (28):

$$\text{Chlorophyll a} = (12.21 \times A663 - 2.81 \times A646)$$

$$\text{Chlorophyll b} = (20.13 \times A646 - 5.03 \times A663)$$

$$\text{Total chlorophyll} = \text{Chl a} + \text{Chl b} \quad \text{(Eqn. 3)}$$

This study used a randomized block design (RBD) consisting of two factors: four rice varieties and three salt stress treatments (0 mM, 100 mM and 200 mM), with three replications. The data were analyzed using analysis of variance (ANOVA) to determine the effect of treatment on the measured parameters. If the treatment has a significant effect on the changes in the parameters, then DMRT (Duncan's multiple range test) is continued with a confidence level of 95 %

Results and Discussion

Effect of salinity stress on plant shoot fresh weight

The fresh weight of the shoot is obtained by weighing after harvest. The results of the average fresh weight of shoots of rice plant varieties after salt stress treatment are shown in Fig. 1A and B. Four rice varieties tested on day 0 of salt stress showed that the fresh weight values of shoots were not significantly different as no salt stress occurred (Fig. 1A). At the same time, on day 3, the addition of salt stress caused a significant difference in the fresh weight of shoots between the control treatment and salt stress treatment of all tested rice varieties (Fig. 1B). The inpari30 variety presents a better level of tolerance than other varieties, this can be seen in the fresh weight of the shoot produced by this variety after suffering salt stress, which is not very different from the treatment without salt stress.

Plants can produce assimilates from the process of photosynthesis and transform them into food reserves, which can be observed by the increase in the size of plant organs, shoot dry weight can describe the amount of food reserves translocated to the shoot.

Effect of salinity stress on shoot dry weight

Shoot dry weight was measured after oven-drying for 3 days. On day 0, there were no significant differences among the rice varieties, as no salt stress was applied. By day 3, salinity had a negative impact, particularly in Pamelen, Inpago10 and Gilirang, which showed a marked decrease in shoot dry weight (Fig. 1 C and D In contrast, Inpari30 showed no significant difference between control and salt-stressed plants, suggesting more stable dry biomass under salinity.

Effect of salinity stress on root fresh weight

Root fresh weight was measured after harvesting. On day 0, there were no significant differences among varieties. By day 3, most varieties showed a notable reduction under salt stress (Fig. 1E and F). The largest reductions were recorded in Pamelen and Inpago 10, while Gilirang maintained higher root fresh weight under both 100 mM and 200 mM NaCl. The Gilirang variety showed the highest tolerance in this parameter, with minimal difference between control and salt-stressed roots.

Effect of salinity stress on root dry weight

There are no significant differences were observed on root dry weight at day 0. By day 3, the addition of salt led to a reduction in root dry weight across all varieties (Fig. 1G and H). The reduction was most severe at 200 mM NaCl. The Gilirang and Inpari30 varieties exhibited higher tolerance, with smaller differences in root dry weight under salt stress.

Effect of salinity stress on plant water content

Water content is a critical indicator of plant stress response. On day 0, all varieties had similar water content. By day 3, salinity stress significantly reduced water content in all varieties (Fig. 1I and J). The magnitude of reduction increased with salinity level. Inpari30 and Gilirang maintained relatively stable water content under salt stress indicating better tolerance, while Pamelen and Inpago 10 showed greater reductions.

Effect of salinity stress on electrolyte leakage

Salinity stress damages the plasma membrane, causing electrolyte leakage (EL), which is a key indicator of plant stress (29). Electrolyte leakage (EL) levels were similar across rice varieties on day 0 but increased significantly by day 3 under salinity stress (Fig. 1K and L). The increase was highest in Pamelen and lowest in Inpari 30. Higher salinity levels resulted in higher EL values for all varieties. Among the varieties tested, Inpari 30 showed the best tolerance, with lower EL levels after salinity exposure.

Effect of salinity stress on plant height

The response of rice varieties under normal conditions and salinity stress can be seen in Fig. 2. Plant height was obtained by taking measurements using imagej. The results of the average height of rice varieties on day 0 and day 3 after undergoing salt stress treatment are shown in Fig. 3 A and B. On day 3, the addition of salt stress showed a negative impact on plants with a significant difference in plant height between control and salt stress treatments of all rice varieties tested. The reduction in height was more evident at 200 mM NaCl. The Inpari30 variety is a rice variety that has better tolerance than other varieties due to having minimal difference in plant height between the control treatment and salt stress.

Effect of salinity stress on root length

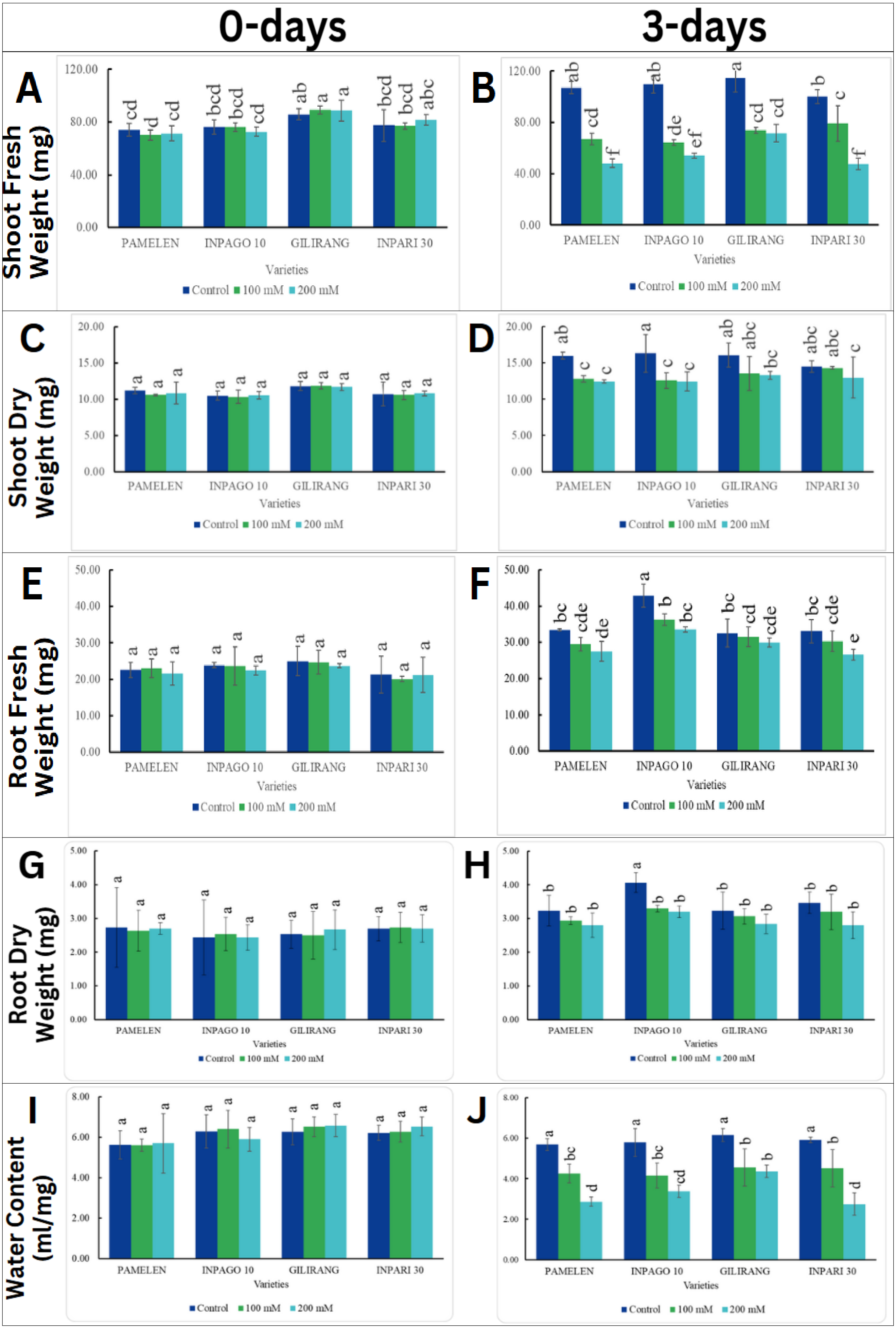
Root length is an important parameter for identifying the impact of salinity stress on plants because it experiences direct contact (30). Root length is obtained by measuring the length of plant roots using imagej. The results of the average root length of rice varieties subjected to salt stress are shown in Fig. 3 C and D. On day 3, plants with a shorter root length difference when they received the salt stress treatment was shown in all varieties tested. The Inpari30 variety shows better tolerance than other varieties because it does not suffer a significant decrease in root length due to salt stress.

Effect of salinity stress on chlorophyll pigments

Leaves are important organs in photosynthesis and the chlorophyll content in leaves can provide an overview of the efficiency of plant photosynthesis (31). Chlorophyll was measured to see the impact of salinity stress on the photosynthesis process of plants (32). The average results of chlorophyll a, chlorophyll b and total chlorophyll of rice plant varieties given salinity stress are shown in Fig. 4a-c, the rice varieties tested on the 3rd day of salinity stress have shown a negative impact on plants with a difference in lower chlorophyll content when given salinity stress treatment from all rice varieties tested. The inpago10 variety has higher tolerance with chlorophyll a, chlorophyll b and total chlorophyll levels not experiencing a significant decrease in salinity stress.

The results of the study showed that salinity stress at concentrations of 100 mM and 200 mM NaCl had a significant negative impact on the morphological and physiological growth and chlorophyll pigment content of four rice varieties, with the effect increasing with increasing salt concentration. In general, the decrease in fresh and dry weight of shoots and roots, plant height, root length, water content and chlorophyll content, as well as increased electrolyte leakage, indicates metabolic disorders and cell damage caused by excessive accumulation of Na^+ and Cl^- ions. Differences in responses among varieties indicate varying adaptability to salinity stress. Inpari 30 consistently exhibited higher tolerance levels than the other varieties, as evidenced by smaller reductions in growth parameters and lower electrolyte leakage. Inpari30's tolerance may be due to its ability to maintain Na^+/K^+ balance through ion exclusion and vacuolar sequestration. Excessive of Na^+ ions can be reduced by increasing its excretion. Na^+ ions leave the cell, limiting the uptake of Na^+ ions and accumulating Na^+ ions in the vacuole so that ionic balance can be maintained (1)

Results show that salt stress greatly affects the water content of the plant, so plants under salt stress exhibited reduced shoot fresh weight and root fresh weight. Salt stress can lead to osmotic disturbances such that water uptake is less than optimal and results in decreased shoot fresh weight and root fresh weight as the amount of water available to them is limited (33). High salinity levels also cause turgor pressure in the leaves to decrease, leading to stomatal closure and reduced stomatal conductance, which then disrupts the photosynthesis process (34). This will impact on the reduction of the fresh shoot weight of each variety, which indicates the water and organic matter content of the plant. The existence of different responses between varieties may be due to differences in the genetic characteristics of each variety, resulting in different adaptive responses to salt stress (35). Excess Na^+ and Cl^- uptake disrupts the uptake of essential nutrients (Ca^{2+}, K^+, NO_3^-), inhibiting protein



0-days
3-days
A
B
C
D
E
F
G
H
I
J
Shoot Fresh Weight (mg)
Shoot Dry Weight (mg)
Root Fresh Weight (mg)
Root Dry Weight (mg)
Water Content (ml/mg)
120.00
80.00
40.00
0.00
20.00
15.00
10.00
5.00
50.00
40.00
30.00
5.00
4.00
3.00
2.00
1.00
8.00
6.00
PAMELEN
INPAGO 10
GILIRANG
INPARI 30
Varieties
Control
100 mM
200 mM

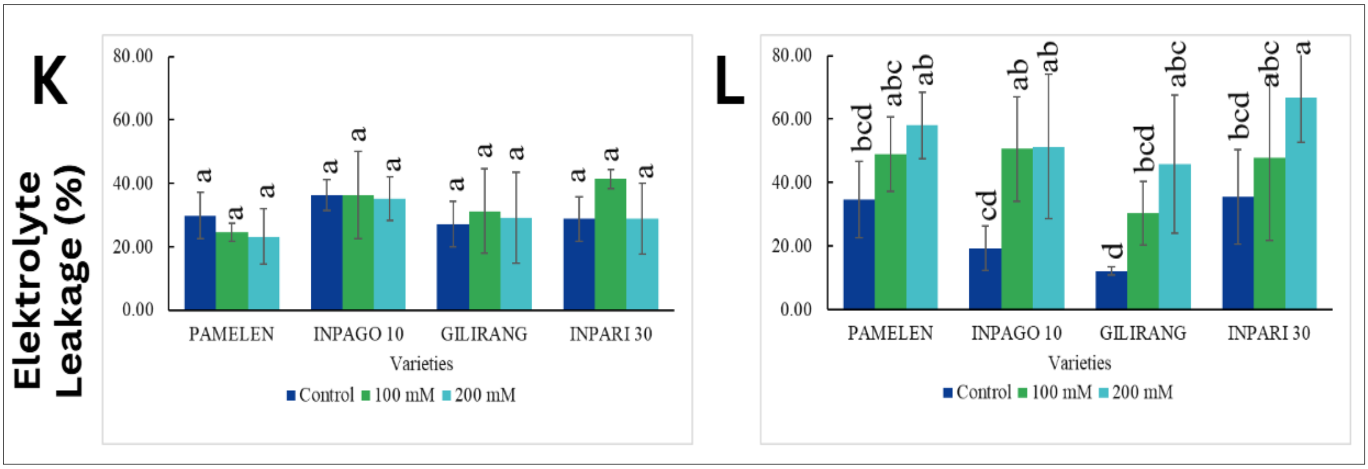


Fig. 1. Effect of salinity stress on physiological parameter. (A, B) Shoot fresh weight; (C, D) Shoot dry weight; (E, F) Root fresh weight; (G, H) Root dry weight; (I, J) Water content; (K, L) Electrolyte leakage.

Fig. 2. Response of rice varieties under normal conditions and salinity stress of 100 mM and 200 mM.

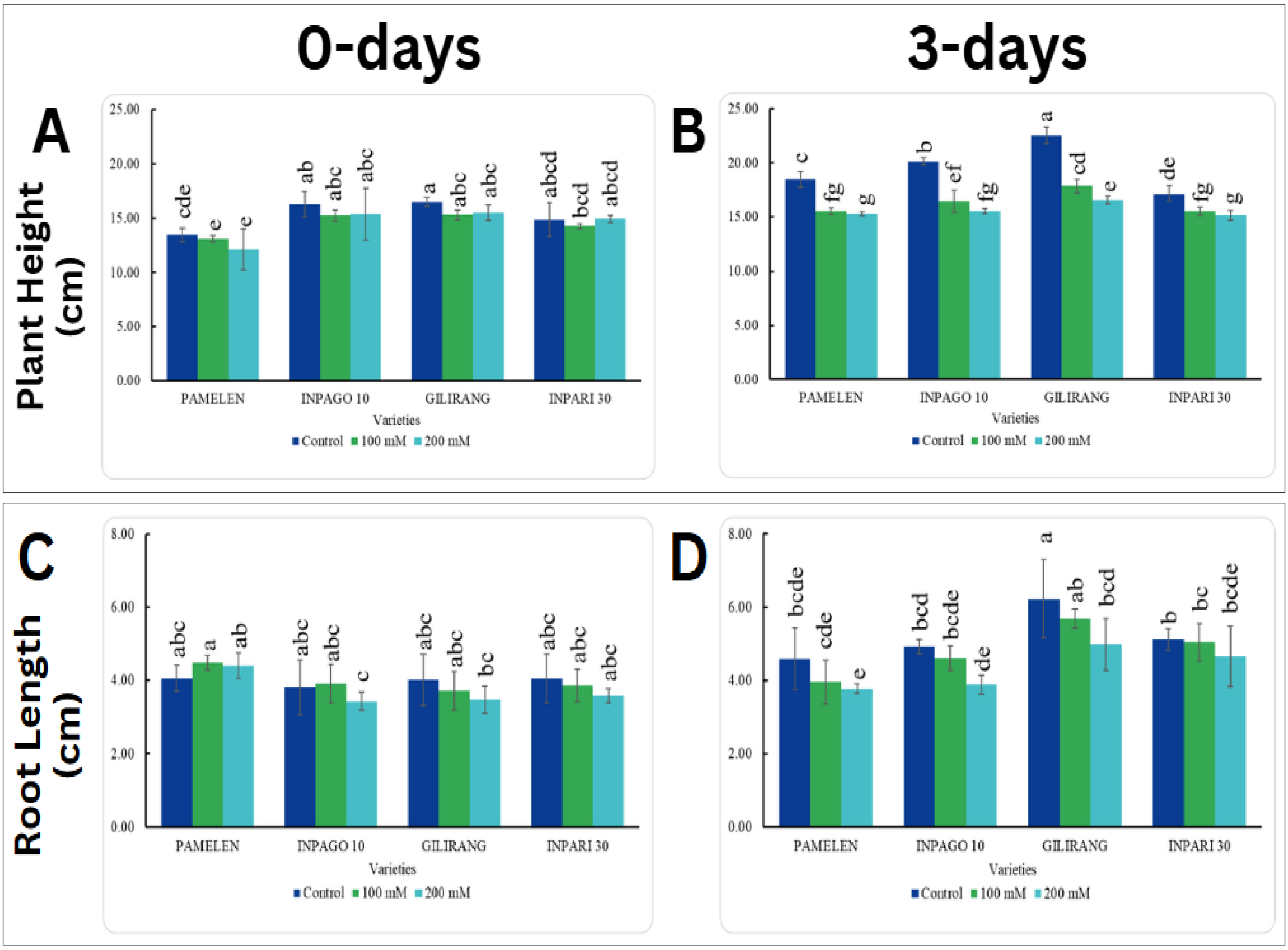


Fig. 3. Effect of salinity stress on morfological parameter. (A, B) Plant height; (C, D) Root length.

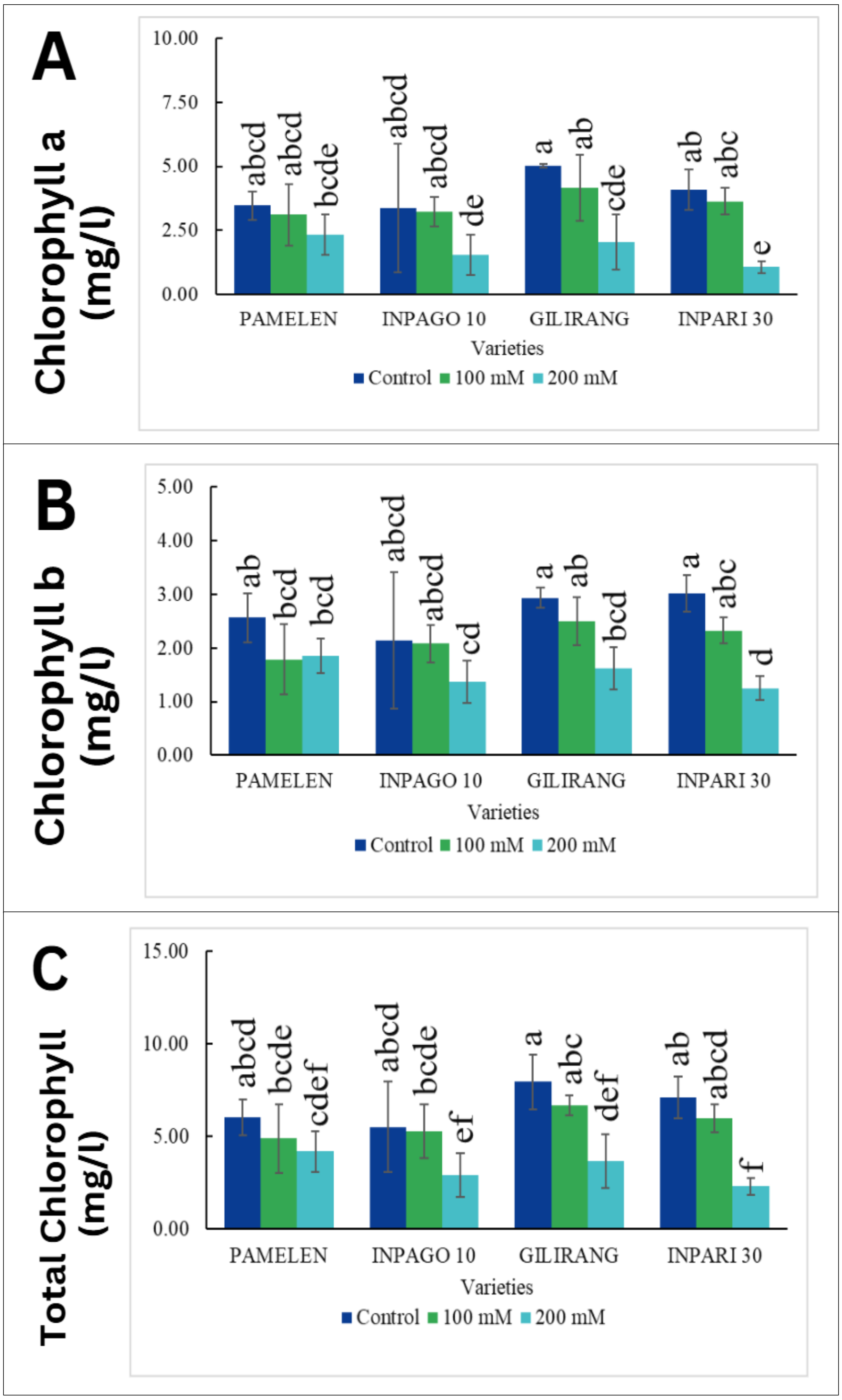


Fig. 4. Effect of salinity stress on chlorophyll contents (A) Chlorophyll a, (B) Chlorophyll b and (C) Total Chlorophyll.

synthesis, photosynthesis and enzyme activity and damage to organelles in cells thereby inhibiting plant growth and affecting the reduction in root fresh weight (36). Salinity stress can cause a decrease in the dry weight of plant shoot, this is caused by high salt levels which disrupt the photosynthesis process due to the accumulation of Na^+ and Cl^- ions in chloroplasts, reducing assimilate production and translocation to shoots (37, 33). High salinity induces osmotic and ionic stress, limiting shoot growth and promoting early senescence, resulting in a decrease in shoot dry weight (38). Salinity increases osmotic pressure, reducing water availability and impairing absorption (39, 40). Due to reduced water uptake and photosynthesis, thus affecting the metabolic process and cause a decrease in the root dry weight. High salt levels can trigger excessive ROS production, damaging chloroplasts, membranes and enzymes, thereby disrupting cellular metabolism (41-43).

Salinity stress causes increased osmotic pressure and decreased water potential, leading to water loss and membrane damage and will have an impact on reducing the plant's ability to absorb water and nutrients necessary (44). Excessive Na^+ and Cl^- accumulation further impaired water absorption, resulting in decreased water content (45). Salinity stress causes membrane damage and disrupted cell function. Salinity alters membrane structure and composition, leading to oxidative stress, which raises EL (46, 47). It also causes an imbalance in reactive oxygen species (ROS), resulting in membrane lipid damage, water loss and eventual cell death. Reduced potassium levels under salt stress further worsen EL, as potassium helps stabilize membranes (48). High EL impairs plant metabolism and growth (49).

Salinity stress causes disruption of mineral supply which induces ion changes, inhibition of photosynthesis process in plants which reduces carbohydrate supply and high salt levels can reduce tissue expansion turgor due to inhibited water absorption (50). This can influence the decrease in plant height in each variety, which corresponds to the inhibited metabolic capacity in the plant body. Salt stress can also cause leaf colour changes to yellow and discoloration due to degradation of chlorophyll levels, causing the leaves to turn green, which also correlates with disruption of the photosynthesis process that requires chlorophyll (33). Roots are an important part that absorbs water and nutrients from the planting medium (51). Salt stress can inhibit plant root growth because cell division and elongation are greatly affected by salt stress, thereby inhibiting root growth. Plants under salt stress produce the hormone ethylene as an adaptation, but excessive production of ethylene due to salt stress results in inhibition of plant growth and development, preventing roots from experiencing higher growth (52). Salinity stress that increases ions such as Na+, Ca2+ and Cl- causes a decrease in root diameter, number and length (53).

Plants that experience decreased chlorophyll levels due to salinity stress are considered a common symptom of oxidative stress and this is due to inhibition of chlorophyll synthesis and activation of chlorophyll degradation by the chlorophyllase enzyme. Decreased chlorophyll content indicates a photoprotection mechanism by reducing light absorption in plants (54). Salinity stress which causes an increase in certain ions such as Na+ and cl- causes other ions such as K+ to decrease and has an impact on reducing chlorophyll and damaging the thylakoid membrane (55), Mg^{2+} ions which are the main components of the formation of chlorophyll molecules are also reduced and are correlate with a decrease in chlorophyll pigment content. In addition, the decrease in total chlorophyll content due to high salinity stress is also caused by damage to the chloroplast membrane which has an impact on less chlorophyll accumulation and decreased photosynthesis efficiency (56).

The correlogram in Fig. 5 illustrates the Pearson correlation coefficients among eight plant traits: Plant Height (PH), Root Length (RL), Shoot Fresh Weight (SFW), Shoot Dry Weight (SDW), Root Fresh Weight (RFW), Root Dry Weight (RDW), Water Content (WC) and Electrolyte Leakage (EL). Several parameter pairs demonstrate consistent correlation trends across both figures, Fig. 5A and Fig. 5B, indicating stable relationships regardless of the treatment conditions. Notably, PH and RL consistently exhibit a strong positive correlation in both figures, suggesting that taller plants tend to develop longer roots, regardless of environmental stress. Similarly, SFW and SDW maintain a positive relationship in both conditions, reflecting that greater shoot biomass is generally correlate with increased dry matter accumulation. The relationship between RFW and RDW also remains strongly positive across figures, indicating a proportional increase in dry mass with fresh root biomass. In addition, SFW is positively correlated with both RFW and WC in both figures, raising the suggestion that more vigorous shoot growth is accompanied by increased root biomass and better water status. In terms of negative correlations, PH and Electrolyte Leakage (EL) consistently show an inverse relationship, implying that taller plants tend to exhibit lower membrane damage, indicating a better stress tolerance. Similarly, WC and EL are negatively correlated in both figures, suggesting that higher water content within plant tissues is correlates with reduced electrolyte leakage and thus better membrane stability. These consistent trends highlight key physiological relationships that are maintained under varying environmental or experimental conditions.

Conclusion

Based on the research that has been carried out, it was concluded that salinity stress at 100 mM and 200 mM NaCl significantly causes negative impacts on rice plants. The four rice varieties tested in the vegetative phase experienced morphological, physiological and chlorophyll pigment reduction, especially under 200 mM salinity stress treatment. Based on the results of research and statistical analysis conducted, the Inpari 30 variety shows the highest tolerance ($p < 0.05$) in terms of fresh shoot weight, dry shoot weight, dry root weight, water content, plant height, root length and electrolyte leakage. In contrast, Pamelen was the most sensitive variety, showing the greatest reductions across most traits. However, field validation under saline conditions is necessary before recommending its large-scale adoption.

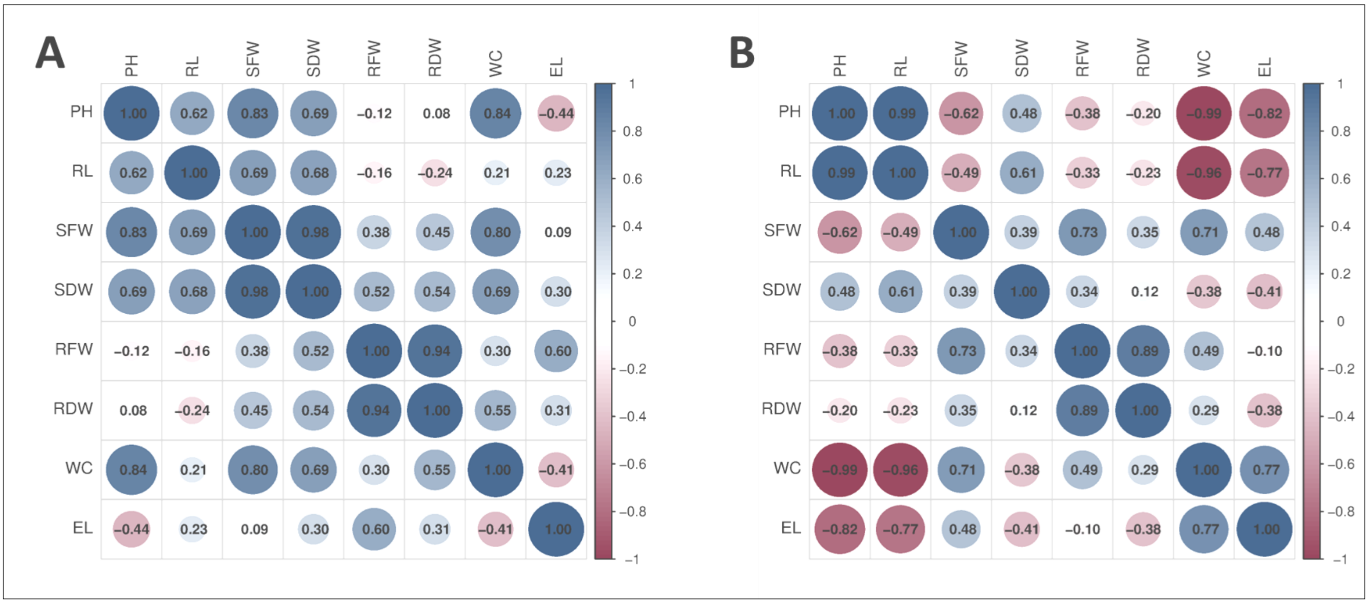


Fig. 5. The correlogram of performance (Δ). (A). Performance comparison between 100 mM to 0 mM; and (B). Performance comparison between 200 mM to 0 mM. PH: Plant height, RL: Root length, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, WC: Water content, EL: Electrolyte leakage.

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Authors' contributions

MQJ contributed to research design, conceptualization, writing of the manuscript, formal analysis and plant growth analysis. AYPH contributed to formal analysis and plant growth analysis. TN carried out statistical and formal analyses. WM contributed to plant growth analysis and revision of the manuscript. TBS contributed to conceptualization, research design, results and discussion, writing, revision of the manuscript and overall coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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