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Limited Salt Tolerance of Indonesian Rice Varieties Biosalin-1-Agritan and Biosalin-2-Agritan at Early Seedling Stage under NaCl Stress

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Abstract

This study evaluated the salt tolerance of 2 salt tolerant rice varieties, Biosalin-1-Agritan and Biosalin-2-Agritan under NaCl concentrations ranging from 0 to 50 part-per-thousand (ppt) to assess their potential for large-scale cultivation on saline soils. Plant height, root length, fresh weight and dry weight were measured at 5 and 12 days after NaCl exposure, indicating both varieties maintained stable growth at NaCl levels up to 10 ppt, but growth metrics declined significantly at higher salinity levels. Notably, Biosalin-2-Agritan showed marginally better tolerance than Biosalin-1-Agritan, with resilience in some growth traits. Despite these observations, the limited resilience of both varieties under high salinity suggests that further genetic improvements, such as sodium azide-induced mutagenesis, are necessary to enhance their performance. This study provides essential insights into the salt tolerance of these rice varieties, supporting ongoing efforts to expand agricultural productivity in saline environments and strengthen food security in Indonesia.

Keywords: Abiotic stress, High-salinity tolerance, Mutant, Rice varieties seedling growth

Introduction

Rice is a vital staple crop in Indonesia, supporting the dietary needs of more than 90 % of the population [1]. With a population of around 270.2 million [2], Indonesia faces substantial rice demand, driven by population growth and an average annual per capita consumption of 74.78 kg [3]. This translates to a national requirement of approximately 20.21 million tons annually. This significant dependence on rice highlights the critical need to maintain and improve rice production to secure the nation's food supply. The limited availability of arable land presents a major obstacle to meeting rising demand, requiring innovative strategies to boost productivity. One such approach involves utilizing marginal lands, such as saline soils, for agricultural purposes. Indonesia's geographic conditions offer significant yet largely underutilized potential for expanding agriculture into saline lands. Between 1990 and 2018, the country's coastline increased from 90,586.25 to 91,363.65 km, a total extension of 777.40 km [4]. This change includes a loss of 5,995.52 km of natural coastline and a gain of 6,771.92 km in artificial coastline over the past 28 years. Indonesia has approximately 440,300 ha of saline land, with 140,000 ha classified as saline and an additional 304,000 ha categorized as slightly saline [5]. Salinity in these areas, characterized by elevated concentrations of Na^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-} in soil solutions [6], poses a significant challenge to rice cultivation.

Soil can be classified into 3 main types based on electrical conductivity (EC), pH, exchangeable sodium percentage (ESP), sodium adsorption ratio (SAR) and physical condition [7]. Saline soils have an EC above 4 dS m^{-1} (equivalent to 2.11 ppt), a pH below 8.5, an ESP under 15, and a SAR below 13, generally maintaining a normal physical structure. Sodic soils have an EC below 4 dS m^{-1}, a pH above 8.5, an ESP over 15, and a SAR above 13, leading to poor physical structure due to high sodium levels, which can negatively impact soil structure. Saline-sodic soils show high EC above 4 dS m^{-1} and an ESP over 15, with SAR values above 13. Although their pH is usually below 8.5, some saline-sodic soils can exceed this threshold. Despite their high sodium levels, saline-sodic soils typically retain a normal physical structure. Saline soils contain high levels of soluble salts, which can inhibit plant growth, although many crops experience adverse effects even at lower EC levels [8]. Soil salinity disrupts plant physiology, causing osmotic stress and ionic toxicity that stunts growth, reduces yields and threatens the survival of rice [9]. With limited arable land, utilizing saline soils for agriculture offers a valuable opportunity to increase food production, though it requires careful management to reduce salinity's harmful effects. In Indonesia, significant research efforts have focused on developing soil amelioration strategies, such as incorporating organic materials like livestock manure [10] and gypsum [11], to improve soil structure and decrease salinity. These amendments have demonstrated potential in enhancing the physical and chemical properties of saline soils, making them more favorable for plant growth.

In addition to soil amelioration efforts, breeding and deploying salt-tolerant rice varieties offers a promising strategy for addressing the challenges of saline agriculture. However, the high-yielding rice breeding programs of the 1960s and 1970s reduced genetic diversity, narrowing the genetic base of rice varieties. This limited diversity now restricts the progress of breeding programs [12,13]. To overcome this, the National Research and Innovation Agency (BRIN) developed 2 salt-tolerant rice varieties, Biosalin-1-Agritan (Decree of the Minister of Agriculture of the Republic of Indonesia No. 894/HK.540/C/06/2020) and Biosalin-2-Agritan (Decree of the Minister of Agriculture of the Republic of Indonesia No. 895/HK.540/C/06/2020), which have shown tolerance to salinity stress at the seedling stage. These varieties were developed through gamma irradiation of callus cultures from their respective parent varieties, Ciherang (22.468 Gy) for Biosalin-1-Agritan and Inpari 13 (23.124 Gy) for Biosalin-2-Agritan, enabling rice cultivation on saline soils. While Biosalin-1-Agritan and Biosalin-2-Agritan rice varieties have demonstrated resilience during the early stages of growth, little is known about how they perform under higher salinity levels. The findings indicate stable growth at salinity levels up to 10 ppt NaCl. This study aims to examine the growth potential of these both varieties when exposed to different concentrations of NaCl, representing a range of saline conditions. Specifically, we evaluated plant height, root length, fresh weight and dry weight as indicators of growth performance under increasing salinity levels. Insights gained from evaluating the resilience of these salt-tolerant varieties in controlled saline environments will inform their suitability for large-scale cultivation on saline lands.

Materials and methods

Plant materials and germination conditions

Two salt-tolerant rice varieties, Biosalin-1-Agritan (selection number CH-1) and Biosalin-2-Agritan (selection number II-13-78), were used in this study. Biosalin-1-Agritan has a 1,000-grain weight of 23.96 g, yield potential of 8.75 tons/ha, an amylose content of 20.07 %, a flowering time of 82 days after sowing (DAS), and a harvest time of 113 DAS. Biosalin-2-Agritan has a 1,000-grain weight of 24.24 g, yield potential of 9.06 tons/ha, an amylose content of 20.57 %, a flowering time of 74 DAS, and a harvest time of 107 DAS. Seeds of both varieties were obtained from the National Research and Innovation Agency (BRIN). Seeds from each variety were surface-sterilized with a 1 % (v/v) NaOCl solution for 10 min, thoroughly rinsed 5 times with distilled water, and then soaked in distilled water for 24 h at room temperature. Subsequently, the seeds were germinated in plastic boxes lined with moistened cotton paper and incubated in a growth room at 25 °C with a 12-hour light/12-hour dark photoperiod and 70 % relative humidity. The 12-hour light/12-hour dark photoperiod was chosen to provide neutral conditions that neither induce flowering nor favor the vegetative growth phase. This duration minimizes photoperiod effects, allowing us to focus solely on the tested variables, as demonstrated by Zhang *et al.* [14]; Hidayah *et al.* [15].

NaCl stress treatment

The rice seeds of Biosalin-1-Agritan and Biosalin-2-Agritan germinated by day 5, after which NaCl treatments at varying concentrations (0 as control, 10, 20, 30, 40 and 50 parts per thousand (ppt)) were applied 2 days later, based on modifications of salinity stress protocols by Farooq *et al.* [16], who used 0 - 25.52 ppt, and Garg *et al.* [17], who tested 0 - 47.93 ppt. The treatments were administered in 2 phases. In the first phase, beginning on day 2 after germination, seedlings were exposed to NaCl stress for 5 days, reaching 7 days post-germination at the time of the first observation. In the second phase, NaCl stress was continued for an additional 7 days, with the second observation conducted at 14 days post-germination. The 2 growth stages were chosen to capture distinct phases of rice development under NaCl stress. The first phase represents an early seedling stage when the plants are highly sensitive to environmental stresses. The second phase represents a slightly later developmental stage when the plants may begin to exhibit adaptive mechanisms or cumulative effects of stress. By analyzing responses at these stages, we aim to provide a more comprehensive understanding of the dynamic changes in rice seedlings under salinity stress. Each treatment involved 15 plants per variety, with 3 biological replicates per treatment. Data represent the mean per plant (cm/plant; mg/plant). The NaCl solutions were refreshed every 2 days to maintain stable salt concentrations.

Growth parameter measurements

After NaCl treatments, the phenotypic differences between the Biosalin-1-Agritan and Biosalin-2-Agritan rice varieties were documented. The experiment was conducted once with 2 treatment and observation phases. Photographs were taken 7 days after germination following 5 days of NaCl stress (first observation), and again 14 days after germination following 12 days of NaCl stress (second observation). Each photograph included a 1 cm scale bar to indicate size. Plant height (cm) was measured from the base of the stem to the tip of the longest leaf, and root length (cm) was measured from the base of the stem to the tip of the longest root. Both plant height and root length measurements were performed using ImageJ software (https://imagej.net/ij/), which was calibrated using the 1 cm scale bar in each photograph to ensure accurate pixel-based measurements. Leaf greenness was assessed during each observation phase using the Royal Horticultural Society (RHS) Colour Chart by visually matching the green color of the leaves to the closest shade in the chart under natural daylight conditions. This method ensured consistent and standardized comparisons. Fresh weight (mg) was determined by weighing the whole seedlings immediately after harvest using an analytical balance (Ohaus Pioneer PA214, Ohaus Corp., NJ, USA), which has a resolution of 0.1 mg and was regularly calibrated according to the manufacturer's specifications to maintain accuracy. For dry weight (mg), seedlings were dried under natural sunlight for 7 days until a constant weight was achieved, ensuring reliable measurements.

Statistical analysis

The growth measurements for each variety and treatment group were recorded as means ± standard error (SE). All data were analyzed using analysis of variance (ANOVA), and significant differences among treatments were identified using Duncan's multiple range test at $p < 0.05$. Different letters within the same figure panel indicate significant differences. Statistical analyses were conducted using R version 4.4.2 (https://www.r-project.org/) using the agricolae package, and graphs were generated with the dplyr, tidyr, and ggplot2 packages. Pathway analysis was conducted using structural equation modeling (SEM) in the lavaan package and visualized with the semPlot package.

Results and discussion

Growth responses during the first phase of NaCl stress

During the initial phase of observation, rice seedlings of both Biosalin-1-Agritan and Biosalin-2-Agritan showed variable responses to increasing NaCl concentrations over the observation period (**Figure 1**). The growth response of Biosalin-1-Agritan and Biosalin-2-Agritan rice varieties under different NaCl concentrations demonstrated a significant decrease in plant height with increasing salinity (**Figure 2(A)**). At NaCl concentrations of 0 to 10 ppt, both varieties maintained relatively stable plant heights. However, a notable decline in height was observed from 20 ppt onward, with a more pronounced reduction at 40 and 50 ppt. Biosalin-2-Agritan showed a slightly better ability to retain plant height compared to Biosalin-1-Agritan, suggesting that Biosalin-2-Agritan may have a higher tolerance to salinity in terms of vertical growth. This observation demonstrates a greater tolerance to salinity in both Biosalin-1 and Biosalin-2 varieties than what was reported by Rodríguez-Coca *et al.* [18], who observed reduced growth in rice under salt stress levels of 5 dS m^{-1} (8.96 ppt) to 7.5 dS m^{-1} (13.05 ppt). The ability to retain height more effectively under high salinity may reflect a superior capacity to maintain cell turgor and elongation processes under osmotic stress [19]. Fresh weight showed a clear decline as salinity increased (**Figure 2(B)**). At NaCl concentrations up to 20 ppt, both varieties displayed a relatively stable fresh weight, suggesting that these levels of salinity did not significantly impact growth. However, at 50 ppt, a marked reduction in fresh weight was observed in both varieties, with Biosalin-2-Agritan showing a slight advantage in maintaining shoot fresh weight. This result is in line with the findings of Jharna *et al.* [20], where rice shoot fresh weight consistently decreased as salinity increased, with a reduction of 70.02 % at 8 dS m^{-1} (13.86 ppt) and up to 96.42 % at 16 dS m^{-1} (26.19 ppt).

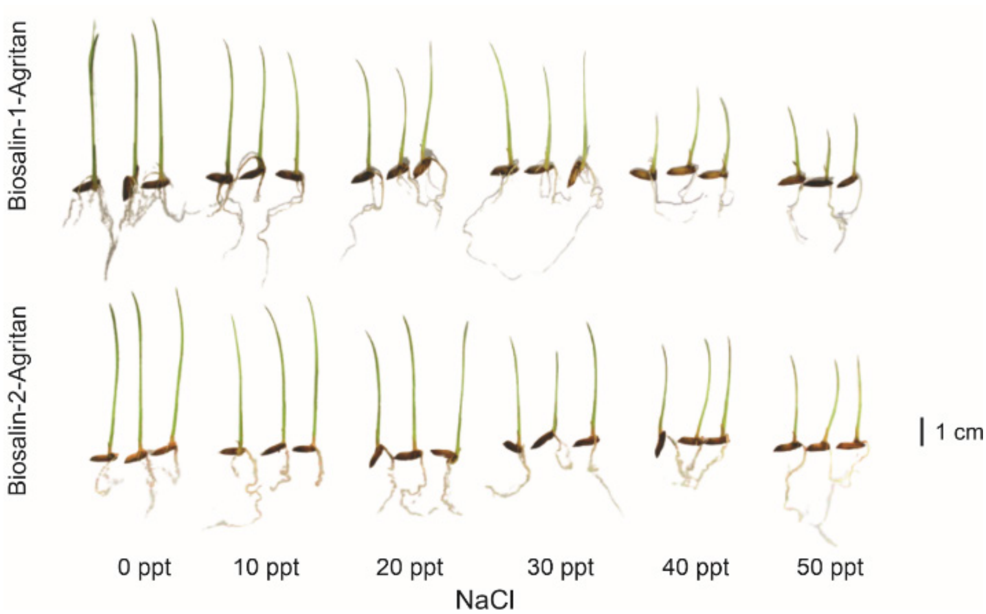

Figure 1 Phenotypic responses of Biosalin-1-Agritan and Biosalin-2-Agritan rice seedlings to different NaCl concentrations (0, 10, 20, 30, 40 and 50 ppt) observed on day 7 after germination, following 5 days of NaCl treatment. Scale bar = 1 cm.

Root length was also affected by salinity but exhibited a unique response pattern (**Figure 2(C)**). At 30 ppt NaCl, Biosalin-1-Agritan showed a significant increase in root length compared to other treatments, while Biosalin-2-Agritan remained relatively stable. This may indicate that Biosalin-1-Agritan adapts to moderate stress conditions by elongating its roots to enhance water uptake. However, at higher NaCl concentrations (30 - 50 ppt), the root length of both varieties decreased, indicating that salinity at these levels inhibits root development. Moderate salinity can also stimulate root elongation as an adaptive strategy for accessing deeper water sources in date palm seedlings [21]. However, as salinity levels increase, excessive ionic toxicity and osmotic stress inhibit root growth, which is a common response across rice varieties [22]. In terms of dry weight, both varieties exhibited relatively minor variations across NaCl concentrations, with significant differences only appearing at 20 - 40 ppt (**Figure 2(D)**). At this concentration, the dry weight of Biosalin-2-Agritan remained slightly higher, suggesting that Biosalin-2-Agritan may possess adaptive mechanisms to maintain dry biomass under high salinity stress. The ability of Biosalin-1-Agritan to maintain higher dry weight at 30 and 40 ppt suggests that it may possess better mechanisms for carbon allocation or osmoregulatory processes under saline conditions. This advantage in maintaining biomass has also been observed in other salt-tolerant rice varieties, as reported by Ferreira *et al.* [23].

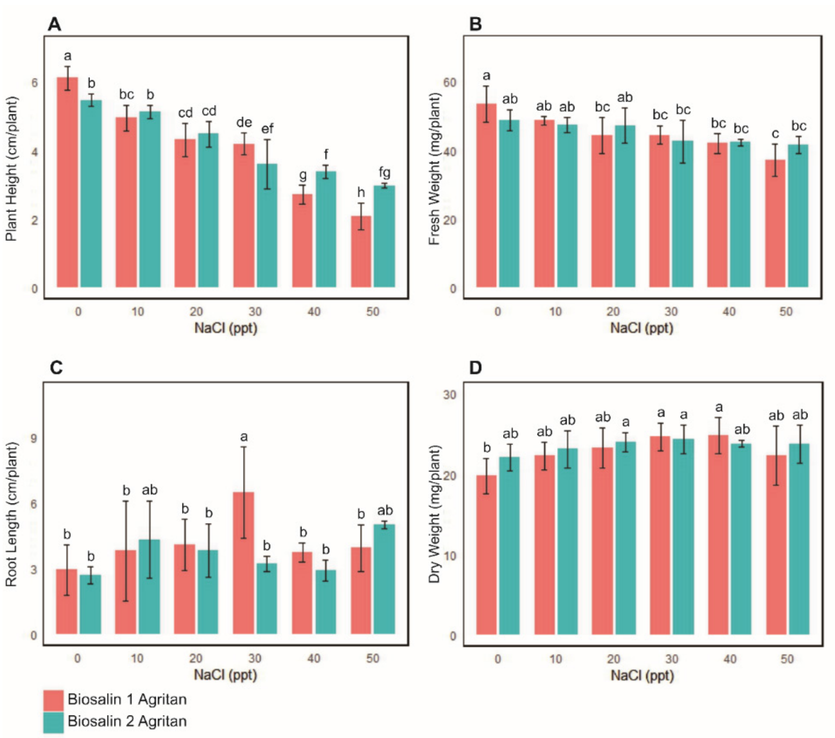

Figure 2 Growth responses of Biosalin-1-Agritan and Biosalin-2-Agritan rice varieties observed on day 7 after germination, following 5 days of treatment under different NaCl concentrations (0, 10, 20, 30, 40 and 50 ppt). (A) plant height (cm/plant), (B) fresh weight (mg/plant), (C) root length (cm/plant) and (D) dry weight (mg/plant). Different letters within the same figure panel indicate significant differences ($p < 0.05$) among treatments according to Duncan's multiple range test.

Growth responses during the second phase of NaCl Stress

Similar to the first phase, the second phase highlights distinct differences in salinity tolerance between the 2 varieties (**Figure 3**). Both exhibited resilience, maintaining healthy green shoots (RHS 149C, RHS 141D based on the Royal Horticultural Society colour chart) under salinity levels up to 10 ppt. By 20 ppt, they both showed significant browning, indicating their susceptibility to salt stress. As salinity levels increased, both varieties suffered severely, with widespread chlorosis. These observations are consistent with the findings of Alharbi *et al.* [24], who reported that visible signs of chlorosis and browning in rice seedlings occur when plants fail to maintain ionic balance and chlorophyll integrity under high salinity stress. Furthermore, both varieties significantly reduced plant height as NaCl concentration increased (**Figure 4(A)**). At 0 ppt, plant height reached its maximum, with no significant difference between the varieties. However, as salinity increased, plant growth was inhibited, significantly reducing height for both varieties. By 50 ppt, plant growth was inhibited to about a quarter of the control level. This reduction in plant height under increasing salinity levels is well-documented in previous studies, such as those by Li *et al.* [25], who found that rice growth is severely stunted at salinity levels 3 - 6 ppt. The reduction in height may be attributed to osmotic stress and ion toxicity, which interfere with cellular expansion and division. The more gradual decline in height observed in Biosalin-1-Agritan suggests that it may not have suitable mechanisms that help maintain cellular homeostasis for a more extended period under moderate salinity stress.

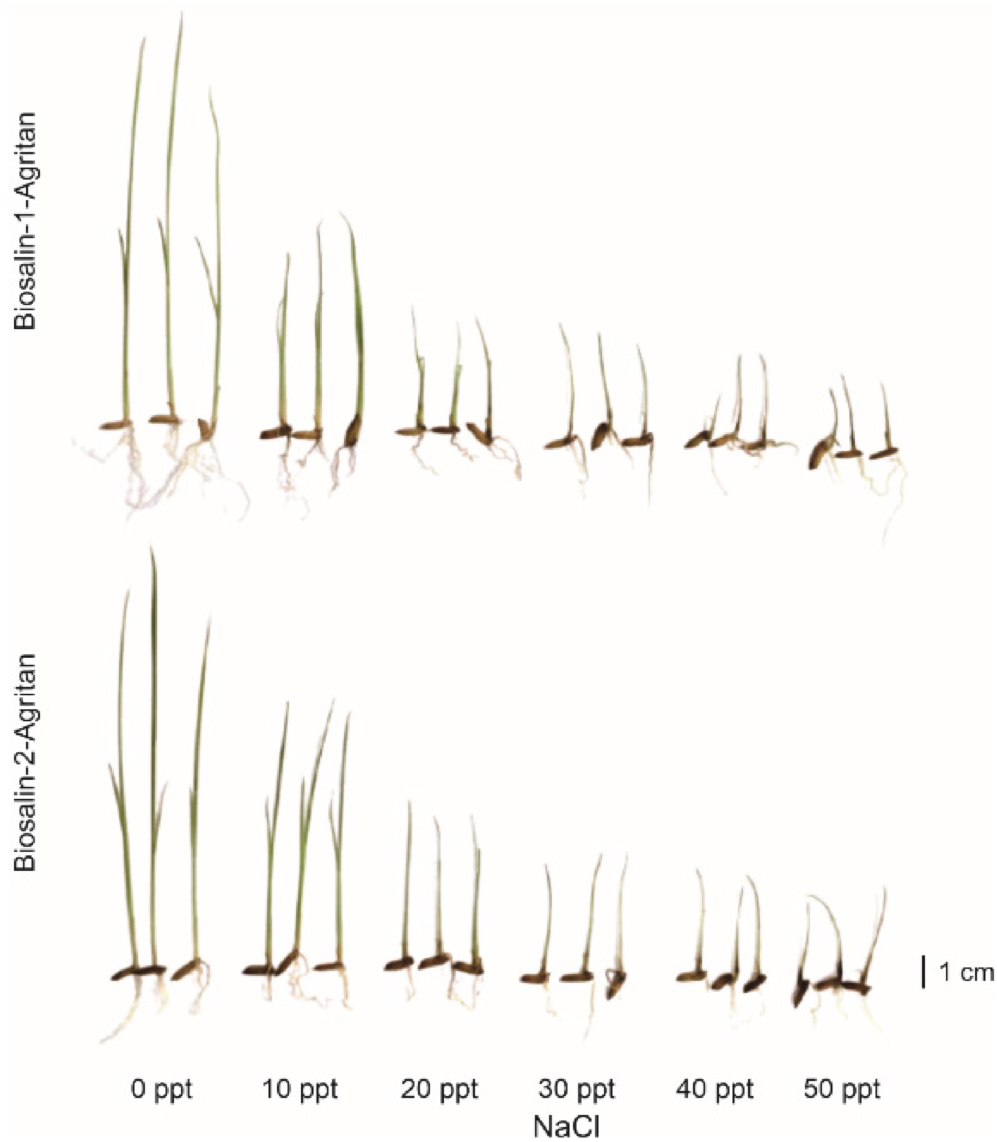

Figure 3 Phenotypic responses of Biosalin-1-Agritan and Biosalin-2-Agritan rice seedlings to different NaCl concentrations (0, 10, 20, 30, 40 and 50 ppt) observed on day 14 after germination, following 12 days of NaCl treatment. Scale bar = 1 cm.

Fresh weight results followed a similar trend, with a significant decrease observed as NaCl concentration increased (**Figure 4(B)**). At 0 and 10 ppt, both varieties showed comparable fresh weights, with no significant difference between them. However, as NaCl levels reached 20 ppt and above, fresh weight began to decline more noticeably. This trend mirrors findings by Subekti *et al.* [26], who noted that fresh weight in rice varieties starts to drop significantly at salinity levels around 10.5 dS m^{-1} (6 ppt, high salinity) due to water deficit and reduced nutrient uptake. Root length also showed a significant response to increasing NaCl concentrations (**Figure 4(C)**). At lower salinity levels (0 and 10 ppt), both varieties exhibited similar root lengt, with Biosalin-2-Agritan slightly outperforming Biosalin-1-Agritan. However, as salinity stress intensified from 20 to 50 ppt, root lengths of both varieties decreased. Salinity stress typically results in reduced root growth, as shown in studies by Herawati *et al.* [27], where rice root systems became shorter under 15.62 dS m^{-1} (9 ppt, high salinity) due to ionic imbalances and the toxicity of accumulated salts. The initial advantage of Biosalin-2-Agritan in root length could indicate a faster root development under mild stress, but its eventual decline at higher salinity suggests that it lacks the deeper adaptive responses necessary to cope with prolonged or more severe stress conditions. Furthermore, both rice varieties exhibited a consistent pattern in dry weight accumulation across increasing salinity levels (**Figure 4(D)**). At 0, 10, 40 and 50 ppt NaCl, there were no significant differences in dry weight between the 2 varieties. However, as salinity increased to 20 and 30 ppt, exhibited significantly higher dry weights compared to the lower salinity levels. The superior dry weight accumulation in both varieties at intermediate salinity levels suggests that it may have better carbon partitioning efficiency and stress-response mechanisms, allowing it to sustain biomass production [28].

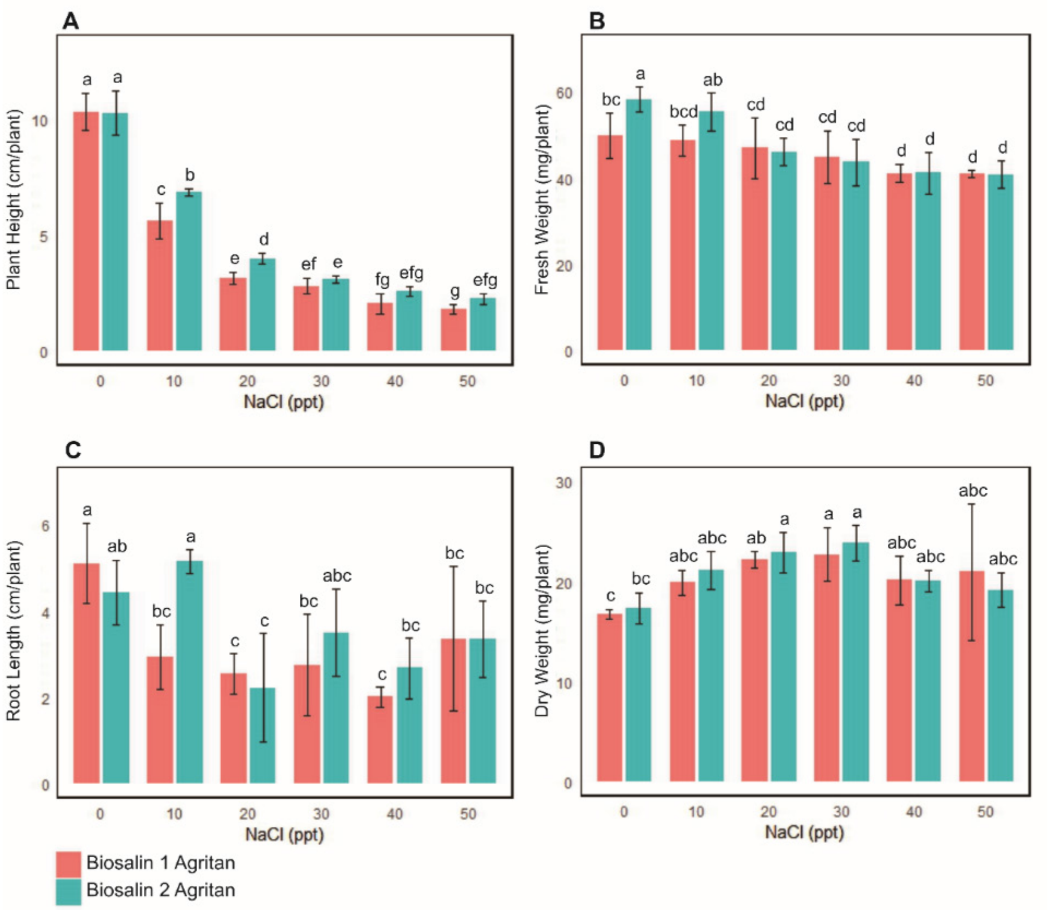

Figure 4 Growth performance of Biosalin-1-Agritan and Biosalin-2-Agritan rice varieties observed on day 14 after germination, following 12 days of NaCl treatment under different NaCl concentrations (0, 10, 20, 30, 40 and 50 ppt). (A) plant height (cm/plant), (B) fresh weight (mg/plant), (C) root length (cm/plant) and (D) dry weight (mg/plant). Different letters within the same figure panel indicate significant differences ($p < 0.05$) among treatments according to Duncan's multiple range test.

Impact of NaCl stress and seedling age on variety responses and future directions

The path analysis reveals complex interactions (**Figure 5**), in which NaCl stress shows a strong negative direct effect on plant height (path coefficient = −0.82), indicating its substantial impact on growth inhibition. Plant height exerts a moderately positive effect on root length (0.15), but NaCl stress itself has a negligible direct effect on root length (−0.01). Similarly, root length has no direct influence on seedling age, but seedling age negatively affects root length (−0.73), indicating that older seedlings may prioritize shoot development under stress conditions. These findings suggest that salinity stress predominantly affects the shoot system while having secondary effects on root development. Seedling age positively influences fresh weight (0.98) and directly impacts dry weight (0.65), with an indirect contribution via fresh weight (−0.15). This positive association underscores the role of seedling vigor in mitigating salinity's adverse effects. Conversely, NaCl stress negatively affects fresh weight (−0.15) but exhibits a modest positive effect on dry weight (0.25), possibly reflecting physiological adaptations, such as osmotic adjustments, that contribute to dry matter accumulation. Fresh weight shows a weak negative effect on root length (−0.24), emphasizing that biomass allocation under stress might favor shoots over roots. Plant height is weakly correlated with fresh weight (−0.05), suggesting that taller plants do not necessarily produce more biomass under salinity stress. NaCl stress does not directly influence fresh weight via plant height but may alter biomass allocation patterns. The analysis highlights the direct and indirect pathways by which salinity stress impacts plant growth parameters, with the most pronounced effects observed on plant height and biomass production. Overall, after 5 days of NaCl treatment, both rice varieties showed noticeable declines in plant height, fresh weight, root length and dry weight as NaCl concentrations increased. After a prolonged NaCl treatment period of 12 days, these adverse effects on growth parameters became even more pronounced, with significant reductions observed at salinity levels exceeding 10 ppt. This indicates that both varieties have limited tolerance to high salinity stress. These results are in agreement with the findings of Wankhade *et al.* [29]; Zhang *et al.* [30], who highlighted the impact of prolonged salt exposure on reducing rice growth, particularly at salinity levels above the tolerance threshold. While both varieties exhibit reduced performance under salt stress, Biosalin-2-Agritan's ability to maintain growth metrics slightly better than Biosalin-1-Agritan suggests it may have a marginally higher tolerance limit.

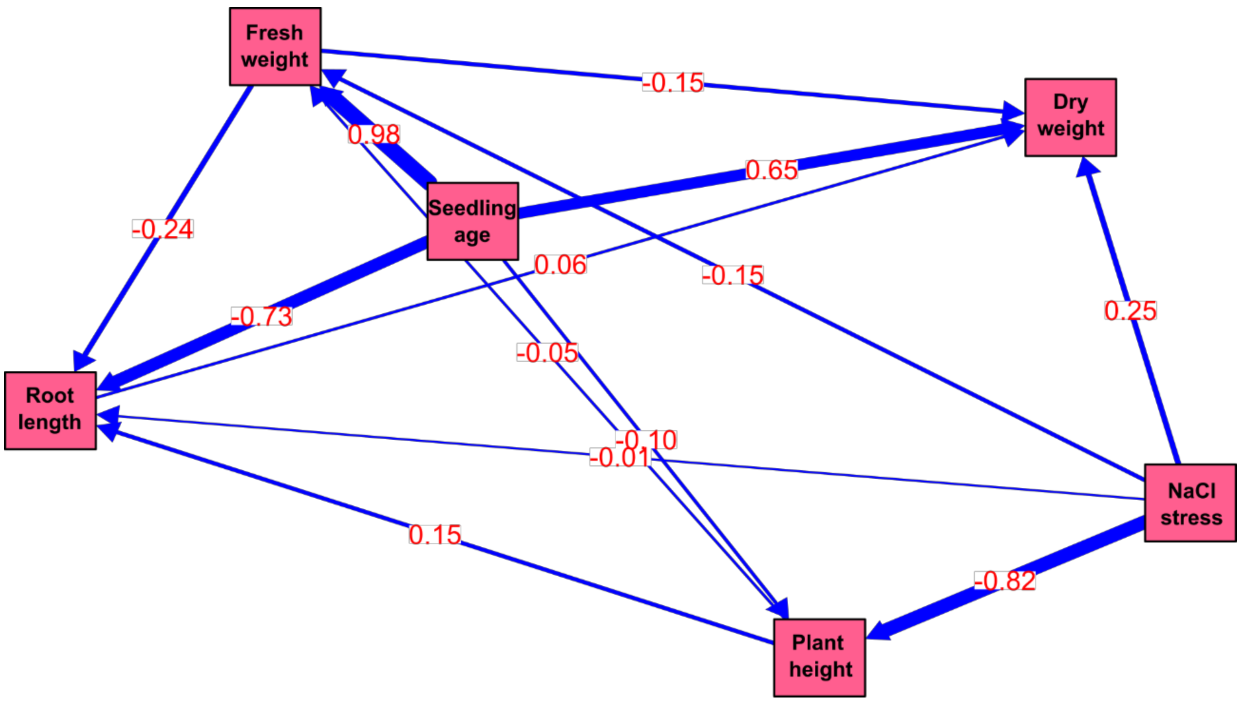

Figure 5 Causal relationships of NaCl stress and seedling age on variety responses. The arrows represent the direction and type of correlation (positive or negative), with their thickness indicating the strength of the relationship.

At the Teluk Awur field site in Jepara, soil salinity is generally moderate, ranging from 5 to 10 dS m^{-1} (2.68 - 5.63 ppt). However, the salinity of the irrigation river, which can reach 72 dS m^{-1} (49.51 ppt), poses an additional challenge. High salinity in the irrigation water can elevate field salinity even further, with water salinity in the field sometimes reaching between 90 and 100 dS m^{-1} (64.15 - 72.66 ppt) (unpublished data). At these extreme salinity levels, neither variety performed well, highlighting the need for further genetic improvements to enhance salt tolerance for practical agricultural use in high-salinity environments. Similar conclusions were drawn by previous researchers, who emphasized the necessity of developing rice varieties with enhanced genetic tolerance to salt stress for field application in saline-prone areas [31-33]. Developing such varieties may be achievable through genetic improvement methods, such as mutagenesis using sodium azide

[34,35]. Studies on sodium azide mutagenesis have shown that induced mutations can modify specific genomic regions in rice without compromising broad agronomic traits [36]. This makes it a potential strategy for further development. Continued research and development of these varieties could ultimately provide valuable resources for increasing rice productivity in saline-affected regions.

Conclusions

The Biosalin-1-Agritan and Biosalin-2-Agritan rice varieties exhibit limited tolerance to high salinity stress, maintaining relatively stable growth up to 10 ppt of NaCl. However, salinity levels beyond this threshold, particularly at 20 ppt and higher, significantly reduce growth parameters such as plant height, fresh weight, root length and dry weight. While Biosalin-2-Agritan shows slightly better resilience in some growth traits compared to Biosalin-1-Agritan, it is unlikely that either variety would perform well under the challenging conditions at the Teluk Awur field site in Jepara. These extreme salinity conditions are anticipated to severely limit the growth of both varieties. Therefore, further genetic improvements are necessary to enhance the salt tolerance of these rice varieties for practical field applications in highly saline environments.

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