Effect of seed priming with salicylic acid on germination and early seedling growth of 'Sembada Hitam' black rice subjected to saline conditions

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ABSTRACT

Background and Objective: Black rice, 'Sembada Hitam', has become popular as a functional food because it contains anthocyanin and other minerals. Salicylic acid (SA) can sustain plants and help them grow better under environmental stresses, including salinity. This research was conducted to evaluate the effect of SA applied as seed priming on the germination and early seedling growth of rice under salinity.

Methodology: The design of this experiment was 4 × 4 factorial in a completely randomized design. The first factor was levels of the NaCl, namely 0 (control), 50, 100, and 150 mM. The second factor was different levels of SA, namely 0 (control), 0.5, 1, and 2 mM. The rice seeds were soaked in SA for 24 hours, and then placed in a medium containing NaCl. Twenty rice seeds were germinated with three replicates. Seed germination was monitored daily for a week, germination rate index (GRI), number of roots, root length, shoot length, fresh weight, dry weight, and seedling vigor index (SVI) were determined on the 15th day.

Main Results: The results revealed that salinity inhibited seed germination and seedling growth. The elevated concentrations of NaCl caused the lower germination percentage (GP) and the early growth of seedlings. The adverse effects of salinity were ameliorated in those seeds primed with SA. Seed primed with 0.5 mM SA showed the highest GP under 100 mM NaCl of 80 ± 2.53% ($P < 0.001$). While 1 mM of SA under 50 mM NaCl possesses the highest shoot length (13.63 ± 0.12 cm; $P < 0.001$) and fresh weight (86 ± 0.001 mg; $P < 0.001$). Primed with 2 mM SA under 150 mM NaCl had a lower GP of 28.33 ± 7.64% than those primed with SA of 0.5 mM or 1 mM with GP of 45 ± 5% and 43.33 ± 7.64%, respectively.

Conclusions: Seeds priming with 1 mM SA positively affected germination and early seedling growth of 'Sembada Hitam' black rice subjected to salinity and it is recommended as an appropriate concentration for ameliorating adverse effects of salinity.

Keywords: Germination percentage, salicylic acid, salt stress, seed priming, seedling growth, Sembada Hitam

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INTRODUCTION

Rice (*Oryza sativa* L.) has become the main diet for over half of the world's population. People in Asia normally consume rice as a primary carbohydrate source (Fukagawa and Ziska, 2019). Rice has been cultivated in many countries, and its genetic diversity increased through either conventional or modern breeding programs (Kumar *et al*., 2020). Black rice has become popular nowadays as a functional food because it contains high anthocyanin and minerals that have beneficial effects on human health (Kumar *et al*., 2020). Black rice 'Sembada Hitam' is one of the pigmented rice cultivars that was developed in Sleman, Special Region of Yogyakarta, Indonesia (Kristamtini *et al*., 2014). Black rice possesses a pericarp, aleurone layer, or endosperm that are red-purple in color, indicating anthocyanins content. It also contains dietary fiber that is good for lowering blood sugar content (Hernawan and Meylani, 2016; Kumar *et al*., 2020). Anthocyanins found in black rice have various bio-activities such as anti-allergic, anti-aging, anti-obesity, anti-cancerous, anti-diabetic, and others (Kumar *et al*., 2020).

Black rice production, however, faces many challenges due to environmental stresses such as salinity, drought, heat, cold, and their many combinations (Devireddy *et al*., 2021). Salt stress severely reduced crop yield. It has been reported that worldwide, salt stress caused a $27 billion loss yearly in the agricultural sector (Jayakannan *et al*., 2015). In many countries, including Indonesia, agricultural lands tend to become more saline, and it was caused by inappropriate drainage practices or inadequate irrigation in agriculture (Jayakannan *et al*., 2015; Shaikh-Abol-Hasani and Roshandel, 2019). An increase in soil salinity reduces nutrient and water availability for crop plants, decreasing soil fertility and affecting the ecological structure of communities (Wang *et al*., 2001). Salinity stress affects the growth and development of plants, including their physiology and metabolism, as well as disrupting ionic balance and osmotic conditions in plant tissue, and it may lead to plant senescence (Singh and Sengar, 2014). Salt stress also greatly reduced the relative water content in black gram and mungbean (Hasan *et al*., 2019). The excessive Na^+ content in the water and soil is toxic to the plant, affecting the balance of Na^+ and K^+, and suppressing the supply of one or more nutrients (Almeida *et al*., 2017). Previous research has shown that salt stress inhibited seed germination and seedling growth of sesame (Ahmad *et al*., 2019), inhibited early seedling growth of cotton (Mottaleb *et al*., 2017), maize (Gautam and Singh, 2009), and Moldavian balm (Shaikh-Abol-Hasani and Roshandel, 2019). Among the cereal crops, rice is the most sensitive and has low tolerance to salt stress (Almeida *et al*., 2017). In an effort to overcome the negative impacts of salinity on agricultural land, many methods are being developed. One of the potential protectants to alleviate the negative effects of salinity stress in plants is by application of salicylic acid (Farzana *et al*., 2020).

Salicylic acid (SA) is categorized as a phenolic compound, and it has an impact on several physiological aspects in plants (Seyfferth and Tsuda, 2014). This substance is involved in several functions, such as germination of the seed, plant growth, thermo-genesis, flowering induction, ions uptake, ethylene biosynthesis, stomatal movement, and leaf abscision (Hayat *et al*., 2013). Plant development and response to abiotic and biotic stress can be regulated by SA (Hayat *et al*., 2013; Yusuf *et al*., 2013). SA ameliorates the stress condition and maintains the plant's ability to grow well, causing drought tolerance of the wheat seedlings (Movaghatian and Khorsandi, 2013). A useful method for increasing the proportion and rate of germination, seedling growth, and seedling uniformity is through seed priming, it can promote normal and improved germination percentage as well as seedling emergence in various crops, such as wheat, sesame, tomato, lemongrass, and Moldavian balm (Ghoohestani *et al*., 2012; Idrees *et al*., 2012; Ahmad *et al.*, 2019; Shaikh-Abol-Hasani and Roshandel, 2019; Abdi *et al*., 2022). Seed germination and seedling establishment are the most important phases of the plant life cycle because it has a significant impact on the final density of plants (Shatpathy *et al*., 2018). The emergence of the coleoptile's white tip and its subsequent expansion are indicators of germination. The moment the tip of a seedling appears above the water's or soil's surface is known as seedling emergence, and this encompasses both germination and post-germination growth (Yoshida, 1981). Seed germination is part of the growth cycle, which is important for plant species (Movaghatian and Khorsandi, 2013). Germination can be influenced by internal and external factors. The internal factors affect seed and seedling characteristics, while external factors include abiotic factors (seedbed physical and chemical components and their interaction with climate) and biotic factors (seedbed biological components) (Lamichhane *et al*., 2018). Salinity is one of the abiotic stressors that affect seed germination. The amount of various salts, including NaCl, magnesium sulfates, calcium sulfates, and bicarbonates, in the soil and water is known as salinity (Hoang *et al*., 2016).

The evaluation of SA as a protectant under salt stress for Indonesia's local rice cultivar 'Sembada Hitam' remains unclear. There is no data regarding the germination and seedling growth of black rice cultivar 'Sembada Hitam' subjected to saline conditions and treated with SA. The purpose of this research was to assess the effects of seed priming using SA on the germination percentage, germination rate index (GRI), the number of roots, root length, shoot length, fresh and dry weight, and seedling vigor index (SVI) of the black rice 'Sembada Hitam' subjected to saline conditions.

MATERIALS AND METHODS

Experimental Design

This research was conducted at the Laboratory of the Plant Physiology, Faculty of Biology, Universitas Gadjah Mada from August to September 2022. The seeds of black rice 'Sembada Hitam' were used in this research. The experiment design used in this research was 4 × 4 factorial in a completely randomized design (CRD) with three replicates. The treatments consisted of salinity stress using sodium chloride (NaCl) solution at four levels of concentration (0, 50, 100, or 150 mM) as the first factor. This range of salinity stress treatment was based on research conducted by Idrees *et al*. (2012) and Shaikh-Abol-Hasani and Roshandel (2019). Seed priming with SA was conducted using four levels of concentration (0, 0.5, 1, or 2 mM) as the second factor (Youssef *et al*., 2017).

Treatments with Sodium Chloride (NaCl) and Salicylic Acid

Prior to the treatment, the black rice 'Sembada Hitam' seeds were sterilized using 5% hypochlorite solution for 5 minutes and then rinsed with ddH_2O (Tania *et al*., 2021) for 10 minutes and then put in the tissue paper to decrease the water content. The seeds were subsequently soaked in SA of 0 (as control using ddH_2O), 0.5, 1, or 2 mM, where the SA solution was prepared in a glass beaker of 100 mL. The priming was carried out for 24 hours at room temperature (25 °C), then air-dried by putting the seeds in the tissue paper at room temperature (25 °C) (Moghaddam *et al*., 2020). The levels of salinity were measured using an electrical conductivity (EC) meter. The NaCl was dissolved in ddH_2O, and the salinity levels were measured by putting the EC meter equipment in the solution until the value of electrical conductivity remained constant. The value of electrical conductivity showed at the equipment could be converted into the salinity levels as reported by Khare *et al*. (2015). For instance, 9.6 ± 0.5 dS/m corresponds to a concentration of 100 mM, representing medium salinity; ±5 dS/m corresponds to 50 mM, indicating low salinity; and ±15 dS/m corresponds to 150 mM representing high salinity. Then, the plastic cups were prepared by placing two grams of cotton sheets as growth media, which were then watered with 20 mL of NaCl at concentrations of 0 (as control using ddH_2O), 50, 100, or 150 mM. Twenty primed seeds were germinated in each plastic cup and for each treatment combination with three replicates. The seeds' germination and salinity levels of the growing media were monitored daily, and NaCl was applied as the requirement.

Seed Germination and Early Seedling Growth Measurement

The seed germination was counted daily for a week. The characteristics of a normal seedling start from seed germination, as the radicle starts to emerge with a length of 2 mm or greater (Moghaddam *et al*., 2020). The seed coat ruptured, plumule, and radicle came out are the characteristics of seed germination. Germination was expressed in percentage. After 15 days, three seedlings were selected per cup to measure the number of roots, root length, shoot length, fresh weight (FW), and dry weight (DW). The root length was measured from the stem base until the tip of the root, and the shoot length was measured from the stem base until the longest leaf of the seedling. The measurement was carried out using a ruler. The fresh and dry weights were measured using an analytical scale. The dry weight was determined after seedlings were dried in an oven at 72°C for 48 hours.

The germination percentage (GP), germination rate index (GRI), and seedling vigor index (SVI) were calculated using the following equations:

$$GP = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds placed in germination}} \times 100$$ (Tania *et al*., 2021)

$$GRI = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \cdots + \frac{\text{Number of germinated seeds}}{\text{Day of final count}}$$ (Tania *et al*., 2021)

Seedling vigor index (SVI) = (Root length + Shoot length) × GP (Shatpathy *et al*., 2018)

Statistical Analysis

Data were collected, tabulated, and statistically analyzed. Data were analyzed with the first factor being NaCl concentrations (0, 50, 100, or 150 mM) as salinity stress, and the second factor was SA concentrations (0, 0.5, 1, or 2 mM), and the interaction between two factors was also analyzed with two way-analysis of variance (ANOVA). The mean value between treatments was compared using Duncan's multiple range test (DMRT) at a 0.05 probability level to determine the difference among the mean of treatments. SPSS statistics software was used to perform the statistical analysis.

RESULTS AND DISCUSSION

Germination Percentage

Figure 1 shows the effect of seed priming with SA followed by salt stress on the germination percentage of black rice 'Sembada Hitam'. In the control treatment, seed germination reached 100% regardless of the different concentrations of SA applied as a priming agent. Salinity caused a decline in the germination percentage of those seed priming with ddH_2O. Higher concentrations of NaCl applied caused a higher reduction in seed GP of black rice 'Sembada Hitam'. Salt stress with high concentrations of NaCl (100 and 150 mM of NaCl) caused a significant difference in GP compared to the control and the seedlings treated with low salt stress (NaCl 50 mM). Under 50 mM of NaCl treatment, seeds primed with 0.5 or 1 mM of SA reached 93.33% and 95% GP, respectively, whereas the GP of those seeds without SA priming only reached about 85%. In higher concentration of salt stress (150 mM of NaCl), the seeds treated with SA of 0.5 or 1 mM also showed a significant increase in GP (45% and 43.33%, respectively) compared to those seeds without SA priming (31.67% GP) or seed primed with 2 mM SA (28.33% GP) with $P < 0.001$. Priming with SA of 0.5 and 1 mM showed significant differences in the height of seedings subjected to 50, 100, and 150 mM of NaCl, compared to the control. It has been widely reported that salt stress will cause an increase in abscisic acid (ABA) level (Torun *et al*., 2022), whereas ABA can also inhibit the biosynthesis of gibberellin as well as biosynthesis of amylase, both are required for seed storage breakdown during germination process (Hu *et al*., 2024).

The result of this experiment showed that salinity inhibited the germination of black rice 'Sembada Hitam'. The percentage of black rice that germinated and the germination rate index were both considerably decreased by high salt stress levels (100 and 150 mM). This result is similar to a study reported by Vibhuti *et al*. (2015), who found that in rice cultivar 'Narendra 1', salt stress (20 dS/m) reduced seed germination to 65%. According to Shaikh-Abol-Hasani and Roshandel (2019), *D. moldavica* germination percentage was dramatically reduced in response to salt stress of 100 or 150 mM NaCl when compared to the control. Several rice cultivars, including 'Kuthiru', 'Kuttusan', 'Orkazhama', 'Chovvarian', 'Orthadian', 'Ezhome-1', and 'Ezhome-2', have lower chlorophyll contents when subjected to salinity stress (Khan *et al*., 2019).

A useful method for encouraging seed germination is seed priming, which produces healthy, robust seedlings as well as faster, better germination and emergence in a variety of crops (Shaikh-Abol-Hasani and Roshandel, 2019). It has been suggested that SA can ameliorate the negative effect of salt stress during seed germination (Torun *et al*., 2022). In this experiment, SA applied as a seed priming agent of 0.5 or 1 mM dramatically elevated the black rice's germination percentage as compared to the control (non-saline) condition. This experiment revealed that priming with SA 0.5 or 1 mM significantly increased the GP of black rice 'Sembada Hitam' compared to control (without SA treatments). While, under 150 mM of NaCl, priming with SA 2 mM did not impact significantly to the GP.

Germination Rate Index

Figure 2 shows the impact of SA treatments and salt stress on the seed germination rate index of black rice 'Sembada Hitam'. Saline conditions tend to decrease the GRI where the GRI was 1.35 ± 0.2 compared to the control condition with a GRI of 8.78 ± 0.3 ($P < 0.001$). Priming with SA 1 and 2 mM tends to increase the germination rate of black rice under control by 9.50 ± 0.2 and 9.39 ± 0.1, respectively. Under salt stress conditions, SA priming tends to increase the GRI except at 150 mM salinity, priming black rice seeds with 2 mM SA reduced the GRI.

The seed priming strategy lessen the detrimental impacts of salt stress. According to Souri and Tohidloo (2019), SA treatment in tomato seeds might mitigate the bad effects of salinity stress by lowering the concentration of Na^+ by increasing the proline and soluble sugar concentrations when the plants are exposed to 100 mM NaCl. Salicylic acid, known as stress hormonal, that involve in plant defense mechanisms under abiotic stress such as salinity. There are complex mechanisms for plant tolerance under salt stress. Seed priming with SA suppressed reactive oxygen species (ROS) accumulation and prevented lipid peroxidation, also involved in an antioxidant defense mechanism by increased proline, sugar, and abscisic acid production (Ellouzi *et al*., 2023). Salicylic acid treatment was also found to reduce the detrimental effects of salinity stress on the development of two wheat cultivars: 'PAN3497' and 'SST806' (Abdi *et al*., 2022), mainly through glycine betaine (GB) accumulation. Additionally, SA is also reported to lessen the effects of drought on rice and sorghum by maintaining the plant's water status (Jangra *et al*., 2019; Liu *et al*., 2022). In ginger plantlets exposed to salt stress, SA enhanced the ion balance and strengthened the antioxidant system (Hundare *et al*., 2022). According to Alamri *et al*. (2018), SA priming enhanced wheat seed germination under salinity-stressed conditions, primarily through the ability of SA to reduce the ROS formation such as malondialdehyde content and H_2O_2 and increase the proline and total soluble carbohydrates content (Chutipaijit *et al*., 2011). The germination rate in sesame was greatly affected by varying doses of SA employed as seed priming (Ahmad *et al*., 2019). The germination rate of the rice cultivar 'Nipponbare' was raised to 26.32%,14.74%, and 13.68% by applying 0.1, 0.5, and 1 mM of SA because it regulates Na^+/K^+ balance and maintains the balance between GAs and ABA homeostasis (Liu *et al*., 2022).

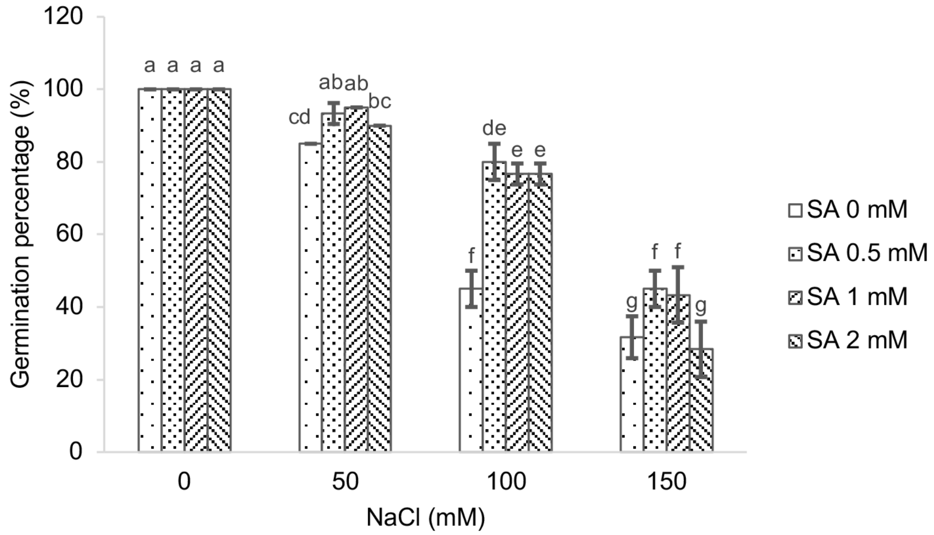

Figure 1 Germination percentage (%) of black rice 'Sembada Hitam' seeds after salicylic acid (SA) treatments (0, 0.5, 1, or 2 mM) under saline conditions (0, 50, 100, or 150 mM of NaCl). Different letters on the top of the bar show significant differences at P < 0.05.

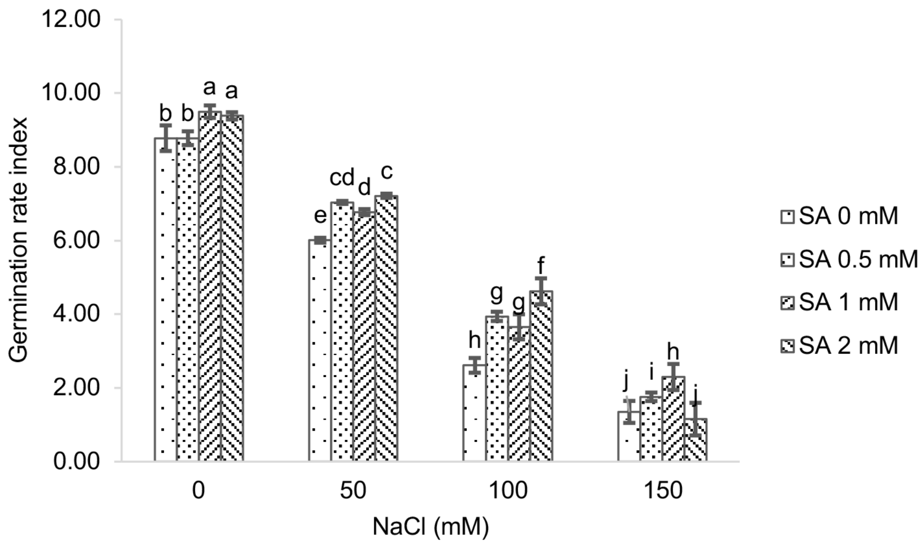

Figure 2 Germination rate index of black rice 'Sembada Hitam' seeds after salicylic acid (SA) treatments (0, 0.5, 1, or 2 mM) under saline conditions (0, 50, 100, or 150 mM of NaCl). Different letters on the top of the bar show significant differences at P < 0.05.

Number of Roots

Figure 3 shows that under control conditions, seedlings in which seeds were previously primed with SA of 0.5 or 1 mM possessed a higher root number (8 roots) compared to those seeds without SA priming (7 roots) with $P < 0.001$. The number of roots significantly decreased in seedlings grown under various NaCl concentrations. The higher NaCl concentrations applied, the less root number was formed. Under 50 mM of NaCl, priming with SA 0.5 or 1 mM caused an increased root number compared to those without SA priming or SA of 2 mM. Under 100 mM of NaCl, priming with SA tends to increase the number of roots. A similar finding was found in seedlings grown under 150 mM of NaCl, in which priming seeds with SA 1 mM increased root number by 5 ($P < 0.001$).

This study found that black rice ‘Sembada Hitam’ grown under salt stress had considerably fewer roots overall, as well as shorter roots and shorter shoots compared to non-saline conditions. The findings of this study are similar to other reports in which, under salinity stress, tomato plants (Ghoohestani *et al*., 2012) and *Oryza sativa* L. (Shatpathy *et al*., 2018) having seed primed with SA exhibited longer root lengths than non-SA treatment. Under water stress conditions, SA induces antioxidant responses from dehydration damage (Shatpathy *et al*., 2018). The length of *D. moldavica* seedlings was found shortened by 16% and 64%, respectively, under salt stress at 100 and 150 mM of NaCl (Shaikh-Abol-Hasani and Roshandel, 2019), this is due to a salinity increase in the lipid peroxidation and ion leakage. This study found that salt stress decreased the weight of black rice ’Sembada Hitam’, both fresh and dry weights. Priming with SA may lessen the negative consequences of salt stress in reducing both fresh and dry weight. The better growth obtained in those seedlings treated with SA amid salinity conditions is probably due to reducing the detrimental effects of oxidative stress by decreasing lipid peroxidation production and ion leakage (Shaikh-Abol-Hasani and Roshandel, 2019). When compared to non-SA priming, seed priming with SA improved the fresh weight of seedlings in *Lathyrus sativus* (Moghaddam *et al*., 2020). SA increased the vigorous and larger seedlings by enhancing the chlorophyll, dissolved sugars, and protein content of wheat plants, also SA involved in the growth regulator, namely cell division and cell elongation through the aid of compounds such as auxin (Hamid *et al*., 2010).

Root Length

The effects of salinity and priming seeds with SA on the root length of black rice ‘Sembada Hitam’ is displayed in Figure 4. The root length of black rice ‘Sembada Hitam’ varied significantly depending on the NaCl and SA concentrations. In control condition, SA of 0.5 or 1 mM increased root length, however, priming seeds with 2 mM SA did not cause any significant difference in root length. Under 50 mM saline conditions, the average root length of black rice seedlings was greater, even in those seedlings without SA priming. Priming with 0.5 mM SA increased the average root length, but higher SA priming reduced the root length. Under higher salinity conditions (100 or 150 mM NaCl), only priming with 0.5 mM SA increased root length (7.67 and 5.77 cm, respectively), while higher SA concentration applied as seed priming tended to reduce the average root length ($P < 0.001$).

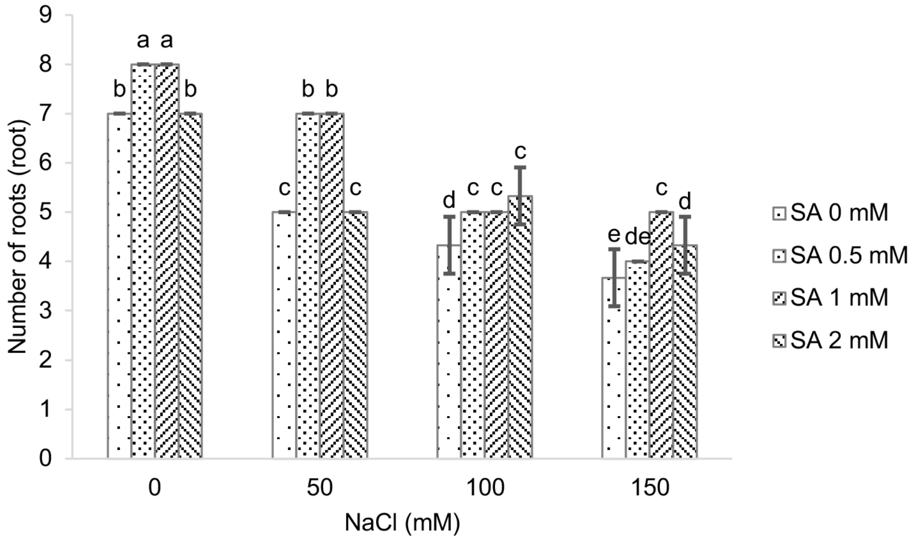

Figure 3 Number of roots of black rice 'Sembada Hitam' seeds after salicylic acid (SA) treatments (0, 0.5, 1, or 2 mM) under saline conditions (0, 50, 100, or 150 mM of NaCl). Different letters on the top of the bar show significant differences at P < 0.05.

Shoot Length

The effects of NaCl and SA treatment as seed priming on the shoot length of black rice 'Sembada Hitam' seedlings are displayed in Figure 5. In the control condition, SA priming significantly reduced the average shoot length of black rice 'Sembada Hitam'. On the contrary, under saline conditions, either 50, 100, or 150 mM, SA priming tends to increase in the average shoot length. The greater concentration of SA applied as a seed priming agent, either 0.5 or 1 mM, the higher average shoot length, but SA of 2 mM caused the average shoot length to decrease and become similar to those treated with SA of 0.5 mM. Priming with 1 mM SA gave the highest shoot length in NaCl stress of 50, 100, and 150 mM as 13.63 ± 0.12, 11.17 ± 0.29, and 10.27 ± 0.46 cm, respectively (P < 0.001).

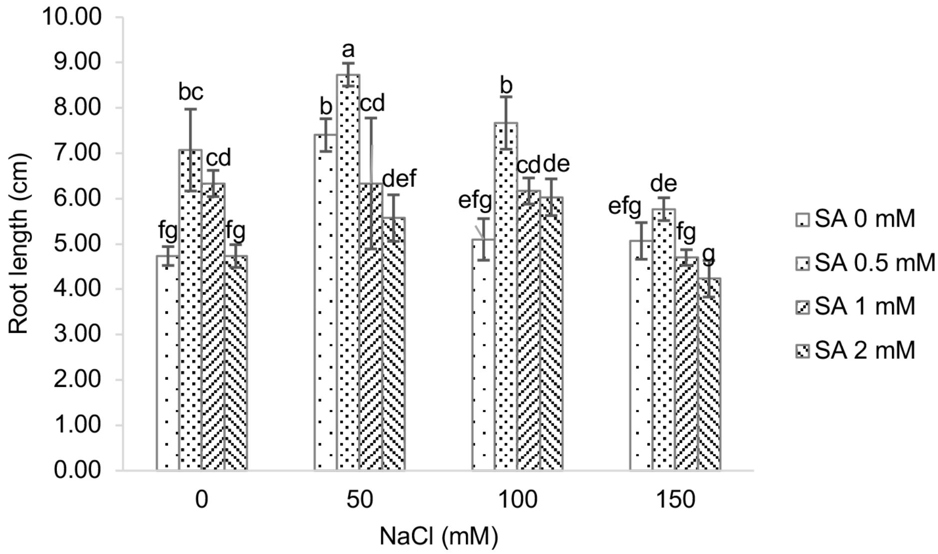

Figure 4 Root length (cm) of black rice 'Sembada Hitam' seeds after salicylic acid (SA) treatments (0, 0.5, 1, or 2 mM) under saline conditions (0, 50, 100, or 150 mM of NaCl). Different letters on the top of the bar show significant differences at P < 0.05.

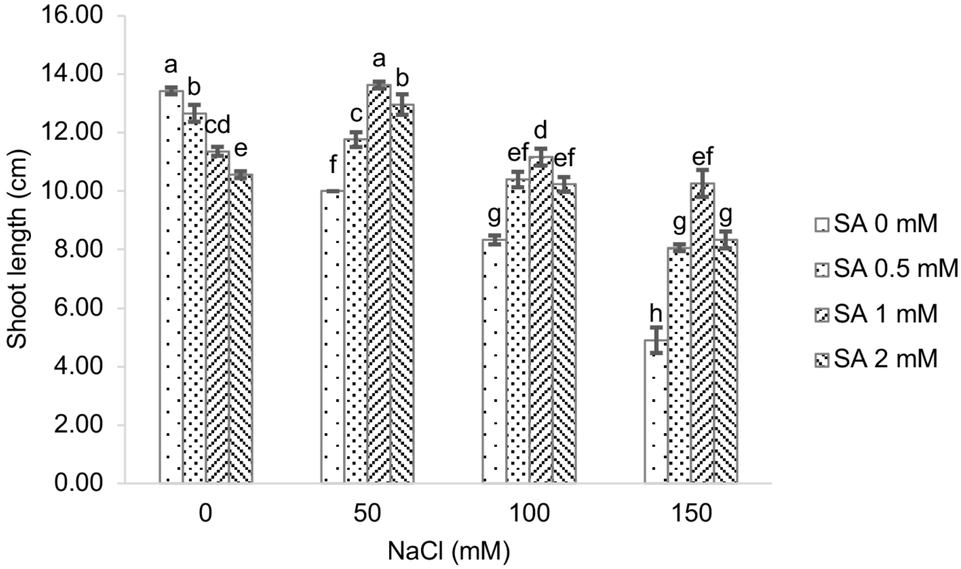

Figure 5 Shoot length (cm) of black rice 'Sembada Hitam' seeds after salicylic acid (SA) treatments (0, 0.5, 1, or 2 mM) under saline conditions (0, 50, 100, or 150 mM of NaCl). Different letters on the top of the bar show significant differences at $P < 0.05$.

Fresh and Dry Weight

The effects of salinity and seed priming with SA on the fresh weight and dry weight (mg) of black rice 'Sembada Hitam' seedlings are displayed in Figure 6. The FW of black rice 'Sembada Hitam' seedlings tends to decrease along with the increase in NaCl treatment if the plants are not treated with SA. Seedling FW under 0, 50, 100, and 150 mM NaCl without SA treatment were 73, 62, 54, and 59 mg, respectively. Priming with 1 mM SA increased the FW of seedling (86 mg) under 50 mM NaCl treatment compared to control (62 mg). While, priming with 0.5 or 1 mM SA resulted in higher FW under 50, 100, and 150 mM NaCl compared to plant did not treat with SA.

Priming with SA under 0 and 50 mM NaCl did not give a significant difference to the DW of the seedlings. Under 100 mM NaCl, priming with 0.5 mM SA gave the highest DW of seedlings (22 mg), while under 150 mM NaCl, 1 mM SA gave the highest DW of black rice (22 mg). Under control conditions, only priming with 1 mM SA increased the seedling's DW. In saline conditions, either 50, 100, or 150 mM NaCl, priming with SA showed no significant difference in seedling's DW among different concentrations of SA applied as a seed priming agent, except in 150 mM NaCl treatment, SA of 1 mM slightly increased seedling's dry weight (22 mg) compared to non-SA-treated plant (19 mg; $P < 0.001$).

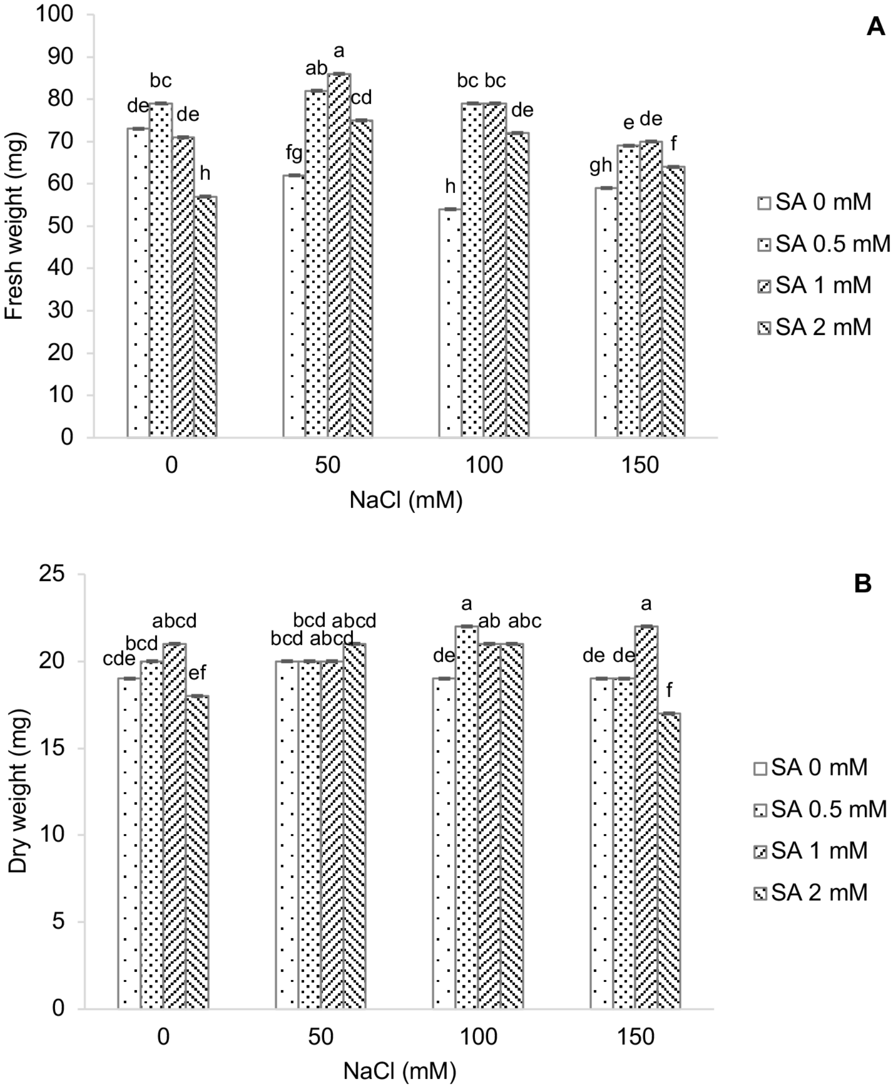

Figure 6 Fresh (A) and dry (B) weight (mg) of black rice ‘Sembada Hitam’ seeds after salicylic acid (SA) treatments (0, 0.5, 1, or 2 mM) under saline conditions (0, 50, 100, or 150 mM of NaCl). Different letters on the top of the bar show significant differences at $P < 0.05$.

Seedling Vigor Index

The seedling vigor index of black rice ‘Sembada Hitam’ grown under different salinity and SA treatments is shown in Figure 7. In the control (non-saline) condition, SVI of black rice ‘Sembada Hitam’ was relatively similar despite different SA concentrations previously applied as priming agents. In salt stress conditions, those seedlings without SA priming tend to have a smaller vigor index. The reduction in vigor index becomes greater concomitantly as salinity increases. However, in seedlings subjected to NaCl of 50, 100, and 150 mM and having 1 mM SA as priming agent, the vigor index increased significantly (19.89 ± 2.44, 13.16 ± 0.43, and 7.33 ± 0.28, respectively) compared to seedlings without priming with SA (13.83 ± 0.69, 6.66 ± 0.95, and 2.78 ± 0.28, respectively; $P < 0.001$). There was an interaction between salt stress using

NaCl and SA treatments for all traits (germination percentage, germination rate index, number of roots, root length, shoot length, fresh weight, dry weight, and seedling vigor index) as shown in Table 1. The growth performance of seedlings is illustrated in following Figure 8.

Table 1 Analysis of variance (two-way ANOVA) of germination and early seedling growth of black rice 'Sembada Hitam' under salt stress (0, 50, 100, and 150 mM of NaCl) treated with salicylic acid (SA) 0, 0.5, 1, and 2 mM

Sources of variance	df	Germination percentage	Germination rate index	Number of roots	Root length	Shoot length	Fresh weight	Dry weight	Seedling vigor index
Salinity	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SA	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Salinity × SA	9	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The research revealed that salt stress reduced the seedling vigor index of black rice 'Sembada Hitam'. NaCl of 50, 100, or 150 mM reduced the SVI to 13.83, 6.66, and 2.78, respectively, compared to the non-saline condition, which had an SVI of around 16.93. SA significantly increased the SVI of back rice 'Sembada Hitam' in saline conditions. Increasing salt concentrations declined SVI in rice, from 11.5 to 1.1 (Vibhuti *et al*., 2015). Previous research by Moghaddam *et al*. (2020) revealed that primed seeds with 0.2 mM SA increased 23.4% of SVI of *Lathyrus sativus*. In wheat (*Triticum aestivum*), salt stress with 150 mM NaCl dramatically decreased the root and shoot length, germination percentage, germination index, and seed vigor index, the mechanism of plant defense under salinity stress such as regulating stomata (transpiration) (Tania *et al*., 2021).

Abiotic stressors, such as salt stress, cause signal transduction, which modifies many physiological, metabolic, and molecular responses and controls plant growth and development. Receptors, including histidine kinases, receptor-like kinases, G-protein-coupled receptors, receptors to ROS, and other metabolites and signaling molecules, are typically the ones that perceive stress, salt stress-induced signal transduction (Devireddy *et al*., 2021). The buildup of Na^+ ions in the tissue is exacerbated by salt stress. By resulting in ion imbalances and the deactivation of numerous functioning proteins, Na^+ buildup restricts survival and growth (Devireddy *et al*., 2021). According to Roy *et al*. (2014), the effects of salt stress also included osmotic stress, nutritional imbalances, oxidative damage, a reduced ability to scavenge ROS, and a drop in photosynthetic activity. Plants respond to salt stress using two different sensory mechanisms: the histidine kinase receptor protein HK1 and the alteration of Ca^{2+} levels in the cytosol caused by salt stress. Proteins like the calcineurin B-like interacting protein kinase and calcineurin B-like proteins are activated by it. In order to initiate gene transcription, it activates the hyperosmotic signal. Plant response to salt stress, signaling pathways, including the salt-overly-sensitive pathway, hormone and ROS, and MAPK signaling were triggered, which led to the activation of stress-related genes (Zhu, 2016). Another mechanism for coping with salt stress is the reduction of salt entry into the plant tissue and cytoplasm, as well as the level of salt stress through molecular and biochemical changes (Gautam and Singh, 2009). Although SA can counteract the negative effects of salt stress, its ameliorative benefits under salinity stress were lessened when its concentration increased (Idrees *et al*., 2012; Shaikh-Abol-Hasani and Roshandel, 2019). At the molecular, cellular, biochemical, and physiological levels, SA plays roles during salinity stress; in a rice cultivar, NaCl 100 mM drastically lowered photosynthetic and content of total protein, while the mechanism of SA for salt tolerance including lowered activity of antioxidant enzymes, lowered ROS accumulation, and improved protein and photosynthetic (Khan *et al*., 2019).

Black rice 'Sembada Hitam' seed germination and seedling growth were strongly impacted by salinity with a NaCl concentration of 100 mM. However, the negative effects of salinity stress can be mitigated by seed priming at a concentration of 1 mM SA. Under 50, 100, and 150 mM salinity stress, black rice 'Sembada Hitam' seedling development characteristics are increased by SA with a concentration of 1 mM. Otherwise, 2 mM of SA generally reduced the germination and early seedling growth of rice. It is economically beneficial to use the SA in low concentrations.

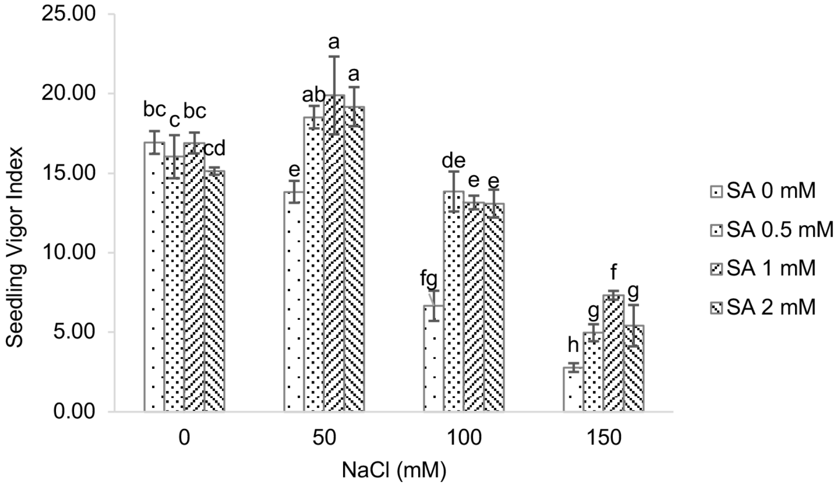

Figure 7 Seedling vigor index (SVI) of black rice 'Sembada Hitam' seeds after salicylic acid (SA) treatments (0, 0.5, 1, or 2 mM) under saline conditions (0, 50, 100, or 150 mM of NaCl). Different letters on the top of the bar show significant differences at $P < 0.05$.

Figure 8 Morphological of black rice 'Sembada Hitam's seedlings treated with 0 (A), 0.5 (B), 1 (C), and 2 mM (D) salicylic acid under salt stress (0, 50, 100, and 150 mM NaCl). The white bar shows 5 cm in length.

CONCLUSIONS

The experiment's findings showed that salinity stress impeded the germination and early seedling growth of black rice 'Sembada Hitam'. Priming with 1 mM of SA enhanced germination and early seedling growth of black rice 'Sembada Hitam'under salinity-stressed conditions. It can be inferred that in black rice 'Sembada Hitam', SA at low concentrations produced more beneficial effects than salicylic acid at high concentrations in ameliorating salt stress. This research can be used as a basic application in the field of salicylic acid as amelioration in saline conditions, especially in marginal areas with high salinity levels. More research is needed to explore the mechanism of salicylic acid as an ameliorant for salt stress in another rice phase, including vegetative, generative, and ripening, and examine the other parameters such as biochemical analysis (enzymatic antioxidant and pigment content).

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