PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(2): 1-11
https://doi.org/10.14719/pst.3548

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Effects of salicylic acid on growth, photosynthesis pigment, proline and endogenous hormones content of black rice grown under salt stress

Hana Widiawati[1], Sukirno Sukirno[1], Aziz Purwantoro[2], Sri Koerniati[3] & Kumala Dewi[1*]

[1]Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

[2]Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

[3]Research Centre of Genetic Engineering, National Research and Innovation Agency Republic of Indonesia, Bogor 16911, Indonesia

Email: kumala.dewi@ugm.ac.id

OPEN ACCESS

ARTICLE HISTORY
Received: 03 April 2024
Accepted: 15 October 2024
Available online
Version 1.0 : 10 April 2025
Version 2.0 : 15 April 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Hana W, Sukirno S, Aziz P, Sri K, Kumala D. Effects of salicylic acid on growth, photosynthesis pigment, proline and endogenous hormones content of black rice grown under salt stress. Plant Science Today. 2025; 12(2): 1-11. https://doi.org/10.14719/pst.3548

Abstract

Salinity is one of the abiotic stresses that inhibits plant growth and development. One of the mechanisms by which plants tolerance to salinity is through synthesizing salicylic acid (SA). This research was aimed to evaluate the impact of SA on growth, photosynthesis pigments, proline accumulation and endogenous hormone levels of black rice 'Sembada Hitam' subjected to saline conditions. Black rice seeds were germinated in a plastic tray containing growth media and seedlings of 3 weeks old were transplanted into a plastic chamber with similar growth media. Sodium chloride of 0 mM (control), 50 mM, 100 mM or 150 mM were applied 1 month after planting, whereas plants were sprayed with different concentration of SA, namely 0 mM (control), 0.5 mM, 1 mM or 2 mM at 25, 50, 75 and 90 days after planting. Five replicates were prepared for each treatment combination. Several growth parameters such as plant height, root length, number of tillers and flag leaf area and physiological parameters such as chlorophyll, carotenoids, proline and endogenous hormones content were determined. The results indicated that salinity inhibited the growth parameters of 'Sembada Hitam' rice. Elevated sodium chloride levels resulted in reductions in plant height, root length, number of tillers and flag leaf area. Application of SA mitigated the adverse effects of salinity by enhancing plant height, root length, number of tillers and flag leaf area. The presence of SA also led to increase levels of Indole-3-Acetic Acid (IAA), Gibberellins (GA_3), Cytokinins (CKs), Jasmonic Acid (JA) and endogenous Salicylic Acid (SA), while reducing Abscisic Acid (ABA) levels in black rice under saline conditions.

Keywords

abiotic stress; biochemical; physiological; salinity; Sembada Hitam

Introduction

Climate change has many effects on agricultural sectors. Greenhouse gases like carbon dioxide (CO_2) trap and absorb the heat that is radiated from the Earth's surface, increasing temperature and influence groundwater for agricultural sector (1). Sea water extrusion influences the agricultural land in the sea coastal region. Increasing the earth's temperature also causes high soil evaporation. Furthermore, it can increase salinity level of the soil by releasing soluble cations (Ca^{2+}, K^+, Na^+ and Mg^{2+}) and soluble anions such as Cl^-, SO_4^{2-} and HCO^{3-} (2). Salinity causes serious threats to the land, contaminating groundwater for agricultural, deteriorating the environment, lowering the

productivity of crops and causing the food security issues (3). The salinity levels are shown in the electrical conductivity (EC) capability, which can be measured by EC meter equipment. The salinity level is normally divided into low (2-4 dS/m), medium (4-8 dS/m) and high salinity (>8 dS/m) (4). Rice normally can only tolerate soil with a maximum EC of 3 dS/m, causing rice as a susceptible crop among cereal plants (5). The amount of salt in the soil causes it to become either saline or sodic soil. Sodic soil has a high exchangeable sodium content but a low total salt content; sodic soils have weak tilth due to the dispersal of soil particles caused by the combination of high sodium levels and low total salts (6). Saline soil contains some water-soluble salts in amounts that can harm seed germination and plant growth (6). Saline soil has electrical conductivity (EC) >4 dS/m and pH <8.5, while sodic soil has EC <4 dS/m and pH >8.5 (alkali) (7).

Plants as sessile organism have endogenous hormones for maintaining growth and development even under stressful conditions, including salinity (8). Salinity impacts plant growth and development by decreasing the potential osmotic, leading to osmotic stress and increasing the production of reactive oxygen species (ROS) (9). Reactive oxygen species (ROS) are generated in cells during salt stress, causing an oxidative damage to various biological constituents, such as proteins, lipids and DNA (10). It has been suggested that high salinity levels can impede the growth and development of plants because salinity induces ionic toxicity and osmotic stress. Ion imbalance is caused by excess Na^+ accumulation (ion toxicity). In contrast, osmotic stress decreased the water potential in the soil environment and inhibited water and nutrient absorption (11). Plants facing salinity stress can experience nutrient deficiency due to a decline in osmotic potential, ion toxicity as the result of changes in the balance of Na^+ and K^+ ions or by limiting the transport of one or more nutrients (12). According to a study, pigmented rice is an excellent option for developing cultivars that can withstand abiotic stress (13), particularly salt stress because it contains high proline and anthocyanin contents that has protective response to salinity.

Pigmented rice contains beneficial compounds, such as anthocyanins that play role as antioxidants, so it is considered as a functional food. Many pigmented rice cultivars are developed in Indonesia; one of the cultivars is black rice 'Sembada Hitam' which was cultivated in Sleman Regency, Special Region of Yogyakarta (14). It has been reported that black rice showed electrophysiological tolerance at 150 mM NaCl (15). In black rice 'Jelitheng' it has been found that salinity treatment increased the levels of Na^+ and K^+ in leaves but decreased stomatal opening width, plant height and 100 grains weight (16). Salinity stress (150 mM of NaCl) decreased yield in four pigmented rice cultivars, however, foliar application of spermidine prior to salt stress treatment resulted in an improvement in yield and yield components such as total phenolic content, anthocyanins, proanthocyanins and antioxidant activities (17). In other study, it has been suggested that SA play a role in reducing the toxic effect of salt stress in rice. During salinity induced stress, the application of SA (0.5 mM or 1.0 mM) significantly increased shoot length, chlorophyll and biomass compared to those plants treated with salinity stress only (18).

Salicylic acid is a phenolic substance synthesized in plants and it serves as a growth regulator, involved in several plant metabolic processes as well as a primary defence mechanism (19, 20). Salicylic acid enhances plant resilience to abiotic stress such as drought, high temperatures, salinity, UV radiation, ozone and heavy metal stress (21). In wheat cv. 'Kundan', it has been found that SA increased osmolyte (proline and total soluble sugars) and lessening water stress's detrimental effects on photosynthesis (22). Salicylic acid (SA) has many functions for plant tolerance to abiotic and biotic stress. Under dual stress (drought and salt stress) in rice cultivars 'Japonica' and 'Indica', the application of SA significantly increased antioxidant enzyme activities, reduced rice H_2O_2 and MDA and maintained rice growth (23).

A variety of plant hormones, including Auxin (IAA), Gibberellin (GA_3), Cytokinins (CKs), Salicylic Acid (SA), Jasmonic Acid (JA) and Abscisic Acid (ABA), are involved in regulating growth, metabolism and plant response to the environmental changes (24). It has been suggested that salinity stress also affected the plant hormones content. Previous research found that in saline condition, barley leaf has a higher content of CKs, ABA and ethylene compared to control, whereas the Jasmonic Acid (JA) decreased (25). However, there is no data on the endogenous hormones content of black rice 'Sembada Hitam' grown under saline conditions and treated with SA. The purpose of this study was to assess the effect of salicylic acid on growth, pigment levels, proline and endogenous hormones content of black rice 'Sembada Hitam' grown under salt stress.

Materials and Methods

Preparation and Treatments

This research was carried out in the greenhouse of Sawitsari Research Station and Laboratory of Plant Physiology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia. Black rice cultivar, 'Sembada Hitam,' was obtained from Rice Research Agency of Indonesia. The planting media used in this study were soil and organic fertilizer/ compost 3:1 (v/v). The characteristics of the soil and organic fertilizer, namely texture (sand, dust and harsh), pH, electric conductivity (EC), organic carbon (C), total nitrogen (N), available potassium (K), available phosphor (P) and cation exchange capacity (CEC) were summarized in Table 1. below. The growth media characteristics were analyzed at Balai Pengkajian Teknologi Pertanian (BPTP) [Agricultural Technology Research Center], Yogyakarta.

'Sembada Hitam' rice seeds were rinsed using tap water and then germinated in a plastic tray containing growth medium. Seedlings were maintained for 20 days and then transplanted into a plastic chamber containing growth media which consists of soil and organic fertilizer. One seedling was planted in each plastic bucket. Factorial randomized design with 5 replicates were used. Salicylic

Table 1. The characteristics of mixed of soil and organic fertilizer (compost) used in the research

Parameter	Unit	Amount
Texture		
Sand	%	69
Dust	%	22
Harsh	%	9
pH	-	7.02
EC	µs/cm	54
Organic-C	%	1.88
Total N	%	0.09
Available K	ppm	200
Available P	ppm	37
CEC	cmol (+)/kg	6.37

acid of 0 mM, 0.5 mM, 1 mM or 2 mM was sprayed on the leaves as much as 10 mL/plant for the first time at 25 days after planting (DAP) and subsequently 15 mL/plant at 50 DAP, 75 DAP and 90 DAP. Salicylic acid application was carried out at 08.00 to 10.00 am. Salt stress (NaCl) was applied for the first time at 30 DAP. Rice plants that have been transplanted were watered with 1 L of water (control) or NaCl solution of 50 mM (5 dS/m) as low saline condition, 100 mM (10 dS/m) as medium saline condition or 150 mM (15 dS/m) as high saline condition. Salinity level was maintained by adding NaCl solution each concentration every week.

Physiological parameters

The photosynthetic pigment including total chlorophyll and carotenoid levels of black rice leaf was determined using spectrophotometer method (26). Samples for chlorophyll and carotenoids analysis were taken from leaf at 60 DAP. Leaf sample of 0.05 g was crushed in a mortar and pestle by adding liquid N_2. The sample was then diluted with 5 mL of 80% acetone. The leaf extract was filtered using a Whatman filter paper No. 3 and the filtrate was then mixed with 80% acetone to achieve a final volume of 5 mL. The absorbance of the extract was measured at 663 nm and 646 nm for chl a and b) and at 663 nm, 646 nm and 470 nm for carotenoid using a UV-Vis spectrophotometer *NanoVue*^{TM}. The total chlorophyll and carotenoid were reported in mg/g leaf fresh weight (FW). Calculation for total chlorophyll and carotenoid content are as follow:

$$\text{Chlorophyll a (µg/mL)} = 12.25\ A_{663} - 2.79\ A_{646} \quad \text{(Eqn. 1)}$$

$$\text{Chlorophyll b (µg/mL)} = 21.50\ A_{646} - 5.10\ A_{663} \quad \text{(Eqn. 2)}$$

$$\text{Total chlorophyll (µg/mL)} = 7.15\ A_{663} + 18.71\ A_{646} \quad \text{(Eqn. 3)}$$

$$\text{Carotenoid (µg/mL)} = \frac{1000\ A470 - 1.82\ Ca - 85.02\ Cb}{198} \quad \text{(Eqn. 4)}$$

Proline content was quantified using spectrophotometer method (27), with certain modifications using ninhydrin reagent (acid-ninhydrin: glacial acetic acid: phosphoric acid). Proline content analysis was carried out in leaf of 60 DAP. Leaf sample of 0.25 g was weighed and crushed using a mortar and pestle thoroughly with 5 mL of 3% sulfosalicylic acid solution. The extract was then filtered using a Whatman No. 3. filter paper. Subsequently, 1 mL of filtrate was combined with 1 mL of ninhydrin acid and 1 mL of glacial acetic acid in a conical tube. The mixture was then immersed in a water bath of 95 °C for 1 hr. The reaction was halted by placing a conical tube in an ice box for 5 min. After that, 2 mL of toluene was added into the solution and sample was agitated vigorously for 15-20 sec until 2 distinct layers were formed. The top layer formed (red colour) was taken and its absorbance was measured at 520 nm wavelength using a UV-Vis Spectrophotometer *NanoVue*^{TM}. The proline content was reported as milligrams per gram leaf fresh weight (mg/g FW). Calculation for prolin content was showed below.

$$\text{µmol proline/g FW} = \frac{[(\text{µg proline /]mL X ml toluene})}{115.5\ \text{µg / µmol] / [(g sample)/5]}} \quad \text{(Eqn. 5)}$$

Endogenous hormones profiling of 'Sembada Hitam' black rice was conducted using High-Performance Liquid Chromatography (HPLC). Endogenous hormones content was determined in rice leaf of 70 DAP. Leaf sample of 10 g was sliced into small sizes, then sample was put into an Erlenmeyer, immersed in 50 mL of 80% methanol (v/v) and sample were incubated overnight. The extract was evaporated until 20 mL was left, the concentration of methanol was lowered to 60% by adding double distilled water and the pH of sample was adjusted to 2.5. The sample was then partitioned 3 times with ethyl acetate. The ethyl acetate fraction obtained from each partition was collected and then partitioned 3 times with 5% $NaHCO_3$. The aqueous phase of each partition was collected, pH was adjusted to 2.5 using H_2SO_4 and then partitioned again 3 times using ethyl acetate. Ethyl acetate fraction was collected and dried at room temperature. Methanol absolute (2 mL) was added to the extract and extract was filtered using a syringe connected with a 0.02 mm nylon filter. The content of hormones, namely Auxin (IAA), Gibberellin (GA_3), Cytokinin (CKs), Salicylic Acid (SA), Jasmonic Acid (JA) and Abscisic Acid (ABA), was analysed using the High-Performance Liquid Chromatography method *(Shimadzu Model LC 10A).* Column used was a Shim-pack VP ODS 5 µm 150 x 4.6 mm, with mobile phase acetic acid 0.3% and isocratic mobile phase method. The flow rate was 1 mL/min and the injection volume was 10 mL.

Further analysis for auxin (IAA), an isocratic solvent solution containing 0.3% v/v acetic acid was used to perform the auxin analysis and auxin occurrence in the sample was monitored at 486 nm. After that, the thermostat for the column was adjusted to 25 °C and the flow rate for the separation process was set to 1 mL/min. In the meantime, the GA_3 analysis was conducted at 206 nm using an isocratic elution of a solvent that contained 25% v/v acetonitrile. During the separation process, the column thermostat was consistently set at 30 °C, while maintaining a flow rate of 0.8 mL/min. Cytokinin (CKs) were analysed and shown to be present at 269 nm using an isocratic solvent solution with 8% v/v acetonitrile. A column thermostat with a 15 °C setting and a 1 mL/min flow rate were used throughout the separation. Additionally, the ABA analysis was carried out at 260 nm using an isocratic elution of a solvent that contained acetonitrile and 0.1% v/v H_3PO_4

(45:55 v/v). During the separation process, a column thermostat was set at 25 °C, with a flow rate of 0.6 mL/min. Salicylic Acid (SA) and Jasmonic Acid (JA) were measured at 305 and 407 nm using an isocratic solvent solution that included 82% v/v MeOH (excitation and emission detectors). During the separation, 30 °C was the setting for a column thermostat and 1 mL min^{-1} was the flow rate. The endogenous hormones content was reported as nanograms per gram of leaf fresh weight (ng/g FW) (28).

Plant growth parameters

The growth parameters were monitored, encompassing plant height and root length, number of tillers and assessment of flag leaf area. The plant height measurement was taken from the base stem until the tip of the longest leaf, while the length of the root was calculated from the base stem until the longest root. The number(s) of tillers was calculated for the first, second and third tillers. The leaf area (A) was determined based on the measurements of leaf length (L) and leaf width (W) using the equation

$A = 0.75 \times L \times W$ (29) (Eqn.6)

In which A = area of the flag leaf (cm^2)

L= length of the flag leaf (cm)

W= width of the flag leaf (cm)

These growth parameters were observed at 120 days after planting (DAP).

Statistical analysis

Data were analysed statistically, with the first factor being salinity (NaCl concentrations) and the second being salicylic acid concentrations (30). Data were calculated using Microsoft Excel software. The growth and physiological data were analysed using analysis of variance (Two Way ANOVA) followed by Duncan's Multiple Range Test (DMRT) with a significant level (α) = 0.05. Statistical analysis was conducted using SPSS. The phytohormone content was explained descriptively.

Results

Data on growth parameters obtained in this experiment included plant height, root length, number of tillers and flag leaf area. From Table 2, it can be observed that salinity treatments of 50 mM, 100 mM or 150 mM significantly reduced the plant height of black rice 'Sembada Hitam' compared to control. In contrast, the application of salicylic acid (SA) at 2 mM significantly increased plant height compared to control. In black rice plants treated with 50 mM NaCl, application of SA up to 2 mM did not result in any differences in plant height compared to the control. However, in plants subjected to 100 mM or 150 mM NaCl, the application of 1 mM SA significantly increased the average of plant height compared to those plants treated with 0.5 mM of SA and those without SA application. Additionally, the application of 2 mM did not alter the plant height compared to application of 1 mM SA in plants subjected to 150 mM NaCl.

Table 2. Average of plant height (cm) of black rice 'Sembada Hitam' subjected to salinity and treated with SA

Treatment(s)	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	NaCl 150 mM	Mean
SA 0 mM	121.4 ± 3.58^{fg}	113.2 ± 3.83^{de}	100.8 ± 4.02^{c}	75.0 ± 3.24^{a}	102.60 ± 18.31^{w}
SA 0.5 mM	124.0 ± 3.08^{fg}	119.2 ± 4.09^{ef}	98.4 ± 5.37^{c}	87.4 ± 3.78^{b}	107.25 ± 15.82^{x}
SA 1 mM	126.8 ± 5.36^{gh}	119.8 ± 2.05^{f}	108.4 ± 1.67^{d}	96.8 ± 4.32^{c}	112.95 ± 12.18^{y}
SA 2 mM	131.2 ± 4.97^{h}	112.6 ± 2.30^{d}	99.2 ± 11.39^{c}	96.4 ± 7.44^{c}	109.85 ± 15.64^{xy}
Mean	125.85 ± 5.46^{s}	116.20 ± 4.49^{r}	101.70 ± 7.34^{q}	88.90 ± 10.17^{p}	(+)

Note: Numbers followed with identical letter exhibited no statistically significant difference between treatments based on the DMRT test at a 95% confidence level. (+): there is an interaction, (-): no interaction

Table 3. shows that high levels of salinity (100 mM or 150 mM of NaCl) significantly reduced the root length compared to the control and to plants treated with NaCl of 50 mM. Conversely, the application of SA did not affect the root length of black rice plants grown without salinity treatment. Specifically, the application 0.5 mM, 1 mM and 2 mM of SA did not impact root length in plants subjected to 50 mM and 100 mM of NaCl. However, in plants subjected to severe salinity (150 mM of NaCl), the application of 1 mM and 2 mM SA significantly increased root length compared to those plants treated with 0.5 mM SA or without SA.

Based on Table 4, saline conditions significantly reduced the number of tillers in black rice 'Sembada Hitam' compared to the control. In plants grown without NaCl, the application of SA did not result in any significant difference in the number of tillers. However, in plants treated with 50 mM, 100 mM or 150 mM NaCl, the application of 1 mM and 2 mM of SA tended to mitigate the adverse effects of salinity, resulting in a number of tillers that was similar to or even increased compared to those plants without SA application.

According to Table 5, salinity treatment tends to reduce flag leaf area of black rice compared to the control. Higher salinity levels (50 mM, 100 mM or 150 mM of NaCl)

Table 3. Average root's length (cm) of black rice 'Sembada Hitam' subjected to salinity and treated with SA

Treatment(s)	NaCl0 mM	NaCl50 mM	NaCl100 mM	NaCl150 mM	Mean
SA 0 mM	28.6 ± 1.34^{def}	28.6 ± 2.7^{def}	23.2 ± 5.89^{bc}	13 ± 3.87^{a}	23.35± 7.42^{x}
SA 0.5 mM	28 ± 4.5^{cdef}	32.2 ± 1.92^{f}	24 ± 2.35^{bcd}	15.4 ± 4.45^{a}	24.9± 7.14^{y}
SA 1 mM	24.4 ± 0.89^{bcd}	31.2 ± 2.49^{ef}	27.4 ± 2.88^{cdef}	22.4 ± 5.6^{b}	26.35± 4.63^{x}
SA 2 mM	30.6 ± 1.95^{ef}	28.8 ± 2.77^{def}	27.2 ± 2.59^{cde}	21.6 ± 2.8^{b}	27.05± 4.17^{w}
Mean	27.9 ± 3.31^{qr}	30.2 ± 2.78^{r}	25.45 ± 3.91^{q}	18.1 ± 5.69^{p}	(+)

Note: Numbers with followed with identical letter exhibited no statistically significant difference between treatments based on the DMRT test at a 95% confidence level. (+): interaction, (-): no interaction

Table 4. Average number of tillers of black rice 'Sembada Hitam' subjected to salinity and treated with SA

Treatment(s)	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	NaCl 150 mM	Mean
SA 0 mM	11.20 ± 1.09^{g}	6.20 ± 0.45^{b}	6.00 ± 0.71^{b}	3.60 ± 0.55^{a}	6.75 ± 2.92^{z}
SA 0.5 mM	11.80 ± 0.45^{g}	9.60 ± 0.55^{de}	6.00 ± 1.00^{b}	4.80 ± 0.45^{ab}	8.05 ± 2.93^{y}
SA 1 mM	11.00 ± 0.00^{fg}	9.80 ± 0.84^{def}	7.80 ± 1.79^{c}	5.20 ± 0.84^{b}	8.45 ± 2.46^{x}
SA 2 mM	10.60 ± 0.55^{efg}	9.00 ± 1.58^{cd}	7.80 ± 0.45^{c}	5.40 ± 2.07^{b}	8.2 ± 2.31^{w}
Mean	11.15 ± 0.75^{s}	8.65 ± 1.73^{r}	6.90 ± 1.37^{q}	4.75 ± 1.29^{p}	(+)

Note: Numbers with identical letter exhibited no statistically significant difference between treatments based on the DMRT test at a 95% confidence level. (+): interaction, (-): no interaction

corresponded with a smaller flag leaf area relative to the control. In plants subjected to non-saline conditions, the application of SA did not significantly affect the flag leaf area. Similarly, SA application did not impact the flag leaf area in plants treated with 50 mM or 150 mM NaCl. However, foliar spraying of 1 mM of SA effectively maintained the flag leaf area in plants exposed to 100 mM NaCl.

The data presented in Table 6 indicate that the application of NaCl and SA did not significantly affect the total chlorophyll content under control conditions. However, in plants subjected to 100 mM of NaCl, the application of 1 mM of SA significantly increased total chlorophyll content compared to the plants without SA. Additionally, the foliar application of 2 mM of SA under non-saline condition resulted in a reduction in chlorophyll content. In general, SA application under saline conditions did not significantly alter the total chlorophyll content of black rice plants. Notably, exceptions were observed in plants exposed to 100 mM or 150 mM of NaCl, where the application of 1 mM or 2 mM of SA respectively, led to an increase in total chlorophyll content.

According to Table 7, under control conditions, the application of SA did not alter carotenoid content. In plants treated with 50 mM of NaCl, SA application had no significant effect on carotenoid content. However, the application of 1 mM of SA tended to increase carotenoid content, particularly in plants subjected to 100 mM of NaCl. Moreover, the application of 0.5 mM, 1 mM or 2 mM of SA significantly increased carotenoid content in the plants exposed to 100 mM and 150 mM of NaCl.

The data is presented in Table 8 show that in the absence of salicylic acid (SA) application, sodium chloride (NaCl) 100 mM and 150 mM significantly increased the proline content compared to non-saline and 50 mM of NaCl condition. Generally, the application of 1 mM of SA reduced proline content at medium and high salinity levels. In contrast, under low salinity conditions (50 mM of NaCl), proline levels increased when plants were treated with 0.5 mM, 1 mM or 2 mM of SA. The application of 0.5 mM SA also generally increased proline levels in the 50 mM of NaCl treatment. In plants exposed to 100 mM and 150 mM of NaCl, the application of 1 mM of SA consistently reduced the proline content compared to the control.

This study evaluates the effects of exogenous Salicylic Acid (SA) under saline conditions on endogenous plant hormones, specifically Auxin (IAA), Gibberellins (GA_3), Cytokinins (CKs), Abscisic Acid (ABA), Salicylic Acid (SA) and Jasmonic Acid (JA) were determined in rice leaves. As illustrated in Fig. 1. IAA content generally enhanced, particularly in plants treated with 100 mM or 150 mM of NaCl, compared to control plants. Under non-saline condition, endogenous IAA levels content was the lowest and SA application had no effect on auxin content. Conversely, plants exposed to 150 mM of NaCl and treated with 1 mM or 2 mM of SA exhibited the highest IAA content.

The endogenous GA_3 content exhibited a pattern similar to that of endogenous IAA. As illustrated in Fig. 2, the results indicate that the endogenous GA_3 in black rice generally increased in plants subjected to salt stress at 100 mM or 150 mM NaCl, compared to the control group. In those plants subjected to low salinity (50 mM NaCl), the

Table 5. Average flag leaf area (cm^2) of black rice 'Sembada Hitam' subjected to salinity and treated with SA

Treatment(s)	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	NaCl 150 mM	Mean
SA 0 mM	38.70 ± 1.31^{k}	28.15 ± 2.67^{defgh}	23.89 ± 2.88^{bcd}	19.46 ± 2.98^{a}	27.55 ± 7.68^{x}
SA 0.5 mM	35.76 ± 2.42^{jk}	29.15 ± 3.29^{fghi}	24.62 ± 2.98^{cde}	21.44 ± 3.75^{abc}	27.74 ± 6.23^{x}
SA 1 mM	30.50 ± 5.99^{ghi}	28.66 ± 4.48^{efgh}	25.91 ± 0.56^{def}	21.29 ± 1.86^{abc}	26.59 ± 5.02^{x}
SA 2 mM	33.32 ± 2.64^{ij}	26.56 ± 1.22^{defg}	31.07 ± 2.49^{hi}	20.15 ± 4.39^{ab}	27.77 ± 5.81^{x}
Mean	34.57 ± 4.5^{r}	28.13 ± 3.05^{q}	26.37 ± 3.64^{q}	20.58 ± 3.21^{p}	(+)

Note: Numbers with identical letter exhibited no statistically significant difference between treatments based on the DMRT test at a 95% confidence level. (+): interaction, (-): no interaction

Table 6. Average total chlorophyll (mg/g FW) of black rice 'Sembada Hitam' subjected to salinity and treated with SA

Treatment(s)	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	NaCl 150 mM	Mean
SA 0 mM	1.21 ± 0.16^{cd}	1.31 ± 0.14^{d}	1.13 ± 0.15^{bcd}	1.05 ± 0.19^{abc}	1.175 ± 0.18^{w}
SA 0.5 mM	1.06 ± 0.09^{abc}	1.20 ± 0.18^{cd}	1.16 ± 0.15^{bcd}	1.17 ± 0.18^{cd}	1.145 ± 0.15^{wx}
SA 1 mM	1.21 ± 0.16^{cd}	1.25 ± 0.12^{cd}	1.51 ± 0.13^{e}	0.97 ± 0.10^{ab}	1.235 ± 0.23^{x}
SA 2 mM	0.94 ± 0.08^{a}	1.14 ± 0.10^{bcd}	1.20 ± 0.08^{cd}	1.32 ± 0.07^{d}	1.149 ± 0.16^{wx}
Mean	1.105 ± 0.17^{p}	1.222 ± 0.14^{pq}	1.250 ± 0.19^{q}	1.126 ± 0.19^{p}	(+)

Note: Numbers with identical letter exhibited no statistically significant difference between treatments based on the DMRT test at a 95% confidence level. (+): interaction, (-): no interaction

Table 7. Average carotenoid content (mg/g FW) of black rice 'Sembada Hitam' subjected to salinity and treated with SA

Treatment(s)	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	NaCl 150 mM	Mean
SA 0 mM	0.44 ± 0.03^{b}	0.54 ± 0.06^{cd}	0.44 ± 0.08^{b}	0.35 ± 0.12^{a}	0.44 ± 0.10^{w}
SA 0.5 mM	0.52 ± 0.02^{bc}	0.62 ± 0.09^{de}	0.61 ± 0.06^{cde}	0.62 ± 0.04^{de}	0.59 ± 0.07^{x}
SA 1 mM	0.57 ± 0.11^{cde}	0.61 ± 0.05^{cde}	0.64 ± 0.07^{e}	0.55 ± 0.06^{cde}	0.59 ± 0.08^{wx}
SA 2 mM	0.56 ± 0.02^{cde}	0.52 ± 0.05^{bc}	0.54 ± 0.03^{cd}	0.56 ± 0.02^{cde}	0.55 ± 0.03^{w}
Mean	0.522 ± 0.07^{p}	0.574 ± 0.07^{p}	0.56 ± 0.09^{p}	0.522 ± 0.13^{p}	(+)

Note: Numbers with identical letter exhibited no statistically significant difference between treatments based on the DMRT test at a 95% confidence level. (+): interaction, (-): no interaction

Table 8. Proline content (µmol/g FW) of black rice 'Sembada Hitam' subjected to salinity and treated with SA

Treatment(s)	NaCl 0 mM	NaCl 50 mM	NaCl 100 mM	NaCl 150 mM	Mean
SA 0 mM	0.32 ± 0.09^{a}	0.23 ± 0.19^{a}	3.12 ± 2.13^{d}	2.31 ± 0.91^{cd}	1.50^{w}
SA 0.5 mM	0.14 ± 0.00^{a}	0.75 ± 0.17^{ab}	1.53 ± 0.48^{abc}	2.01 ± 0.98^{bcd}	1.11^{wx}
SA 1 mM	1.24 ± 0.31^{abc}	0.64 ± 0.04^{a}	0.90 ± 0.05^{ab}	0.48 ± 0.02^{a}	0.82^{w}
SA 2 mM	0.23 ± 0.04^{a}	0.62 ± 0.12^{a}	1.26 ± 0.04^{abc}	0.47 ± 0.03^{a}	0.64^{w}
Mean	0.48^{p}	0.56^{p}	1.70^{q}	1.32^{q}	(+)

Note: Numbers with identical letter exhibited no statistically significant difference between treatments based on the DMRT test at a 95% confidence level. (+): interaction, (-): no interaction

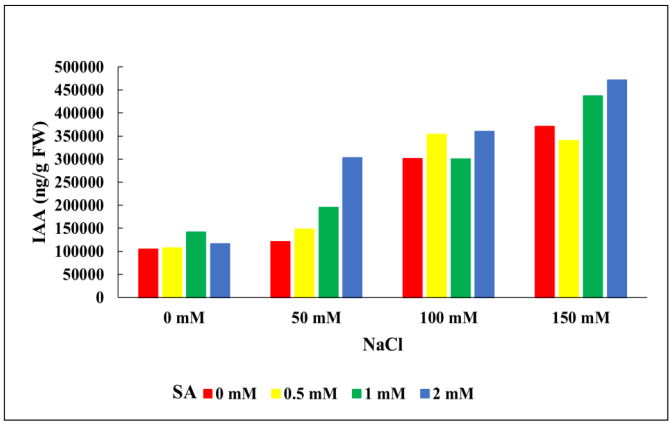

Fig. 1. Endogenous auxin content of black rice 'Sembada Hitam' subjected to salinity and treated with SA.

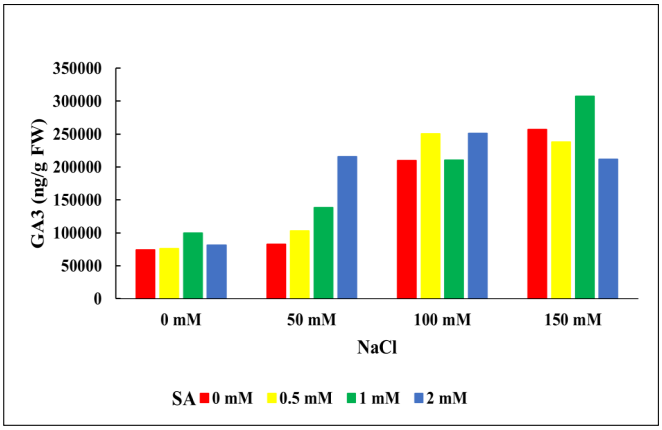

Fig. 2. Endogenous GA_3 content of black rice 'Sembada Hitam' subjected to salinity and treated with SA.

application of 1 mM or 2 mM SA increased the endogenous GA_3 content. However, under moderate or severe salinity, SA application did not significantly affect endogenous GA_3 levels. These findings suggest that SA application can enhance endogenous GA_3 under low salinity conditions.

Fig. 3 illustrates the endogenous ABA content in black rice plants subjected to salinity and SA treatments. Under control conditions (no salinity), SA application tended to reduce endogenous ABA levels. In low salinity conditions (50 mM NaCl), the application of 0.5 mM or 1 mM SA had no effect on endogenous ABA, while 2 mM SA slightly increased endogenous ABA content. At higher salinity levels (100 mM NaCl), SA application reduced endogenous ABA, but under severe salinity (150 mM NaCl), SA application had no significant impact. These results suggest that the effect of SA on endogenous ABA depends on the level of salinity. In this study, SA application notably reduced endogenous ABA under 100 mM NaCl conditions, indicating that SA can mitigate the adverse effects of salt stress and maintain ABA levels similar to those observed under control conditions.

Salicylic acid (SA), a plant hormone classified as a phenolic compound and has crucial role in protecting plants under unfavourable conditions, including salinity stress. This research found that endogenous SA content increased significantly in plants exposed to 100 mM or 150 mM NaCl compared to control (Fig. 4). The application of exogenous SA at 1 mM or 2 mM further enhanced endogenous SA level in plants treated with 50 mM or 150 mM NaCl. However, in plants exposed to 100 mM NaCl, exogenous SA had no significant effect. Similarly, under non-saline conditions, exogenous SA did not alter the endogenous SA levels.

Fig. 5 shows a pattern for endogenous Jasmonic Acid (JA) content similar to that observed for endogenous IAA, GA_3 and SA levels. In this study, the endogenous JA content increased in plants exposed to saline conditions of 100 mM or 150 mM NaCl. The application of 2 mM SA further enhanced endogenous JA levels, particularly in plants subjected to 50 mM or 150 mM of NaCl. Under control condition, however, SA application did not result in any significant increase in endogenous JA content.

The cytokinins (CKs) analysed in this study were categorized into trans zeatin, 9-ribosyl-cis-zeatin, zeatin-O-glucoside, zeatin riboside-O-glucoside, dihydrozeatin, dihydrozeatin riboside and dihydrozeatin-O-glucosdie. Among these, trans zeatin and 9-ribosil-trans-zeatin are the 2 most prominent cytokinins observed. The levels of CKs generally increased after the rice plants were subjected to the saline conditions of 100 mM or 150 mM NaCl. Additionally, salicylic acid (SA) treatments at 1 mM or 2 mM further increased CKs levels in plants exposed to severe salinity (150 mM of NaCl). Under non-saline conditions, CK levels remained consistent across plants treated with different SA concentration. A similar trend was observed in plants subjected to low salinity (50 mM of NaCl), except for a notable increase in CK levels with the application of 2 mM of SA (Fig. 6).

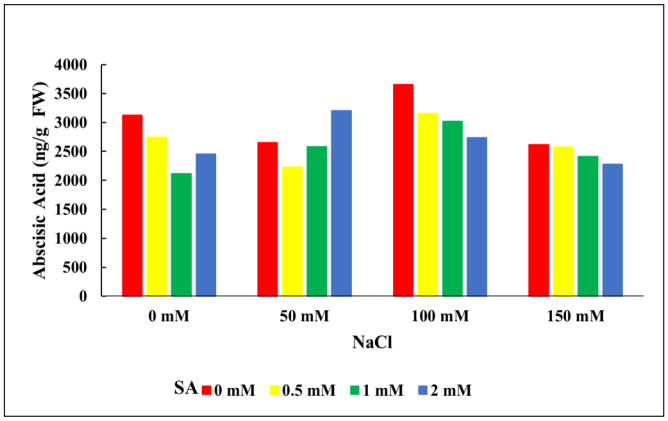

Fig. 3. Endogenous abscisic acid (ABA) content of black rice 'Sembada Hitam' subjected to salinity and treated with SA.

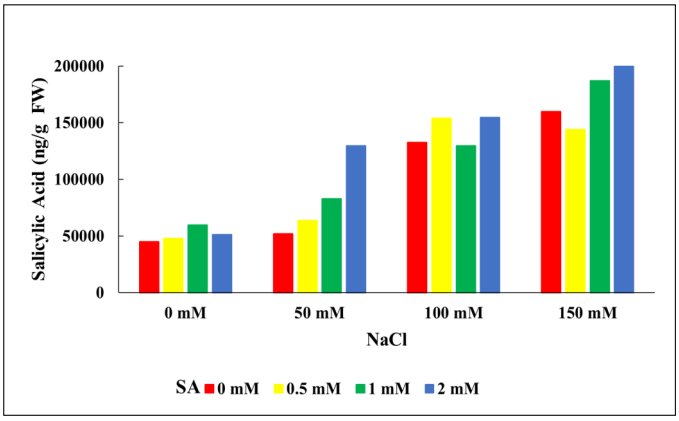

Fig. 4. Endogenous salicylic acid content of black rice 'Sembada Hitam' subjected to salinity and treated with SA.

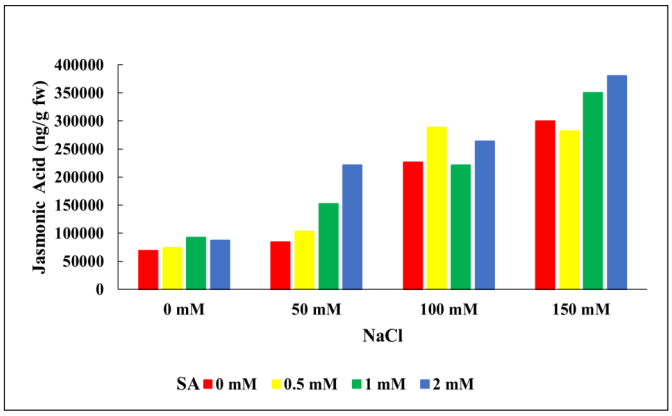

Fig. 5. Endogenous jasmonic acid content of black rice 'Sembada Hitam' subjected to salinity and treated with SA.

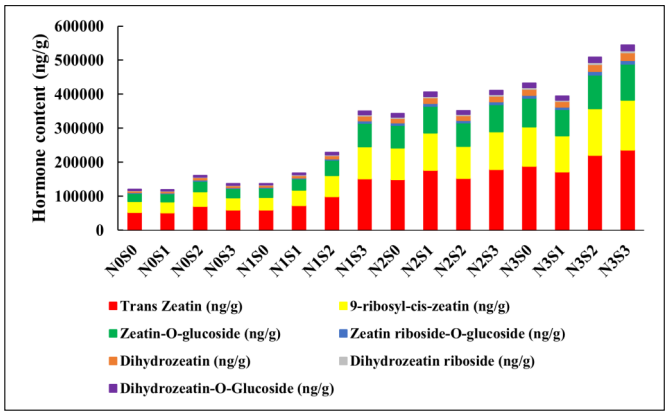

Fig. 6. Endogenous cytokinin content; trans zeatin, dihydrozeatin, 9-ribosyl-cis-zeatin, dihydrozeatin riboside, zeatin-O-aglucoside, dihydrozeatin-O-glucoside, dan zeatin riboside-O-glucoside with treatment N0S0 (NaCl 0 mM, SA 0 mM), N0S1 (NaCl 0 mM, SA 0.5 mM), N0S2 (NaCl 0 mM, SA 1 mM), N0S3 (NaCl 0 mM, SA 2 mM), N1S0 (NaCl 50 mM, SA 0 mM), N1S1 (NaCl 50 mM, SA 0.5 mM), N1S2 (NaCl 50 mM, SA 1 mM), N1S3 (NaCl 50 mM, SA 2 mM), N2S0 (NaCl 100 mM, SA 0 mM), N2S1 (NaCl 100 mM, SA 0.5 mM), N2S2 (NaCl 100 mM, SA 1 mM), N2S3 (NaCl 100 mM, SA 2 mM), N3S0 (NaCl 150 mM, SA 0 mM), N3S1 (NaCl 150 mM, SA 0.5 mM), N3S2 (NaCl 150 mM, SA 1 mM), N3S3 (NaCl 150 mM, SA 2 mM.

Discussion

The observation from this experiment revealed that salinity stress generally reduced the growth parameters, including plant height, root length, number of tillers and flag leaf area. The root length gradually decreased after being subjected to NaCl, as salt stress negatively impacts the epidermal cells of the root (23). As reported by (23), high salt concentration (75-150 mM of NaCl) consistently reduced the primary and lateral root length of *Arabidopsis thaliana*. This result resembles the prior research, which stated that the seedling's length and root length of rice cultivars 'ASD16' and 'BR26' decreased in salinity treatment of 100 mM NaCl compared to control. In comparison, 1 mM SA treatment can improve the seedlings root lengths after plants are subjected to 100 mM NaCl (31). The research revealed, as the previous research, that salt stress reduced growth of rice (31) and pea plants (32). Salt stress influences growth by reducing the plant's dry weight and height and reducing the physiological aspect such as pea plants' chlorophyll a and b (33). Other research revealed that spraying SA could decrease the negative impacts of salt stress in the rice (31) and barley (34). In Table 2, treatment NaCl 50 mM, the plant height was increased after applying SA 0.5 mM and 1 mM because SA could ameliorate the adverse effects of salt stress. However, after applying 2 mM of SA, the plant height was reduced, possibly because the high concentration of SA did not work properly to make the plants grow better. As we know that plant hormone impact plants in low concentrations. In the treatment NaCl 100 mM, the application of 0.5 mM SA still not working to ameliorate plants from salt stress (SA 0 and 0.5 mM have same letter showed no significant difference), but after the SA concentration was increased 1 mM, the plant has better growth.

Also, SA application to the barley can reduce the harmful of salinity to the root and shoot length (34). Roots are the main organs of the plant that responsible for water absorption and nutrients acquisition for plant metabolism (35). The rice seedling stage is followed by the tillering stage, starting from the axil of one of the lowest nodes, the first tiller emerges. The main tillers are those that emerge from the mother tiller. The presence of salt stress also diminishes the number of tillers, subsequently it will cause a decline in yield production. Flag leaf area of black rice decreased as the plants were subjected to saline conditions. Different quantities of salt cause major alterations in the morphological features of leaves, including both the leaf thickness and the mesophyll tissue (36). The occurrence of a reduced leaf area may be attributed to the stress caused by sodium chloride, which hampers the formation of leaf primordia. Certain rice varieties' tillering stages show that leaf area indices and leaf area are additionally suppressed because of sodium stress (37). Decreasing the leaf area also is caused the decrease of cell walls thickness, palisade and spongy tissue, phloem and xylem tissue (38).

Salinity stress trigger signal transduction that alters change the molecular, metabolic and physiological responses and regulates the plant's growth and development. Salt stress will increase build-up of Na^+ ions in the tissue, which in turn restricts the ability to survive and grow due to disruptions in ion imbalances and the deactivation of many functional proteins. Plants can tolerate stress by regulating the transport of Na^+ and K^+, accumulating various osmoprotectants and decreasing ROS activity (39). Salt stress will be responded by plants at the level of pleiotropic, molecular, cellular and organismic, such as ionic homeostasis maintenance, osmotic adjustment, ROS scavenging and nutritional balance (11). The salicylic acid (SA) treatment was suggested to help the rice plant in obtaining water and maintaining the optimum ratio of K^+ and Na^+ under saline conditions. Salicylic acid was considered as ameliorant of the negative effect of salinity and it can enhance salt tolerance in plants (40).

This research showed that salinity stress also affected the physiological aspects, including the total chlorophyll, carotenoid, proline and endogenous hormone content. Salinity stress generally decreased the total chlorophyll and interestingly increased the carotenoid content in high saline conditions. The result of this experiment supports the previous finding that the effects of NaCl with concentrations 25, 50 and 100 mM significantly decreased the chlorophyll a, b and total of radish compared with control plants (41). Also, the combination between salinity stress and application of SA increased the chlorophyll content of radish leaves compare to non-treatment with SA (41). This research revealed that carotenoid content was increased in plant salinity-stress. As a photosynthetic pigment, carotenoid also has role in plant defence by stabilize the lipid membranes and protect the photosystem that can be damage by the form of reactive oxygen species (42).

In this research, it was found that proline content significantly increased in plant stressed compared to control treatment. However, SA foliar application considerably reduced the leaf proline content when plants exposed to the saline conditions, especially at 100 and 150 mM of NaCl. It has been suggested that salinity stress led to an increase in proline content. Previous research found that that in wild rice (*Oryza australiensis*) subjected to 150 mM of sodium chloride for 14 days, the free proline rapidly accumulated and there were an increased in the expression of genes encoding enzymes invoved in proline synthesis such as *OsP5CS1*, *OsP5CS2* and *OsP5CR* (43). Whereas, following SA application the proline content could be reduced even when plants still subjected to saline conditions. Proline is a kind of osmoprotectant that can improve plant survival under salinity stress because proline has antioxidant properties to reduce the damage of thylakoid membrance, act as energy storage and quench ROS (41). As a compatible solution that helps to maintain the leaf's water status in saline conditions, the proline content was increased in barley by 23.1% after the plant was exposed to 150 mM salt stress (25). High salinity reduces the growth and development of plants rapidly as a response to stress and it affects metabolism or water relations (8).

Endogenous hormones content observed in this experiment included Auxin (IAA), Giberellins (GA_3), Cytokinins (CKs), Abscisic Acid (ABA), Jasmonic Acid (JA) and endogenous Salicylic Acid (SA). The plant has strategies to maintain its survival under stress conditions using hormonal regulation (8). An interesting observation from this research is that the content of IAA, GA_3, CKs, JA and endogenous SA increased in plants facing moderate or severe salt stress. Meanwhile, the ABA content increased in moderate saline condition (100 mM NaCl) and decreased in plants subjected to high saline condition (150 mM). Foliar application of SA generally increased the endogenous content of IAA, GA_3, CKs, JA and SA when plants were subjected to moderate to high saline conditions. The endogenous hormones that were determined in black rice plants were from plants that already subjected to salinity treatment for 40 days. It could be that in black rice, there was a feedback mechanism in the biosynthesis of those hormones. The endogenous ABA as stress hormone usually increase rapidly following stress treatment, but as the plants facing longer duration of stress it may be the plants already come to the process of tolerance to salinity stress and plants try to synthesize more growth hormones. Application of SA will help those plants facing severe salinity stress to overcomes unfavourable effects of salt stress by augmenting the phytohormones content. Whereas the effect of SA on the endogenous hormones will depend on its concentration applied in plants experiencing low salinity stress.

Auxin is the primary plant hormone responsible for controlling the growth and development of plants, the overexpression of the auxin-related biosynthesis gene *YUCCA3* caused higher auxin concentration and hypersensitivity to salt stress (8, 44). Auxin accumulation and redistribution may lead to a decrease in plant growth and development when exposed to salt-stress conditions (8). The levels of IAA from this research increased as the consequence of salinity treatment. It showed that long exposure of salt stress could make the rice plants more tolerance. Previous research also stated that in rice tolerant cultivar (Luna Suwarna) has higher root IAA content (1.086 ug/g FW) compared to susceptible cultivar or IR 64 (0.6608 ug/g FW) when plants subjected to 100 mM NaCl (45).

GA_3 is bioactive molecule classified as diterpenoid phytohormone and has also an important role in plant growth and development regulation. GA has roles on the seed germination, lead expansion, stem elongation and flowering (46). GA used as priming for *Zea mays* L., *Lathyrus sativus* L. and *Pisum sativum* has showed the better germination and growth of these plants under salinity stress (46). It showed that GA3 application counteracted the impeded effects of salinity stress. It similar as the results of this research that to overcome the adverse effects of salinity, plant regulate and increase the levels of GA3.

In this research, the SA treatment also increased the levels of endogenous cytokinins in black rice grown under moderate or high salinity stress. Cytokinins are adenine

derivatives that exist in the form of free bases (trans-zeatin, isopentenyladenine, dihydrozeatin, cis-zeatin), as well as their conjugates (ribosides and nucleotides). CKs has roles in improving growth parameters such as cell enlargement, tissue differentiation, plant height and number of tillers (47). This research revealed that as elevated the levels of salinity, it will improve the CKs content in leaf rice. While the foliar application of SA could more improved the CKs content of rice. The finding of this research is in accordance with those reported in barley that the levels of cZ- and iP-types increased under the high salinity stress (300 mM NaCl) in comparison to control. The levels of endogenous CKs in barley plants subjected to NaCl 150 mM increased 16.5% for cZ- and 2.4-fold for iP-types (25).

Abscisic acid (ABA) is a plant stress hormone that functions as an essential signalling for regulating various abiotic stresses, ABA regulates stomatal closure and water deficit (8). The finding in this research also supports the previous research reported by (25), that stated the salinity stress of 150 mM enhanced the ABA levels compared to control and under salinity stress of 300 mM NaCl, SA application decreased the endogenous ABA levels in both the leaves and root organ of barley by 83.5% and 69.1% respectively. Increasing ABA as a stress response can enhance the stomatal closure that led to an inhibition of the water transpiration and decreased water flow to the shoots because of salinity stress (48). Under saline conditions, the levels of ABA and carotenoid increased. Carotenoids serve as precursors of ABA biosynthesis where under saline conditions, the demand for ABA increases, which may, in turn, enhance carotenoid biosynthesis, creating a feedback loop that helps manage stress (49).

Salicylic acid (SA), a phenolic phytohormone, regulates growth and development, photosynthesis, respiration and transpiration, also it enhances a plant's capacity to endure various external and internal stresses. Jasmonic acid and its derivates regulate various biological processes including generative and embryonic development, ageing, sex determination, seed germination, root growth, tuber formation, phototropism and adaptability. The mechanism of SA in increasing plant tolerance depends on growth condition, intensity and duration of stress and plant species (50). Application of SA promoted salt stress tolerance in cowpea cultivars by increasing root's protein for 12.61% (32). Applying SA to the leaves may improve the level of plant tolerance, the black rice roots may not have yet sensed salinity stress and plant growth hormones could be maintained. The long-term duration of salt stress probably caused the black rice 'Sembada Hitam' become more tolerant to salinity stress. Under salt stress, foliar spray of SA has been reported to stabilized photosynthetic activity and increased antioxidant protection as well as yield in maize plants (51). It has also been discovered that exogenous SA with high concentration pose a significant risk to plants; it stopped barley and wheat from growing, reduced Rubisco activity and lower photosynthesis rate (52). Reactive oxygen species (ROS) accumulation disintegrated cellular and sub-cellular membranes, leading to photosynthetic activity. Increasing the synthesis of sugar in the source organ of the plant is suggested to improve rice yields (53). However, the ROS generated by salt stress could decrease the photosynthetic as the vital metabolism in the plants, which influences the carbon skeleton production, leading to a decrease in the growth and productivity of the rice (53).

Conclusion

From results and discussion, it can be inferred that salt stress affects the growth and physiological aspects, including chlorophyll, carotenoid, proline and phytohormone content in black rice. Long term duration of salinity stress generally decreased the growth, chlorophyll and abscisic acid (ABA), whereas it improved the carotenoid, proline and endogenous growth hormones (IAA, GA_3, CKs, JA, SA). The foliar SA application generally reduced the adverse impacts of salinity by enhancing the growth and physiological aspects of black rice 'Sembada Hitam'. This research revealed that salinity stress reduced the growth of black rice 'Sembada Hitam' such as plant height, root length, number of tillers and flag leaf area. Foliar application of SA of I mM or 2 mM can be used as an ameliorant toward severe salt stress in order that growth of black rice 'Sembada Hitam' can be maintained well.

Acknowledgements

This research and publication were supported by the Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research and Technology of the Republic of Indonesia in Masters to Doctoral Scholarship for Undergraduate Excellence (PMDSU) batch VI research grant 2022 with contract number 089/E5/PG.02.00.PT/2022; 1983/UN1/DITLIT/Dit-Lit/PT.01.03/2022 and Enhancing International Publication Program (PKPI-PMDSU) 2024. The authors thanks to Mr. M. Ariesandy for technical assistance in operating HPLC for hormones analysis.

Authors' contributions

HW carried out the laboratory experiments, collect the data and drafted the manuscript. SS, AP, SK, KD participated in design and coordination and draft correction. 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

References

1. Kumar S, Nand V, Narjary B, Kamra SK, Harode PK, Islam A, et al. Evaluating the impact of projected CO_2, temperature and rainfall change on groundwater resources in a rice–wheat dominated cropping region of northwestern India. J Water and Climate Change. 2023;14(7):2323–41. https://doi.org/10.2166/

wcc.2023.062

2. Stavi I, Thevs N, Priori S. Soil salinity and sodicity in drylands: A review of causes, effects, monitoring and restoration measures. Front Environ Sci. 2021;9. https://doi.org/10.3389/fenvs.2021.712831
3. Hayat K, Bundschuh J, Jan F, Menhas S, Hayat S, Haq F et al. Combating soil salinity with combining saline agriculture and phytomanagement with salt-accumulating plants. Critical Rev in Environ Sci and Technol. 2020;50(11):1085–115. https://doi.org/10.1080/10643389.2019.1646087
4. Ryu H, Cho YG. Plant hormones in salt stress tolerance. J Plant Biol. 2015;58(3):147–55. https://doi.org/10.1007/s12374-015-0103-z
5. Khare T, Kumar V, Kishor PBK. Na+ and Cl- ions show additive effects under NaCl stress on induction of oxidative stress and the responsive antioxidative defense in rice. Protoplasma. 2015;252 (4):1149–65. https://doi.org/10.1007/s00709-014-0749-2
6. Akyol TY, Yilmaz O, Uzilday B, Uzilday OR, Türkan İ. Plant response to salinity: An analysis of ROS formation, signaling and antioxidant defense. Turkish J Bot. 2020;44(1):1–13. https://doi.org/10.3906/bot-1911-15
7. Liu C, Mao B, Yuan D, Chu C, Duan M. Salt tolerance in rice: Physiological responses and molecular mechanisms. The Crop J. 2022;10(1):13–25. https://doi.org/10.1016/j.cj.2021.02.010
8. Singh A, Sengar RS. Salinity stress in rice: An overview. Agri and Food Sci, Biol, Environ Sci. 2014;14(2):643–48. Available from:
9. Chutipaijit S, Cha-um S, Sompornpailin K. High contents of proline and anthocyanin increase protective response to salinity in *Oryza sativa* L. spp. *indica*. Australian J Crop Sci. 2011;5(10):1191–98.
10. Hariadi YC, Nurhayati AY, Soeparjono S, Arif I. Screening six varieties of rice (*Oryza sativa*) for salinity tolerance. Procedia Environ Sci. 2015;28:78–87. https://doi.org/10.1016/j.proenv.2015.07.012
11. Nisa RI, Wulandari RA, Kurniasih B. Effect of salinity during seedling stage on the growth and yield of black rice (*Oryza sativa* L. 'Jeliteng'). IOP Conference Series: Earth and Environ Sci.2022;985(1). https://doi.org/10.1088/1755-1315/985/1/012011
12. Chunthaburee S, Sakuanrungsirikul S, Wongwarat T, Sanitchon J, Pattanagul W, Theerakulpisut P. Changes in anthocyanin content and expression of anthocyanin synthesis genes in seedlings of black glutinous rice in response to salt stress. Asian J Plant Sci. 2016;15(3–4):56–65. https://doi.org/10.3923/ajps.2016.56.65
13. Kim Y, Mun BG, Khan AL, Waqas M, Kim HH, Shahzad R, et al. Regulation of reactive oxygen and nitrogen species by salicylic acid in rice plants under salinity stress conditions. PLoS ONE. 2018;13(3). https://doi.org/10.1371/journal.pone.0192650
14. Dempsey DA, Vlot AC, Wildermuth MC, Klessig DF. Salicylic acid biosynthesis and metabolism. The *Arabidopsis* Book. 2011;0156. https://doi.org/10.1199/tab.0156
15. Mir AR, Somasundaram R. Salicylic acid and salt stress tolerance in plants: A review. J Stress Physiol and Biochem. 2021;17(3).
16. Hu Y, Zhi L, Li P, Hancock JT, Hu X. The role of salicylic acid signal in plant growth, development and abiotic stress. Phyton-Intern J Experim Bot. 2022;91(12):2591–605. https://doi.org/10.32604/PHYTON.2022.023733
17. Sharma M, Gupta SK, Majumder B, Maurya VK, Deeba F, Alam A, et al. Salicylic acid mediated growth, physiological and proteomic responses in two wheat varieties under drought stress. J Proteomics. 2017;163:28–51. https://doi.org/10.1016/j.jprot.2017.05.011
18. EL Sabagh A, Islam MS, Hossain A, Iqbal MA, Mubeen M, Waleed M et al. Phytohormones as growth regulators during abiotic stress tolerance in plants. Front in Agron. 2022;4. https://doi.org/10.3389/fagro.2022.765068
19. Torun H, Novák O, Mikulík J, Strnad M, Ayaz FA. The effects of exogenous salicylic acid on endogenous phytohormone status in *Hordeum vulgare* L. under salt stress. Plants. 2022;11(5):618 https://doi.org/10.3390/plants11050618
20. Lichtenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. methods in enzymology. Methods Enzymol. 1987;148(C):350–82. https://doi.org/10.1016/0076-6879(87)48036-1
21. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973;39(1):205–07. Available from: https://link.springer.com/article/10.1007/BF0001806
22. Montgomery EG. Correlation studies in corn, Annual Report No.24. Nebraska360 Agri Experim Station: Lincoln, NB, USA; 1911. 108–59
23. Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley and Sons; 1984. 201
24. Jini D, Joseph B. Physiological mechanism of salicylic acid for alleviation of salt stress in rice. Rice Sci. 2017;24(2):97–108. https://doi.org/10.1016/j.rsci.2016.07.007
25. Mahdavian K. Efect of diferent concentrations of salicylic acid on salt tolerance of barley (*Hordeum vulgare* L.). J Crop Physiol. 2017;36:121–36.
26. Ren M, Li Y, Zhu J, Zhao K, Wu Z, Mao C. Phenotypes and molecular mechanisms underlying the root response to phosphate deprivation in plants. Intern J Molecular Sci. 2023;24 (6): https://doi.org/10.3390/ijms24065107
27. Maryum Z, Luqman T, Nadeem S, Khan SMUD, Wang B, Ditta A, et al. An overview of salinity stress, mechanism of salinity tolerance and strategies for its management in cotton. Front in Plant Sci. 2022;13. https://doi.org/10.3389/fpls.2022.907937
28. Haque MA, Rafii MY, Yusoff MM, Ali NS, Yusuff O, Datta DR et al. Advanced breeding strategies and future perspectives of salinity tolerance in rice. Agron. 2021;11(8): https://doi.org/10.3390/agronomy11081631
29. Devireddy AR, Zandalinas SI, Fichman Y, Mittler R. Integration of reactive oxygen species and hormone signaling during abiotic stress. Plant J. 2021;105(2):459–76. https://doi.org/10.1111/tpj.15010
30. Jayakannan M, Bose J, Babourina O, Rengel Z, Shabala S. Salicylic acid in plant salinity stress signalling and tolerance. Plant Growth Regulation. 2015;76(1):25–40. https://doi.org/10.1007/s10725-015-0028-z
31. Mahdavian K. Application of salicylic acid on chlorophyll, carotenoids and proline in radish under salinity stress. Proceedings of the National Acad of Sci India Section B - Biol Sci. 2023;93(4):809–18. https://doi.org/10.1007/s40011-023-01484-1
32. Simkin AJ. Carotenoids and apocarotenoids in planta: Their role in plant development, contribution to the flavour and aroma of fruits and flowers and their nutraceutical benefits. Plants. 2021;10(11):2321. https://doi.org/10.3390/plants10112321
33. Nguyen HTT, Bhowmik SD, Long H, Cheng Y, Mundree S, Hoang LTM. Rapid accumulation of proline enhances salinity tolerance in Australian wild rice *Oryza australiensis* Domin. Plants. 2021;10 (10): https://doi.org/10.3390/plants10102044
34. Park J, Kim YS, Kim SG, Jung JH, Woo JC, Park CM. Integration of auxin and salt signals by the NAC transcription factor NTM2 during seed germination in *Arabidopsis*. Plant Physiol. 2011;156 (2):537–49. https://doi.org/10.1104/pp.111.177071
35. Saini S, Kaur N, Marothia D, Singh B, Singh V, Gantet P, Pati PK. Morphological analysis, protein profiling and expression analysis of auxin homeostasis genes of roots of two contrasting cultivars of rice provide inputs on mechanisms involved in rice

adaptation towards salinity stress. Plants. 2021;10(8):1544. https://doi.org/10.3390/plants10081544

36. Tsegay BA, Andargie M. Seed priming with gibberellic acid (GA3) alleviates salinity induced inhibition of germination and seedling growth of *Zea mays* L., *Pisum sativum* var. *abyssinicum* A. Braun and *Lathyrus sativus* L. J Crop Sci and Biotechnol. 2018;21(3):261–67. https://doi.org/10.1007/s12892-018-0043-0

37. Mathpal B, Srivastava PC, Pachauri SP, Shukla AK, Shankhdhar SC. Role of gibberellic acid and cytokinin in improving grain Zn accumulation and yields of rice (*Oryza sativa* L.). J Soil Sci and Plant Nutri. 2023;23(4):6006–16. https://doi.org/10.1007/s42729-023-01459-1

38. Sharipova G, Ivanov R, Veselov D, Akhiyarova G, Seldimirova O, Galin I, et al. Effect of salinity on stomatal conductance, leaf hydraulic conductance, HvPIP2 Aquaporin and abscisic acid abundance in barley leaf cells. Intern J Molecular Sci. 2022;23(22):14282. https://doi.org/10.3390/ijms232214282

39. Wani AB, Chadar H, Wani AH, Singh S, Upadhyay N. Salicylic acid to decrease plant stress. Environ Chem Lett. 2017;15(1):101–23. https://doi.org/10.1007/s10311-016-0584-0

40. Sousa PDJ, Silva MT, Costa CJMA, Dos SNGA, De Araújo BAE, Souza CL et al. Effect of salicylic acid on cowpea seedlings under saline stress. Plant Science Today. 2023;11(1):288-95. https://doi.org/10.14719/pst.2237

41. Tahjib-Ul-Arif M, Siddiqui MN, Sohag AAM, Sakil MA, Rahman MM, Polash MAS et al. Salicylic acid-mediated enhancement of photosynthesis attributes and antioxidant capacity contributes to yield improvement of maize plants under salt stress. J Plant Growth Regulation. 2018;37(4):1318–30. https://doi.org/10.1007/s00344-018-9867-y

42. Chen YE, Cui JM, Li GX, Yuan M, Zhang ZW, Yuan S, et al. Effect of salicylic acid on the antioxidant system and photosystem II in wheat seedlings. Biologia Plantarum. 2016;60(1):139–47. https://doi.org/10.1007/s10535-015-0564-4

43. Chen T, Shabala S, Niu Y, Chen ZH, Shabala L, Meinke H et al. Molecular mechanisms of salinity tolerance in rice. Crop J. Institute of Crop Sci. 2021;9(3):pp.506–20. https://doi.org/10.1016/j.cj.2021.03.005

44. Yuvaraj M, Bose SCK, Elavarasi P, Tawfik E. Soil salinity and its management. In: Soil Moisture Importance. Intech Open; 2021. https://doi.org/10.5772/intechopen.93329

45. Ramamoorthy P, Karthikeyan M, Nirubana V. Management of saline and sodic soils. Intern J Agri Sci and Technol. 2021;1(1):24–2. https://doi.org/10.51483/IJAGST.1.1.2021.24-27

46. Batarseh M. Sustainable management of calcareous saline sodic soil in arid environment: the leaching process in the Jordan Valley. J Appl and Environ Soil Sci. 2017;1–9. https://doi.org/10.1155/2017/1092838

47. Susanti NL, Dewi K. The effect of rice husk biochar on the growth and phytohormone profile of Chinese cabbage under drought conditions. Transactions of the Chinese Society of Agri Machinery. 2023;54(7):15–29.

48. Al-Shammari WB, Altamimi HR, Abdelaal K. Improvement in physiobiochemical and yield characteristics of pea plants with nano silica and melatonin under salinity stress conditions. Horticulturae. 2023;9:711. https://doi.org/10.3390/horticulturae9060711

49. Al-Shammari WB, Alshammery K, Lofti S, Altamimi H, Alshammari A, Al-Harbi NA et al. Improvement of morphophysiological and anatomical attributes of plants under abiotic stress conditions using plant growth-promoting bacteria and safety treatments. Peer J. 2024; 12:e17286. http://doi.org/10.7717/peerj.17286

50. Ambavaram MMR, Basu S, Krishnan A, Ramegowda V, Batlang U, Rahman L et al. Coordinated regulation of photosynthesis in rice increases yield and tolerance to environmental stress. Nat Commun. 2014;5. https://doi.org/10.1038/ncomms6302

51. Kristamtini K, Taryono T, Basunanda P, Murti RH. Keragaman genetik kultivar padi beras hitam lokal berdasarkan penanda mikrosatelit. J AgroBiogen. 2016;10(2):69–76. https://doi.org/10.21082/jbio.v10n2.2014.p69-76

52. Zou Y, Zhang Y, Testerink C. Root dynamic strategies in response to salinity. Plant Cell Environ. 2022;45:695–704. https://doi.org/10.1111/pce.14205

53. Sathasivam R, Radhakrishnan R, Kim JK, Park SU. An update on biosynthesis and regulation of carotenoids in plants. South African J Bot. 2021;140:290–302. https://doi.org/10.1016/j.sajb.2020.05.015