

Salicylic acid treatment induced change in the morphological, chlorophyll content, and phytochemical traits in two rice genotypes

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Manuscript received: 24 November 2024. Revision accepted: 15 February 2025.

Abstract. Mulyati Y, Zubaidah S, Adiredjo AL, Rofiq M, Jadid N, Saputro TB, Isnawati, Muhaimin FI, Farapti, Sari GM. 2025. Salicylic acid treatment induced change in the morphological traits and the content of chlorophylls and endogenous salicylic acid in two rice genotypes. *Biodiversitas* 26: 778-788. Salicylic acid (SA) plays a crucial role in rice plant resistance. At the same time, the elicitation mechanisms of its induction are not well understood. The exploration of rice's endogenous SA during the vegetative and generative phases in response to SA elicitation is a new area of study. Similarly, the correlation between SA and rice resistance characteristics is a fresh perspective that this research aims to address. The study focuses on the effect of exogenous salicylic acid (SA) elicitation on plant height, the content of chlorophyll a, b and total chlorophyll, endogenous SA, and the number of productive shoots and filled grains characters in two rice genotypes, namely genotype A (Situbagendit×Cibogo3) and genotype B (Situbagendit×Ciherang8). SA at concentration 0-2.5 mg L⁻¹ was applied to the in vitro shoot culture of the rice varieties. The results showed that genotype B had significant content of chlorophyll a, total chlorophyll, endogenous SA content (generative phase), number of productive shoots, and filled rice grains compared to genotype A. SA concentration of 2 mg L⁻¹ was proven to be the optimal dose that could maximize the resistance and productivity characters of genotype B. Conversely, higher or lower concentrations tended to produce less significant or negative effects. A positive correlation between endogenous SA content and the number of productive shoots was found in genotype B. This study revealed the importance of genetic factors and elicitor concentration in improving rice resistance traits that affect productivity. These findings have practical implications, as they contribute to the development of superior rice cultivars that can support sustainable food security, offering hope for the future of agriculture.

Keywords: Endogenous salicylic acid, exogenous salicylic acid, genotype, rice resistance character

Abbreviations: ANOVA: Analysis of variance, C: Concentration of SA exogenous, Chl. Tot: Total chlorophyll, Chl. a: Chlorophyll a, Chl. b: Chlorophyll b, df: degree of freedom, FG: Filled grain, G: Genotype, HPLC: High-Performance Liquid Chromatography, LSD: Least Significant Difference, MS: Murashigae and Skoog, ns: not significant differences, PH: Plant Height, PL: Panicle Length, PT: Number of productive tillers, SA: Salicylic acid, SA V: Salicylic acid content in the vegetative phase, SA G: Salicylic acid content in the generative phase

INTRODUCTION

Rice is not just a crop but a lifeline for many countries, particularly those that rely on it as a staple food, including Indonesia (Fahad et al. 2015). Stability and increasing rice production are key to preventing a food crisis (Lu et al. 2018). The main challenge in rice production is pest attacks (Fattah et al. 2020; Babendreier et al. 2022), which significantly impact rice production. For example, attacks by brown planthopper pests have caused a decrease in rice yields of up to 80% and have had a significant economic impact on rice production in Asia (Sriram et al. 2024).

Resistant host plants are the primary strategy for maintaining rice yield potential because they are practical, economical, and more environmentally friendly than pesticides (Ling and Weilin 2016). Research has been conducted to obtain resistant rice varieties through identifying and characterizing pest resistance genes, gene mapping, conventional breeding of tolerant plants (Sriram et al. 2024), and insertion of pest resistance genes into susceptible plants (Simon et al. 2023). However, this strategy is faced with problems, including the evolution of new biotypes of pests. It can eliminate the effectiveness of plant resistance that only has one resistance gene, limited

information on the molecular mechanisms underlying plant resistance to pests, and limited genetic sources studied (Sriram et al. 2024). These limitations are the reason for the minimal number of pest-resistant rice varieties, and research studies are still being carried out to obtain pest-resistant rice.

Rice plants have a natural defense mechanism in the form of salicylic acid (SA) (Lu et al. 2018; Pál et al. 2020; Kalaivani et al. 2021). This defense mechanism can be stimulated or enhanced using elicitors, compounds that can increase the production of secondary metabolites, similar to the natural response of plants to pest attacks. Research shows that rice plants exposed to pests can produce more SA than plants that are not exposed (Lu et al. 2018). The study results showed that SA stimulated a significant increase in secondary metabolites compared to 21 other elicitors (Capite et al. 2016; Ali 2020). The use of exogenous SA has been proven to increase defense responses in wheat (Sorahinobar et al. 2016; Ochoa-Meza 2021), cucumber, and watermelon (Wang et al. 2020), as well as against abiotic stress (Pál et al. 2020).

Apart from being responsible for the defense mechanism of rice plants, SA also plays a role in regulating various physiological and metabolic activities, including regulating growth, flowering induction, seed germination, chlorophyll accumulation, photosynthesis, enzyme activation, and plant maturation (Ali et al. 2017; Kohli et al. 2017). This mechanism is responsible for forming rice tillers and determining the harvest yield. Producing productive tillers and grain weight is a compensation mechanism for rice when pests attack, thereby reducing the negative impact on harvest yields (Ochoa-Meza et al. 2021; Bavi et al. 2022). Moreover, elicitation using SA also brings ecological benefits because SA comes from natural sources, so it is eco-friendly, harmless to life, supports sustainable agriculture, is cost-effective, and fast in increasing secondary metabolite production on a commercial scale (Ali 2020; Ding and Ding 2020).

Utilization of superior lines is the primary key to achieving rice food security. This study used two rice genotypes from previous plant breeding by the research team (Adiredjo 2019; Ardiarini et al. 2022). The two rice genotypes are genotype A, a cross between the Situbagendit×Cibogo3 varieties, and genotype B, which results from a cross between the Situbagendit×Ciherang8 varieties. Both are the fourth generation of their parents and are known to have the characteristics of a much higher number of productive tillers, flowering time, and harvest age that are significantly higher than their parents (Ardiarini et al. 2022). Until now, the resistance characters of the two genotypes have not been studied; therefore, the study aims to examine the morpho-physiological, phytochemical, and agronomic characters of the two rice genotypes due to exogenous SA elicitation. The results of this study are expected to find superior rice genotypes that have pest resistance and high productivity characteristics.

MATERIALS AND METHODS

Study period and location

The study was conducted from March to November 2024. In-vitro elicitation treatment, chlorophyll content test, and total phenolic test were conducted at the Laboratory of Tissue Culture Physiology and Plant Microtechnics, Universitas Brawijaya, Malang, Indonesia. Elicitation plants were acclimatized in the greenhouse of the Department of Biology, Universitas Brawijaya. Salicylic acid content was analyzed at the Chemistry Laboratory, University of Muhammadiyah Malang, Malang, Indonesia.

Plant material

This study used two fourth-generation (F4) rice genotypes obtained from the Plant Breeding Laboratory, Department of Agronomy, Faculty of Agriculture, Universitas Brawijaya. Genotype A is a cross between Situbagendit×Cibogo3, and genotype B is a cross between Situbagendit×Ciherang8. Rice seeds were soaked in 90% ethanol solution for 1 minute, then surface sterilized using 100% commercial bleach solution containing 5.2% sodium hypochlorite for 20 minutes; then rinsed using sterile distilled water three times. Seeds were germinated on agar media for 9 days. The shoot part of 9-day-old sprouts was used as an explant in the elicitation treatment.

In vitro elicitation treatment using salicylic acid (SA) elicitor

Rice plant elicitation was carried out in vitro using a salicylic acid elicitor. Sterilized rice seeds grew in Murashige and Skoog (MS) medium supplemented with vitamin B5 (Teh et al. 2018), 30 g L⁻¹ sugar, 13 g L⁻¹ agar, and salicylic acid at concentrations of 0, 0.5, 1, 1.5, 2, and 2.5 mg L⁻¹, with the medium adjusted to pH 5.8. The medium was sterilized in an autoclave at 121°C under 1 atmosphere of pressure for 15 minutes. Cultures were maintained under light conditions at 24°C for 4 weeks, producing one sprout by the end of this period. Each concentration treatment was repeated three times across three bottles containing one explant.

Acclimatization of plantlets and plant maintenance

Acclimatization is a precise process that aims to adapt the in vitro cultured plantlets to the surrounding environment (in vivo conditions). The plantlets are carefully planted in a specific planting media, a meticulously balanced mixture of compost, rice husks, and humus soil (1:1:1). The plantlets are maintained in a 30×25 cm plastic box and covered evenly. In the second week after acclimatization, the acclimatization box lid is opened gradually, ensuring the plantlets adjust to their natural conditions. The plants are then maintained for 3 months, in line with maintained for 3 months, adjusting the age of the rice genotype used. At the age of 1 month, the plants are transferred to a 30 cm diameter plastic bucket filled with mud or rice field soil and given fertilizer 2 times.

Chlorophyll content analysis

Chlorophyll analysis was performed on rice plants 25 days after acclimatization, using the third fully opened leaf from the shoot. A total of 0.3 g of leaves were crushed, homogenized in 15 mL of an 80% acetone solution, centrifuged at 5,000 rpm for 10 minutes, and the supernatant was taken. Absorbance readings were taken using UV-vis spectrophotometry at wavelengths λ 645 and λ 663.

Endogenous SA analysis

The endogenous salicylic acid was measured during the vegetative and generative phases. Plants in the acclimatization phase served as samples for endogenous SA analysis. The analysis targeted plants 25 days after planting (vegetative phase) and during the flowering phase, indicated by the emergence of the first panicle (generative phase). Salicylic acid levels were determined using the HPLC (High-Performance Liquid Chromatography) technique and the Shimadzu CBM 20 A HPLC apparatus model. The HPLC procedure used the salicylic acid method. Salicylic acid was extracted from 0.2 g of rice leaf samples homogenized in liquid nitrogen and extracted with methanol, according to Kim et al. (2018). The sample was then centrifuged at 13,000 rpm for 5 minutes.

Furthermore, the supernatant was filtered using a 0.22 mm filter membrane. The obtained filtrate was added with 2 mL of distilled water (1:4 v v⁻¹ and passed through a VP ODS 5 μ m shim-pack 150 x 4.6 mm. The column was then washed through the mobile phase method using 82% MeOH, then evaporated to dryness under vacuum at 30°C, while the remaining residue was dissolved in 500 mL of 50% methanol. The measurement of salicylic acid levels was determined based on the following equation.

$$\text{Equation (ng/ml)} = \frac{\text{curve area} - 3,7053 \times 1 \times 1,000}{8,4331 \times \text{sample volume } 200}$$

Measurement of plant height, productive tillers, and filled grains in rice plants

At harvest, we measured plant height, productive tillers, and filled grains. Plant height was recorded using a measuring ruler, measuring from the base of the stem (soil surface or planting media) to the tip of the highest panicle. The number of productive tillers was determined through direct observation. Productive tillers were identified as those bearing panicles filled with grain. The number of filled grains was then counted manually, with each grain carefully separated from the empty ones, ensuring a precise count.

Data analysis

The Kolmogorov–Smirnov test confirmed the normality of the data distribution. The normality analysis examined data on plant height, chlorophyll a content, chlorophyll b content, total chlorophyll, endogenous SA content during vegetative and generative phases, panicle length, number of productive tillers, and filled grain. Two-way analysis of variance (two-way ANOVA) using IBM SPSS Statistic 26 software measured the comparison of morpho-physiological,

phytochemical, and agronomic responses between two rice genotypes due to exogenous SA elicitation. The Least Significant Difference (LSD) test at a 5% significance level ($P=0.05$) was applied to identify significant differences between the two research factors. The effect size of each factor (genotype, elicitor concentration, and their interaction) was calculated using Partial Eta Squared (η^2) and interpreted based on Cohen's (1988) guidelines: small ($\eta^2 < 0.06$), medium ($0.06 \leq \eta^2 < 0.14$), and large ($\eta^2 \geq 0.14$). Pearson's correlation coefficients analysis determined the relationship between endogenous SA content and agronomic characters. The performance analytics package operates in R-software.

RESULTS AND DISCUSSION

Two-way ANOVA results

The results of ANOVA for the factors of rice genotype type (G), elicitor concentration (C), and the interaction between genotype type and elicitor concentration (G x C) due to exogenous salicylic acid elicitation treatment are shown in Table 1. The ANOVA results show that the effects of the three test factors are different for each character tested. In the character of plant height, endogenous SA content in the vegetative phase, endogenous SA content in the generative phase, and the number of productive shoots, the ANOVA results for the G x C interaction were significant at the probability level 0.05. In the characters of chlorophyll content, total chlorophyll content, and the number of filled grains, the G x C interaction was insignificant; the significant factor was G only. All research factors were found to have no significant effect on chlorophyll b content and panicle length.

Table 1. Summary of ANOVA results on all quantitative characters tested due to exogenous SA exposure

Trait	Mean square			
	Genotype (G)	The concentration of SA exogenous (C)	G x C	Error
df	1	5	5	24
PH	27.214 ^{ns}	12.624*	34.367*	7.396
Chl. a	11.543*	0.154 ^{ns}	0.086 ^{ns}	0.156
Chl. b	0.004 ^{ns}	0.049 ^{ns}	0.441 ^{ns}	0.041
Chl. Tot	11.189*	0.223 ^{ns}	0.094 ^{ns}	0.247
SA V	53845.888*	34176.794*	21740.278*	460.044
SA G	345660.797*	93452.365*	36548.429*	1641.655
PL	6.084 ^{ns}	10.951 ^{ns}	12.702 ^{ns}	8.555
PT	5.444*	5.578*	4.111*	0.806
FG	20069.444*	2494.133 ^{ns}	9387.578 ^{ns}	4427.667

Notes: df: degree of freedom, PH: plant height (cm), Chl. a: chlorophyll a (mg g⁻¹), Chl. b: Chlorophyll b (mg g⁻¹), Chl. Tot: total chlorophyll (mg g⁻¹), SA V: salicylic acid content in the vegetative phase (ng g⁻¹), SA G: salicylic acid content in the generative phase, PL: panicle length (cm), PT: number of productive tillers, FG: filled grain, *: statistically significant differences at $p \leq 0.05$, ns: not significant differences

Other information obtained from the ANOVA results is the effect size of each variable studied. Based on the partial eta squared value, the G x C interaction greatly influences all tested characters, except for the chlorophyll character (a, b, and total chlorophyll) with a medium influence category. When each variable stands alone, the concentration factor greatly influences all characters except for the grain content character with a medium category. In the genotype variable, the effect is small on the chlorophyll b and panicle length characters and moderate on the plant height character (PH). It has a significant influence on other test characters (Table 2).

Interaction between rice genotype types and exogenous SA

The morphological character studied in this study was plant height. The results of the LSD further test at a significance level of 0.05 showed that the highest results were found in the interaction of genotype A with an exogenous SA exposure level of 1 mg L⁻¹. At the same concentration, rice genotype B responded differently than genotype A. The most significant reaction in genotype B was at 0.5 mg L⁻¹ SA exposure, which was not statistically different from responses at 1.5 mg L⁻¹ and 2.5 mg L⁻¹ (Table 3). Using the LSD grouping, overlapping letters in the table show no significant differences between specific treatments, even if their values surpass the confidence interval (P=0.05). The height of rice genotypes A and B at various levels of concentration exposure is shown in Figure 1.

Table 2. Effect size of genotype type, elicitor concentration, and their interactions on various test characters

Trait	Partial Eta Squared (η^2) and its interpretation								
	PH	Chl. a	Chl. b	Chl. Tot.	SA V	SA G	PL	PT	FG
G	0.133 (M)	0.756 (L)	0.004 (S)	0.653 (L)	0.830 (L)	0.898 (L)	0.029 (S)	0.220 (L)	0.159 (L)
C	0.263 (L)	0.171 (L)	0.199 (L)	0.158 (L)	0.939 (L)	0.922 (L)	0.211 (L)	0.591 (L)	0.105 (M)
G x C	0.492 (L)	0.104 (M)	0.084 (M)	0.073 (M)	0.908 (L)	0.823 (L)	0.236 (L)	0.515 (L)	0.306 (L)

Notes: S (small): $\eta^2 < 0.06$, M (medium): $0.06 \leq \eta^2 < 0.14$, L (Large): $\eta^2 \geq 0.14$. Interpretation follows Cohen's guidelines (1988)

Table 3. Plant height of two rice genotypes at various levels of exogenous SA concentration

Genotype	Exogenous SA concentration (mg L ⁻¹)					
	0	0.5	1	1.5	2	2.5
A	69.400 ^a	72.433 ^a	79.033 ^c	70.933 ^a	74.067 ^b	71.100 ^a
B	73.767 ^a	77.467 ^{bc}	72.267 ^a	74.900 ^{bc}	72.867 ^a	76.133 ^{bc}

Note: Values followed by the same letter are not significantly different at the 0.05 probability level

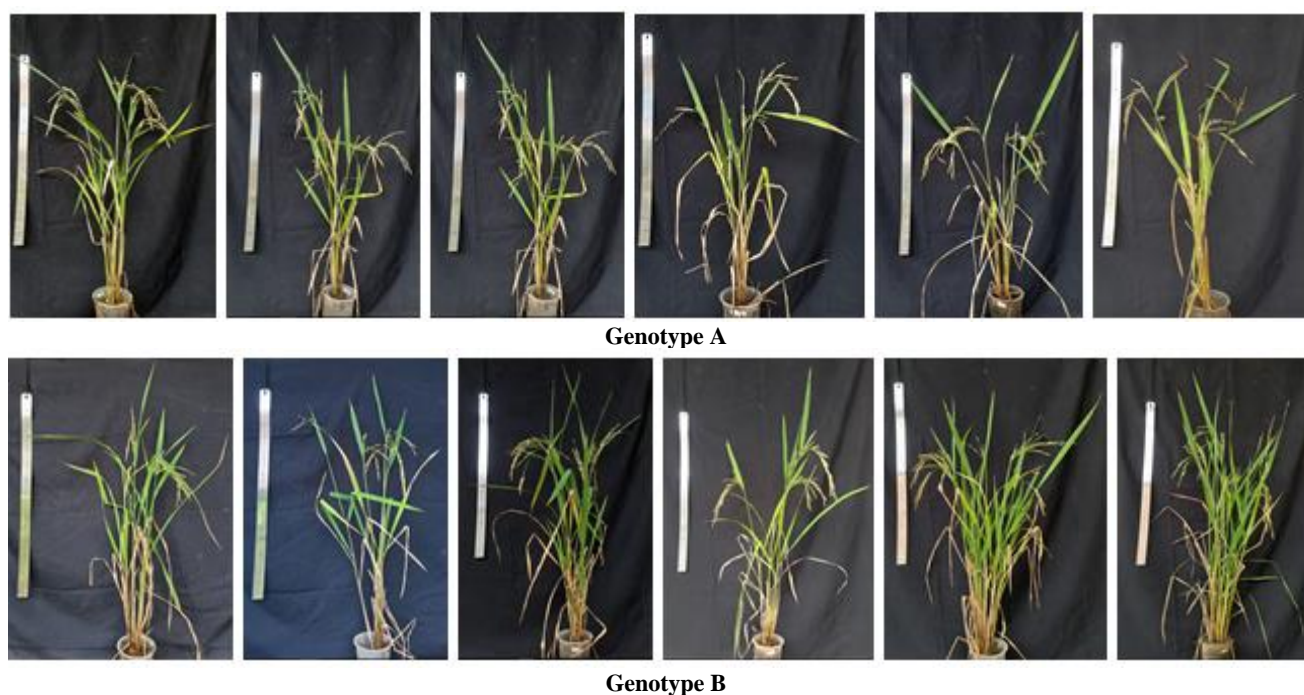


Figure 1. Rice height of genotypes A and B at exogenous SA concentration levels of 0, 0.5, 1, 1.5, 2, and 2.5 mg L⁻¹, respectively, from left to right

The interaction of genotype and exogenous SA concentration also affected the endogenous SA content, measured in two growth phases: vegetative and generative. The two genotypes studied produced endogenous SA in different quantities in natural conditions, showcasing the diversity in plant responses. In the vegetative phase, the endogenous SA content of genotype A was significantly higher than that of genotype B. In contrast, the endogenous SA content of genotype B experienced a spike in the generative phase, significantly higher than that of genotype A.

Exogenous SA exposure was responded to differently by the two genotypes studied in the vegetative and generative phases. In the vegetative phase, the decrease in the endogenous SA content of genotype A occurred at concentration levels of 0.5 mg L⁻¹ and 2.5 mg L⁻¹. At other concentration levels, it had an impact on increasing endogenous SA. A different response was shown by genotype B, where all levels of exogenous SA concentration were

able to increase the endogenous SA content (Table 4). In the generative phase, a negative response was shown by genotype A due to exogenous SA exposure, where all levels of exogenous SA concentrations resulted in a decrease in endogenous SA content.

Meanwhile, in genotype B, exogenous SA concentration treatment below 2 mg L⁻¹ (1.5 mg L⁻¹, 1 mg L⁻¹, and 0.5 mg L⁻¹) caused a decrease in endogenous SA content, while concentration levels above it (2 and 2.5 mg L⁻¹) were responded positively with the detection of an increase in endogenous SA (Table 5). The results of further tests with LSD at a significance level of 0.05 showed that the most significant interaction treatment between genotype and exogenous SA concentration was found in genotype B with a concentration level of 2 mg L⁻¹, both measurements during the vegetative phase and the generative phase. The detailed influence of each interaction is shown in Figure 2.

Table 4. SA content of genotype A and genotype B in the vegetative phase due to exogenous SA exposure

Exogenous SA concentration (mg L ⁻¹)	Replicates	Genotype A			Genotype B		
		RT (min)	Sample curve area	Result (ng g ⁻¹)	RT (min)	Sample curve area	Result (ng g ⁻¹)
0	1	1.506	67.16498	365.29475	1.506	33.56235	168.59332
	2	1.506	59.98432	314.79180	1.506	34.52584	177.41214
	3	1.506	57.26945	311.35569	1.506	39.19525	198.50995
0.5	1	1.506	50.53265	264.41923	1.506	58.38841	317.85993
	2	1.506	46.40054	234.38966	1.506	65.05985	360.17051
	3	1.506	54.35316	297.31887	1.506	63.59871	348.14617
1	1	1.506	63.97814	337.13089	1.506	74.98562	398.54203
	2	1.506	57.76972	314.26364	1.506	79.98562	443.39939
	3	1.506	65.69825	356.85175	1.506	77.68841	421.77613
1.5	1	1.506	68.39528	361.83778	1.506	84.96141	467.73682
	2	1.506	71.64312	383.62338	1.506	83.83985	448.22564
	3	1.506	63.63325	348.34694	1.506	87.11219	484.82445
2	1	1.506	71.94639	389.04100	1.506	102.54147	558.09658
	2	1.506	69.72136	380.01009	1.506	101.16982	535.06377
	3	1.506	76.29885	406.04571	1.506	92.71326	522.50473
2.5	1	1.506	51.84625	279.83191	1.506	86.14698	461.13037
	2	1.506	45.49852	235.99309	1.506	80.46525	446.18736
	3	1.506	51.14987	260.46269	1.506	86.74697	478.01509

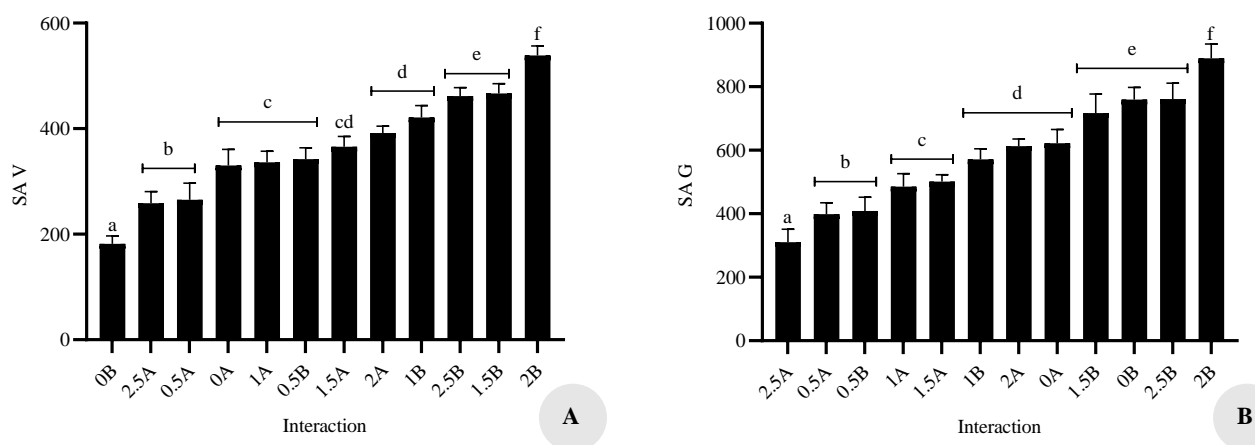


Figure 2. Effect of interaction between genotype and exogenous SA concentration level on endogenous SA content during A. The vegetative and B. Generative phases

Table 5. SA content of genotype A and genotype B in the generative phase due to exogenous SA exposure

Exogenous SA concentration (mg L ⁻¹)	n	Genotype A			Genotype B		
		RT (min)	Sample curve area	Result (ng g ⁻¹)	RT (min)	Sample curve area	Result (ng g ⁻¹)
0	1	1.506	106.62639	575.68016	1.506	127.11178	724.43487
	2	1.506	117.58622	661.96275	1.506	136.09234	740.49539
	3	1.506	114.83845	627.53374	1.506	140.90129	797.48771
0.5	1	1.506	79.59797	436.86310	1.506	76.27482	398.39442
	2	1.506	68.98118	365.11496	1.506	81.43102	456.27443
	3	1.506	70.72334	393.41698	1.506	70.03243	370.99503
1	1	1.506	93.63918	493.72183	1.506	108.06621	606.62519
	2	1.506	79.68064	441.62661	1.506	100.07182	565.70179
	3	1.506	95.22379	521.74496	1.506	102.31845	541.36956
1.5	1	1.506	91.77376	511.92105	1.506	125.14216	705.88263
	2	1.506	93.32366	515.87267	1.506	119.98274	662.89520
	3	1.506	84.83792	476.27401	1.506	138.22609	781.93741
2	1	1.506	112.07433	606.15274	1.506	165.95369	933.95494
	2	1.506	105.97174	594.45053	1.506	155.60782	891.71559
	3	1.506	112.35992	637.83681	1.506	154.53462	843.65068
2.5	1	1.506	62.33566	324.87892	1.506	126.04976	711.15930
	2	1.506	50.66563	265.17013	1.506	143.37637	811.87476
	3	1.506	62.55862	342.10037	1.506	138.25591	759.76473

Table 6. Effect of genotype interaction and exogenous SA concentration on the number of productive shoots

Genotype	Exogenous SA concentration (mg L ⁻¹)					
	0	0.5	1	1.5	2	2.5
A	5.333 ^a	4.333 ^a	5.333 ^a	5.000 ^a	5.333 ^a	4.000 ^a
B	4.333 ^a	4.667 ^a	5.333 ^a	5.000 ^a	9.000 ^c	5.667 ^b

Note: Values followed by the same letter are not significantly different at the 0.05 probability level

Table 7. Effect of genotype on chlorophyll content and number of grains containing

Genotype	Chlorophyll a		Total chlorophyll		Filled grains	
	Mean	Error	Mean	Error	Mean	Error
A	1.151 ^a	.093	1.844 ^a	.117	178.389 ^a	15.684
B	2.283 ^b	.093	2.959 ^b	.117	225.611 ^b	15.684

Note: Values followed by the same letter are not significantly different at the 0.05 probability level

Another character that was significantly affected by the interaction between genotype and exogenous SA concentration was the number of productive shoots. In natural conditions (without exogenous SA treatment), genotype A and genotype B had no significantly different number of productive shoots. Genotype A showed concentrations that were not significantly different at all concentration levels. Meanwhile, in genotype B, the exogenous SA concentration of 2 mg L⁻¹ showed the highest effect among other interaction treatments, followed by the treatment of 2.5 mg L⁻¹. The impact of the interaction between genotype and exogenous SA concentration is shown in Table 6.

The primary influence of genotype factors on the character of chlorophyll content and the number of grains containing

This study found that genotype was the only factor influencing chlorophyll content and the number of filled grains. Genotype affected chlorophyll a and total chlorophyll but did not affect chlorophyll b. The results of the LSD_{0.05}

further test showed that genotype B had content chlorophyll as much as 98.262%, significantly higher than genotype A. Similarly, genotype B had 60.466% more total chlorophyll than genotype A.

In the character of filled grains, this study showed consistent results with the chlorophyll a and total chlorophyll characters. Further test analysis with LSD_{0.05} consistently showed that genotype B was better than genotype A, with a difference between the two of 26.471%. Although the SA concentration factor did not have a significant effect, the mean data showed that a concentration of 2 mg L⁻¹ had the highest impact, and the lowest effect was found at a concentration of 0.5 mg L⁻¹. A summary of the results of further tests of the influence of genotype on the characteristics of chlorophyll content and the number of filled rice grains is shown in Table 7. ANOVA results demonstrated no significant effect of genotype on chlorophyll b content (p=0.761), avoiding further post hoc investigation. Consequently, chlorophyll b data is removed from Table 7, providing parameters with significant genotype effects. A comparison of the production of filled rice grains in

genotypes A and B at various levels of exogenous SA exposure is shown in Figure 3.

Pearson's correlation coefficient

Pearson correlation analysis was conducted to study the relationship between endogenous salicylic acid content (both in the vegetative and generative phases) and rice productivity, in this case, represented by the character of the number of productive shoots. The phytochemical and agronomic characters were selected based on the significant effect of the interaction of the two genotypes with exogenous SA concentrations on the two characters. The results of this analysis are expected to strengthen further the determination of the type of rice genotype that has better resistance properties due to exogenous SA exposure. In genotype A, the analysis results showed that the

endogenous SA content in the vegetative phase did not show a significant correlation with the number of productive shoots (P -two-tailed=0.0659, R -squared=0.2367). A positive correlation was found when correlated with the SA content in the generative phase with a value of P -two-tailed=0.0042, R -squared=0.2764.

Meanwhile, in genotype B, the endogenous SA content in the vegetative and generative phases was positively correlated with the number of productive shoots. The P -two-tailed and R -squared values in the vegetative phase were 0.0038 and 0.4873, respectively. The P -two-tailed and R -squared values in the generative phase were 0.0129 and 0.3896, respectively. The results of Pearson's correlation between endogenous SA content and the number of productive shoots are shown in Figure 4.

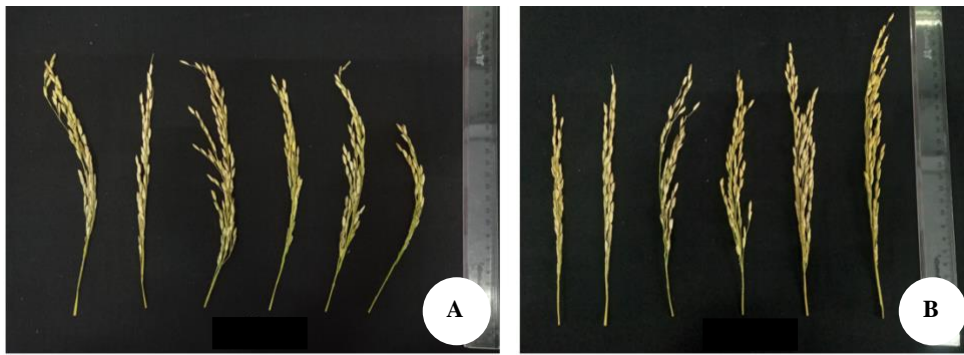


Figure 3. Production of full grains in A. Genotype A and B. Genotype B at various levels of exogenous SA concentration. The SA concentrations from left to right in figures A. and B. are 0, 0.5, 1, 1.5, 2, and 2 mg L⁻¹, respectively

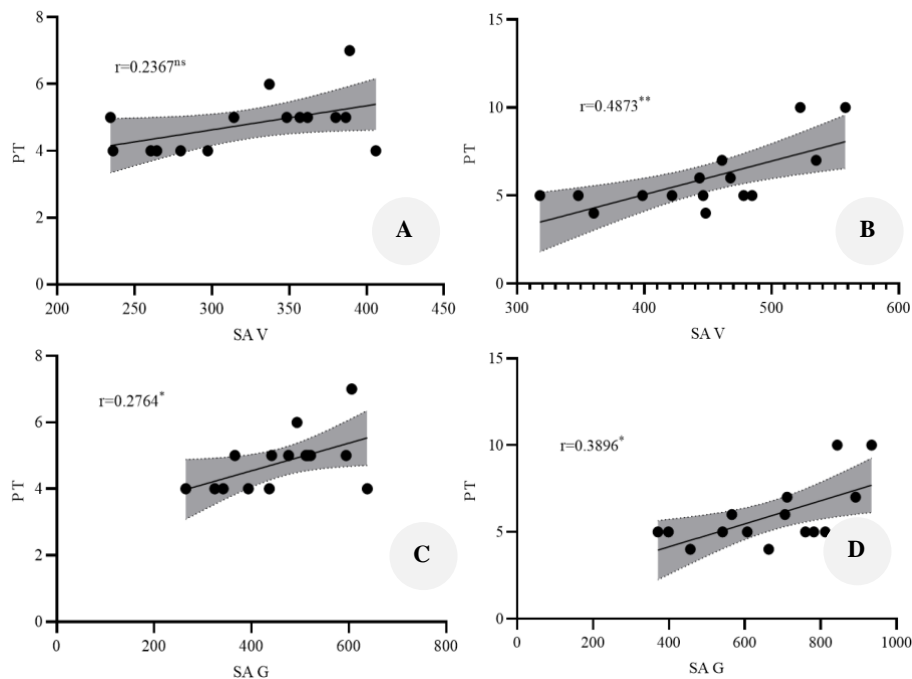


Figure 4. A. Correlation of SA V - PT during the vegetative phase in genotype A, B. Correlation of SA V - PT during the vegetative phase in genotype B, C. Correlation of SA V - PT during the generative phase in genotype A, D. Correlation of endogenous SA V - PT during the generative phase in genotype B. SA V: Salicylic acid content during the vegetative phase, SA G: Salicylic acid content during the generative phase, PT: Number of productive tillers

Discussion

Pest attacks hamper efforts to increase rice productivity to achieve food security. Using resistant host plants is a crucial strategy for food security (Ling et al. 2016). Elicitation is a method currently widely used by researchers to obtain resistant cultivars (El-Beltagi et al. 2022; Kowalska et al. 2022). In this study, two rice genotypes were treated with exogenous SA at various concentration levels to examine its effects on several rice resistance characters. This study also studied the correlation between endogenous SA content due to exposure to exogenous SA and rice productivity.

Genotypes A and B showed different responses due to exogenous SA exposure in this study. The effect of genotype type in responding to exogenous SA exposure has also been reported previously (Sumardi and Fiani 2015; Kalaivani and Sengottayan 2016; Poór et al. 2019; Chen et al. 2022; El Sherbiny et al. 2022). In almost all characters studied in this study, genotype B showed superiority when compared to genotype A. Significantly, genotype B had higher chlorophyll a content, total chlorophyll, endogenous SA in the generative phase, number of productive tillers, and the number of filled rice grains compared to genotype A, only in the character of plant height where genotype A was better than genotype B. Each plant variety has a unique genetic composition, which affects how plants respond to exogenous stimuli, including SA hormones.

Kalaivani and Sengottayan (2016) explained that genes involved in defense signaling pathways, metabolism, and growth can vary between varieties, thus affecting the effectiveness of the response to SA. Genetically, the two genotypes studied have different genetic compositions because they come from crosses of other parents. genotype A results from a cross between Situbagendit×Cibogo3, and genotype B is obtained from a cross between Situbagendit×Ciherang8. Situbagendit is an upland-dryland variety, while Cibogo3 and Ciherang 8 are paddy fields (Adiredjo et al. 2019). Parental differences are likely to affect the genetic composition of the resulting genotypes. This difference allows the two genotypes to have different defense mechanisms against SA exposure (Chen et al. 2022). Based on the research results obtained, genotype B is believed to be more capable of inducing defense pathways triggered by SA. At the same time, genotype A is less responsive, even tending to respond negatively under control conditions or without treatment. Another fact is that genotype B has better physiological conditions with significantly higher chlorophyll a and total chlorophyll content compared to genotype A. The differences in character shown by individual plants are believed to result from different gene expressions (Fadhli et al. 2023; Sudika et al. 2023).

The concentration level of exogenous SA is also an essential factor that influences how an individual responds. Many studies have shown that plant responses to elicitors have dose or concentration-dependent effects, meaning that the effects can vary depending on the dose/concentration used (Poór et al. 2019; Chen et al. 2022; Ahmed et al. 2024). The dose or concentration that significantly affects

the test character is the optimal dose (Ahmed et al. 2024). This study found that a dose of 2 mg L⁻¹ was optimal to stimulate most of the resistance characters of genotype B. These characters are endogenous SA content in the vegetative phase, endogenous SA content in the generative phase, and the number of productive shoots and filled grains. The dose that gave the second effective effect was 2.5 mg L⁻¹. Below a dose of 2 mg L⁻¹, it no longer effectively affected the characters tested. The elicitor may not be enough to trigger a significant defense response at low concentrations. In contrast, the response can be more robust at high concentrations but can also cause negative effects if it is too high (Kalaivani and Sengottayan 2016).

This study revealed that the SA dose of 2 mg L⁻¹ was correct to activate the defense signaling pathway in plants. At this optimal concentration, the elicitor can activate signaling pathways to produce growth hormones, enzymes, and secondary compounds that increase plant resistance. Beyond the optimal dose, if the concentration is too low, this pathway may not be activated effectively, and if it is too high, it can cause physiological stress in plants (Chen et al. 2022; Paul et al. 2024). However, this dose of 2 mg L⁻¹ was only optimal for genotype B. In contrast, in genotype A, this dose had a negative impact on the tested characters. This finding is in line with Chen et al. (2022). The two rice varieties studied, Wanshengyoutianhong-4 (W4) and Huanghuazhan (HZ), were responsive to the lowest SA exposure dose of the given concentration treatments. Low doses are more effective on certain rice varieties (Ding et al. 2016). In contrast, higher SA concentrations significantly inhibited development (Tateda et al. 2014; Trost et al. 2014).

Previous studies have revealed many roles of SA in supporting plant growth (Kalaivani and Sengottayan 2016; Chen et al. 2022; El Sherbiny et al. 2022; Latrianto et al. 2022; Paul et al. 2024). The plant growth studied in this study was plant height. The effect size of the effect of exogenous SA exposure on the height of the two genotypes tested was in the high category (Table 2). Salicylic acid (C₇H₆O₃) is a plant growth regulator of the phenolic acid group that affects growth indirectly (Liang et al. 2022). SA compounds affect plant height by stimulating cell division and differentiation (Paul et al. 2024). SA can interact with auxin to stimulate cell elongation and division, as well as gibberellin, which also triggers plant growth. In addition, other roles of SA in supporting plant growth are increasing root and shoot biomass, improving water and nutrients, and increasing enzyme activity related to water use efficiency, contributing to better growth (Kalaivani and Sengottayan 2016; Chen et al. 2021). Genotype B did not show better results in this character than genotype A. The presence of exogenous SA may be used for the induction of other character signals (Liang et al. 2022), as will be explained further. In addition to affecting plant morphology, SA also plays a role in plant physiological processes.

Regarding chlorophyll characters, Paul et al. (2024) explained that the presence of SA can accelerate the formation of photosynthetic pigments. In this study, exogenous SA significantly affected chlorophyll a and total chlorophyll. The effect is in the medium category (Table 2). Chlorophyll is known to be the primary pigment in the photosynthesis

process, while total chlorophyll is an indicator of photosynthesis efficiency (Singh and Singh 2015; Fakhri et al. 2021).

In this study, the presence of exogenous SA significantly affected the endogenous SA content in both genotypes studied. The effect of exogenous SA on endogenous SA has been studied in wheat (El-Alwany and Banni 2023), maize (El-Mergawi and El-Wahed 2020), cucumber (Kim et al. 2017), *Hordeum vulgare* L. (Torun et al. 2022), and other species Kavulych et al. (2023). Unlike previously reported studies, our study examined the endogenous SA content of rice more specifically, namely in the vegetative and generative phases. This study aimed to determine the role of SA in supporting metabolism for characters responsible for rice defense. In the two genotypes studied, the endogenous SA content increased from the vegetative phase to the generative phase. The difference is, under natural or normal conditions without exogenous SA treatment, the endogenous SA of genotype A is higher than that of genotype B. However, the endogenous SA of genotype B experienced a sharp increase of 316% during the generative phase. The increase in endogenous SA during the Generative also increased, but only 88.48%.

Exogenous SA exposure can activate plant physiological responses (El-Mergawi and El-Wahed 2020). This response activates Systemic Acquired Resistance (SAR) (El-Alwany and Banni 2023). SAR is a plant defense mechanism that can be activated (inducible) (Wang et al. 2018); the study by El-Alwany and Banni (2023), the endogenous SA content increased from days 10, 20, and 30 after exogenous SA exposure. This condition indicates that the effect of exogenous SA treatment is gradual and continuous, strengthening plant defenses over time (time-dependent accumulation). The difference in the increase in endogenous SA levels due to exogenous SA exposure in the two genotypes studied in this study is believed to be due to the influence of different genotype types, as previously explained. However, exogenous SA exposure does not always impact increasing endogenous SA. In genotype A, endogenous SA exposure decreased at all concentration levels, below normal conditions (without exogenous SA treatment). The decrease in endogenous SA levels over time of exogenous SA exposure was also confirmed by El-Mergawi and El-Wahed (2020). The decrease occurred after the peak concentration, which may be due to the transformation of the hormone into its conjugate form. SAG, or salicylic acid O- β -glucoside, is a conjugate form of SA produced as a plant defense response to manage stress (Kim et al. 2017).

In addition to affecting morphology and physiology, salicylic acid also affects rice productivity (Kalaivani and Sengottayan 2016; Paul et al. 2024). The productivity characteristics studied in this study were the number of productive shoots and the number of full grains. Exogenous SA exposure had a significant effect on both characters, with a partial eta squared value (η^2) ≥ 0.14 (Table 2) (Shieh 2015; Norouzian and Plonsky 2018). In both characters, genotype B showed a better response than genotype A. Several metabolic mechanisms are mediated by SA in increasing rice productivity, including increased plant growth, nutrient availability (such as N, P, K, Ca, and Mg),

and disease resistance (Kalaivani and Sengottayan 2016). Support for these metabolic processes can, in turn, support increased rice productivity.

The correlation study between SA content and the number of productive shoots provides evidence that strengthens the opinion that SA plays a vital role in plant defense mechanisms through its compensation mechanism. This finding is also supported by the results of previous studies (Kalaivani and Sengottayan 2016; Singh et al. 2019; Priya et al. 2022). The compensation mechanism by forming a higher number of productive tillers than in normal conditions can increase rice productivity so that plants do not experience yield losses due to unfavorable conditions. Conversely, rice can produce higher grains of rice (Table 7). Both genotypes tested in this study showed the same compensation mechanism. However, the facts show that genotype B's compensation mechanism is better than genotype A's in dealing with exogenous SA exposure. In genotype B, Pearson's correlation coefficient showed significant results in the correlation between endogenous SA content, both in the vegetative and generative phases and the number of productive tillers. These results can be interpreted that the positive role of SA in the vegetative phase positively impacts the formation of agronomic characters (in this study, namely the number of productive tillers and full rice grains) in the generative phase (Figure 4). In the previous discussion, it has been explained that exposure to exogenous SA does not inhibit the growth of genotype B. Its presence triggers an increase in endogenous SA content during the vegetative to generative phases (Tables 4 and 5). Physiologically, genotype B can also produce chlorophyll a and total chlorophyll, which are significantly higher than genotype A. This condition impacts well-maintained metabolic regulation, supports efficient photosynthesis, and ultimately can increase crop yields (Paul et al. 2024). This situation was not found in genotype A. The significance of the correlation between endogenous SA content and the number of productive tillers was only found in the generative phase, not in the vegetative phase. Genotype B could still survive well during the vegetative phase based on plant height data (Table 1). However, its ability to survive decreases along with the decrease in endogenous SA and chlorophyll content, which, in turn, impacts crop yields.

Overall, these findings indicate that the role of SA as an exogenous elicitor significantly impacts various aspects of rice plant growth, physiology, and productivity, especially in genotype B. This genotype consistently showed superiority in defense mechanisms through various compensatory and productivity characters compared to genotype A, which showed a negative response to exogenous SA exposure in several characters. This difference reflects the importance of genetic factors in determining the effectiveness of SA elicitation in plants, especially in efforts to find resistant cultivars. In addition, determining the optimal SA concentration is critical to the success of elicitation, with a concentration of 2 mg L⁻¹ proven to be the most effective for genotype B in improving resistance and productivity characteristics. These findings provide in-depth insights into SA-based defense mechanisms and their application in

developing pest-resistant and high-potential rice varieties, supporting efforts to achieve sustainable food security.

This study shows that salicylic acid significantly improves rice resistance and productivity, but several limitations require consideration. First, this study only examined two rice genotypes, so the results may not fully represent other genotypes. Second, controlled conditions through in vitro elicitation and acclimatization limited the ability to observe plant responses in more complex field conditions. Third, this study did not evaluate the effectiveness of resistance to actual pest attacks, which is important for practical applications. The study also did not test the interaction of SA with other stress factors, such as drought or salinity, and did not explore the effects of SA concentrations outside the studied range. Considering these limitations point to the need for further research to address these aspects and broaden our understanding of the results.

ACKNOWLEDGEMENTS

We want to thank the Research Institute Community Engagement, State University of Malang, Indonesia which funded the research and publication through the JatimPro Grant Scheme 2024, with contract number 5.4.18/UN32.14.1/LT/2024.

REFERENCES

- Adiredjo AL, Damanhuri, Respatijarti. 2019. Experimental hybridization to produce F1 from a cross between gogo-dryland and paddy-field rice cultivars subjected to different environmental conditions in Indonesia. *Indian J Agric Res* 53: 213-217. DOI: 10.18805/IJARE.A-375.
- Ahmed S, Ahmed SF, Biswas A, Sultana A, Issak M. 2024. Salicylic acid and chitosan mitigate high temperature stress of rice via growth improvement, physio-biochemical adjustments and enhanced antioxidant activity. *Plant Stress* 11: 100343. DOI: 10.1016/j.stress.2023.100343.
- Ardiarini NR, Damanhuri, Adiredjo AL. 2022. Genetic variability and gene action for several traits in F4 and F5 population of rice. *Plant Breed Biotechnol* 10 (1): 31-36. DOI: 10.9787/PBB.2022.10.1.31.
- Ali B. 2020. Salicylic acid: An efficient elicitor of secondary metabolite production in plants. *Biocatal Agric Biotechnol* 31: 101884. DOI: 10.1016/j.bcab.2020.101884.
- Ali M, Abbasi BH, Ahmad N, Khan H, Ali GS. 2017. Strategies to enhance 21 biologically active-secondary metabolites in cell cultures of *Artemisia* – current 22 trends. *Crit Rev Biotechnol* 37: 833-851. DOI: 10.1080/07388551.2016.1261082.
- Babendreier D, Tang R, Horgan FG. 2022. Prospects for integrating augmentative and conservation biological control of leaffolders and stemborers in rice. *Agronomy* 12 (12): 2958. DOI: 10.3390/agronomy12122958.
- Bavi K, Khavari-Nejad RA, Najaf F, Ghanati F. 2022. Phenolics and terpenoids change in response to yeast extract and chitosan elicitation in *Zataria multiflora* cell suspension culture. *3 Biotech* 12 (8): 163. DOI: 10.1007/s13205-022-03235-x.
- Capite AD, Lancaster T, Puthoff D. 2016. Salicylic acid treatment increases 1 the levels of 2 triterpene glycosides in Black Cohosh (*Actaea racemosa*) rhizomes. *J Chem Ecol* 3 (42): 13-16. DOI: 10.1007/s10886-015-0655-x.
- Chen G, Zheng D, Feng, N, Zhou H, Mu D, Liu L, Zhao L, Shen X, Rao G, Li T. 2021. Effects of exogenous salicylic acid and abscisic acid on growth, photosynthesis and antioxidant system of rice. *Chil J Agric Res* 82 (1): 21-32. DOI: 10.4067/S0718-58392022000100021.
- Chen L, Feng L, Liang X, Li J, Liao G, Zhu L, Fu K, Fan W, Wang S, Liu J. 2022. Characterizing the mechanism of serotonin alleviates rice resistance to brown planthopper *Nilaparvata lugens* (Homoptera: Delphacidae) nymphs. *Agronomy* 12 (12): 3191. DOI: 10.3390/agronomy12123191.
- Cohen J. 1988. *Statistical Power Analysis for the Behavioral Sciences* (2nd ed.). Hillsdale, NJ: Lawrence Erlbaum Associates Publishers, New York.
- Ding Y, Zhao JH, Nie Y, Fan B, Wu SJ, Zhang Y, Sheng JP, Shen L, Zhao RR, Tang XM. 2016. Salicylic acid-induced chilling- and oxidative-stress tolerance in relation to gibberellin homeostasis, CBF pathway, and antioxidant enzyme systems in cold-stored tomato fruit. *J Agric Food Chem* 64: 8200-8206. DOI: 10.1021/acs.jafc.6b02902.
- Ding P, Ding Y. 2020. Stories of salicylic acid: A plant defense hormone. *Trends Plant Sci* 25: 549-565. DOI: 10.1016/j.tplants.2020.01.004.
- El-Mergawi R, El-Wahed MSA. 2020. Effect of exogenous salicylic acid or indole acetic acid on their endogenous levels, germination, and growth in maize. *Bull Natl Res Centre* 44: 167. DOI: 10.1186/s42269-020-00416-7.
- El-Alwany AM, Banni A. 2023. Spectrophotometric quantification of endogenous salicylic acid in priming wheat tissue with *Puccinia triticina* f. sp. *tritici*. *Alq J Med App Sci* 5 (1): 119-125. DOI: 10.5281/zenodo.6209951.
- El-Beltagi HS, Mohamed HI, Aldaej MI, Al-Khayri JM, Rezk AA, Al-Mssallem, MQ, Sattar MN, Ramadan KMA. 2022. Production and antioxidant activity of secondary metabolites in Hassawi rice (*Oryza sativa* L.) cell suspension under salicylic acid, yeast extract, and pectin elicitation. *Plant Tissue Cult* 58: 615-629. DOI: 10.1007/s11627-022-10264-x.
- El Sherbiny HA, El-Hashash EF, Abou EMM, Nofal RS, El-Mageed ATA, Bleih EM, El-Saadony MT, El-Tarabily KA, Shaaban A. 2022. Exogenously applied salicylic acid boosts morpho-physiological traits, yield, and water productivity of lowland rice under normal and deficit irrigation. *Agronomy* 12: 1860. DOI: 10.3390/agronomy12081860.
- Fadhli N, Farid M, Azrai M, Nur A, Efendi R, Priyanto SB, Nasruddin A, Novianti F. 2023. Morphological parameters, heritability, yield component correlation, and multivariate analysis to determine secondary characters in selecting hybrid maize. *Biodiversitas* 24 (7): 3750-3757. DOI: 10.13057/biodiv/d240712.
- Fahad S, Hussain S, Saud S, Khan F, Hassan S, Amanullah, Nasim W, Arif M, Wang F, Huang J. 2015. Exogenously applied plant growth regulators affect heat-stressed rice pollens. *J Agron Crop Sci* 202 (2): 139-150. DOI: 10.1111/jac.12148.
- Fakhri M, Riyani E, Ekawati AW, Arifin NB, Yuniarti A, Widayawati Y, Saputra IK, Samuel PD, Arif MZ, Hariati AM. 2021. Biomass, pigment production, and nutrient uptake of *Chlorella* sp. under different photoperiods. *Biodiversitas* 22 (12): 5344-5349. DOI: 10.13057/biodiv/d221215.
- Fattah A, Nuraeni S, Rauf AW. 2020. The vegetation model: An environmentally friendly farming system in the management of rice stem borer in South Sulawesi. *Adv Eng Res* 194: 30-34. DOI: 10.2991/aer.k.200325.007.
- Kalaivani M, Maruthi-Kalaiselvi M, Senthil-Nathan, S. 2021. Seed treatment and foliar application of methyl salicylate (MeSA) as a defense mechanism in rice plants against the pathogenic bacterium, *Xanthomonas oryzae* pv *oryzae*. *Pestic Biochem Physiol* 101: 115-126. DOI: 10.1016/j.pmpp.2017.07.001.
- Kalaivani K, Sengottayan SN. 2016. Effect of methyl salicylate (MeSA), an elicitor on growth, physiology, and pathology of resistant and susceptible rice varieties. *Sci Rep* 6: 34498. DOI: 10.1038/srep34498.
- Kavulych Y, Kobyletska M, Romanyuk N, Terek O. 2023. Stress-protective and regulatory properties of salicylic acid and prospects of its use in plant production. *Stud Biol* 17 (2): 173-200. DOI: 10.30970/sbi.1702.718
- Kim Y, Kim S, Shim LS. 2017. Exogenous salicylic acid alleviates salt-stress damage in cucumber under moderate nitrogen conditions by controlling endogenous salicylic acid levels. *Hortic Environ Biotechnol* 58 (3): 247-253. DOI: 10.1007/s13580-017-0111-7.
- Kowalska J, Krzyminska J, Tyburski J. 2022. Yeasts as a potential biological agent in plant disease protection and yield improvement—a short review. *Agriculture* 12: 1404. DOI: 10.3390/agriculture12091404.
- Kohli SK, Handa N, Kaur R, Kumar V, Khanna K, Bakshi P, Singh R, Arora S, Kaur R, Bhardwaj R. Role of Salicylic Acid in Heavy Metal Stress Tolerance: Insight into Underlying Mechanism. Springer, Singapore. DOI: 10.1007/978-981-10-6068-7-7.
- Latrianto A, Solichatun, Pitoyo A, Maylendra CT. 2022. The effect of salicylic acid and Benzyl Amino Purine (BAP) concentrations variation on the growth of protocorm *Dendrobium stocklebuschii* x

- Dendrobium calophyllum* orchids. Pros Sem Nas Masy Biodiv Indon 8 (1): 87-95. DOI: 10.13057/psnmbi/m080112. [Indonesian]
- Ling Y, Weilin Z. 2016. Genetic and biochemical mechanisms of rice resistance to planthopper. Plant Cell Rep 35 (8): 1559-1572. DOI: 10.1007/s00299-016-1962-6.
- Liang B, Wang H, Yang C, Wang L, Qi L, Guo Z, Chen X. 2022. Salicylic acid is required for broad-spectrum disease resistance in rice. Intl J Mol Sci 23 (3): 1354. DOI: 10.3390/ijms23031354.
- Lu HP, Luo T, Fu H, Wang L, Tan Y, Huang J, Wang Q, Ye GY, Getehouse AMR, Lou Y, Shu Q. 2018. Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis. Nat Plants 4: 338-344. DOI: 10.1038/s41477-018-0152-7.
- Norouzian R, Plonsky L. 2018. Eta- and partial eta-squared in L2 research: A cautionary review and guide to more appropriate usage. Second Lang Res 34 (2): 257-271. DOI: 10.1177/0267658316684904.
- Ochoa-Meza LC, Quintana-Obregón EA, Vargas-Arispuro I, Falcón-Rodríguez AB, Aispuro-Hernández E, Virgen-Ortiz JJ, Martínez-Téllez MA. 2021. Oligosaccharins as elicitors of defense responses in wheat. Polymers 13 (18): 3105. DOI: 10.3390/polym13183105.
- Pál M, Szalai G, Lantos E, Nagyéri G, Janda T. 2020. Comparative study of salicylic acid contents in young wheat and rice plants and their anticancer activities in HepG2 and Caco-2 cells. Biol Futura 71 (3): 265-271. DOI: 10.1007/s42977-020-00026-4.
- Paul A, Mriganko K, Pompei D, Bidisha H, Ayekpam R, Nilabh T, Sudeshna R. 2024. Role of salicylic acid in mitigating stress and improving productivity of crops: A review. J Adv Biol Biotechnol 27 (7): 1351-1361. DOI: 10.9734/jabb/2024/v27i71097.
- Poór P, Borbély P, Bódi N, Bagyánszki M, Tari I. 2019. Effects of salicylic acid on photosynthetic activity and chloroplast morphology under light and prolonged darkness. Photosynthetica 57 (2): 367-376. DOI: 10.32615/ps.2019.040.
- Priya, Dhingra M, Singh CB. 2022. Effect of salicylic acid on growth, physiological traits, yield and water productivity of summer mungbean (*Vigna radiata* L. Wilczek) under different irrigation regimes. Ann Plant Soil Res 24 (4): 564-570. DOI: 10.47815/apr.2022.10209.
- Shieh G. 2015. Sample size calculations for precise interval estimation of the eta-squared effect size. J Exp Educ 83 (2): 203-217. DOI: 10.1080/00220973.2014.907227.
- Simon EV, Hechanova SL, Hernandez JE, Charng-Pei Li, Tulek A, Ahn EK, Jairin J, Choi IR, Sundaram RM, Jena KK, Kim SR. 2023. Available cloned genes and markers for genetic improvement of biotic stress resistance in rice. Front Plant Sci 14: 1247014. DOI: 10.3389/fpls.2023.1247014.
- Singh S, Singh BB. 2015. In vitro evaluation of *Dichanthium annulatum* (Marvel grass) grass hay diets supplemented with browse foliage. Indian J Anim Sci 85: 1348-1353. DOI: 10.56093/ijans.v85i12.54395.
- Singh P, Yadav VK, Khan AH, Yadav RK. 2019. To study the response of salicylic acid on growth, physiological traits, yield and yield components of wheat varieties on timely and late sown conditions. Intl J Chem Stud 7 (3): 981-986.
- Sorahinobar M, Niknam V, Ebrahimzadeh H, Soltanloo H, Behmanesh M, Enferadi ST. 2016. Central role of salicylic acid in resistance of wheat against *Fusarium graminearum*. J Plant Growth Regul 35: 477-491. DOI: 10.1007/s00344-015-9554-1.
- Sriram M, Manonmani S, Gopalakrishnan C, Sheela V, Shanmugam A, Swamy KMR, Suresh R. 2024. Breeding for brown plant hopper resistance in rice: Recent updates and future perspective. Mol Biol Rep 51: 1038. DOI: 10.1007/s11033-024-09966-9.
- Sudika IW, Soemeinaboedhy IN, Sutresna IW. 2023. Genetic diversity and gain quantitative characters of maize from index-based selection at two dry lands in Lombok, Indonesia. Biodiversitas 24 (1): 11-19. DOI: 10.13057/biodiv/d240102.
- Sumardi, Fiani A. 2015. Genetic diversity of sandalwood (*Santalum album*) and reintroduction to East Nusa Tenggara. Pros Sem Nas Masy Biodiv Indon 1 (3): 409-412. DOI: 10.13057/psnmbi/m010304. [Indonesian]
- Tateda C, Zhang Z, Shrestha J, Jelenska J, Chinchilla D, Greenberg JT. 2014. Salicylic acid regulates *Arabidopsis* microbial pattern receptor kinase levels and signaling. Plant Cell 26 (10): 4171-4187. DOI: 10.1105/tpc.114.131938.
- Teh CY, Maziah M, Shaharuddin NA, Ho C. 2018. Efficient in vitro multiple shoots regeneration system from rice shoot apex in recalcitrant Malaysian Indica rice cultivars (*Oryza sativa* L.). Indian J Biotechnol 17: 504-513.
- Trost G, Vi SL, Czesnick H, Lange P, Holton N, Giavalisco P, Zipfel C, Kappel C, Lenhard M. 2014. Arabidopsis poly(A) polymerase PAPS1 limits founder-cell recruitment to organ primordia and suppresses the salicylic acid-independent immune response downstream of EDS1/PAD4. Plant J 77 (5): 688-699. DOI: 10.1111/tpj.12421.
- Torun H, Novak OR, Mikulík J, Strnad M, Ayaz FA. 2022. The effects of exogenous salicylic acid on endogenous phytohormone status in *Hordeum vulgare* L. under salt stress. Plants 11 (5): 618. DOI: 10.3390/plants11050618.
- Wang XD, Bi WS, Jing G, Yu XM, Wang HY, Liu DQ. 2018. Systemic acquired resistance, *NPR1*, and pathogenesis-related genes in wheat and barley. J Integr Agric 17 (11): 2468-2477. DOI: 10.1016/S2095-3119(17)61852-5.
- Wang S, Zhou Y, Luo W, Deng L, Yao S, Zeng K. 2020. Primary metabolites analysis of induced citrus fruit disease resistance upon treatment with oligochitosan, salicylic acid and *Pichia membranaefaciens*. Biol Control 148: 104289. DOI: 10.1016/j.biocontrol.2020.104289.