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Antioxidant profile of several local rice varieties from Central Java, Indonesia, under biotic stress conditions of bacterial leaf blight

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Abstract. *Cahyadi MA, Yunus A. 2025. Antioxidant profile of several local rice varieties from Central Java, Indonesia, under biotic stress conditions of bacterial leaf blight. Asian J Agric 9: 368-376.* Central Java harbors diverse local rice germplasm that requires preservation, yet their resistance to bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) remains underexplored. Antioxidant activity has been linked to BLB resistance, indicating its potential as a screening tool for identifying tolerant varieties in breeding programs. This study examined four local rice varieties inoculated with Xoo, focusing on catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), total flavonoids, and total phenolic compounds. Based on lesion length, Mentik Susu (MS) and Rojolele (RL) were resistant, while Merah Segreng (MSeg) and Hitam Mutiara (HM) showed moderate resistance. At 5 days post-inoculation (dpi), the highest CAT activity (0.019 units/mg protein) occurred in MSeg, while at 20 dpi HM showed the highest (0.014 units/mg protein). For POD, RL exhibited the highest activity (0.271 units/mL protein) at 5 dpi, and MSeg at 20 dpi (0.436 units/mL protein). The highest SOD activity at 5 dpi was observed in HM (169.35 units/mg protein), whereas MS peaked at 20 dpi (157.95 units/mg protein). These enzymatic antioxidant levels did not differ significantly among varieties, but non-enzymatic antioxidants—flavonoids and phenolics—showed significant variation, with MS and RL containing 44.30 and 27.09% more flavonoids and 25.88 and 18.91% more phenolics, respectively, than moderately resistant varieties. These findings indicate that non-enzymatic antioxidants contribute more prominently to BLB resistance than enzymatic ones, and that varieties rich in flavonoids and phenolic compounds, such as Mentik Susu and Rojolele, have stronger resistance, highlighting their potential for breeding programs to enhance tolerance against bacterial leaf blight while supporting the conservation and utilization of Central Java's local rice diversity.

Keywords: Antioxidant profile, bacterial leaf blight, biotic stress, local varieties

INTRODUCTION

Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is one of the most serious threats to rice production globally. In Indonesia, BLB leads to yield losses ranging from 21-36% during the rainy season and 18-28% in the dry season (Suparyono et al. 2004). This disease poses a major challenge in Central Java, one of the country's main rice-producing provinces, where stable rice production is essential to meet national food demands (Mistiyah et al. 2018). Consequently, breeding rice varieties with BLB resistance has become a priority to ensure sustainable rice production.

Plants have evolved complex defense mechanisms against biotic stresses, including BLB. One of the earliest and most significant responses to pathogen attack is the production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radicals (-OH), and singlet oxygen ($^1O_2$). When maintained at controlled concentrations, reactive oxygen species (ROS) function as signaling molecules and are also involved in various pathways that contribute to pathogen suppression (Waszczak et al. 2018; Huihui et al. 2020; Sahu et al. 2022). While ROS act as signaling molecules in defense pathways, their excessive accumulation can damage cellular components, including lipids, proteins, and nucleic acids (Choudhury et al. 2017; Maurya 2020). Therefore, plants rely on a tightly regulated antioxidant system to detoxify ROS and mitigate oxidative stress.

The plant antioxidant defense system consists of enzymatic and non-enzymatic components. Key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), play crucial roles in ROS scavenging and redox homeostasis. Several studies have highlighted the importance of these enzymes in enhancing disease resistance. For example, Ramzan et al. (2021) demonstrated that chili (*Capsicum annuum* L.) varieties with high resistance to *Xanthomonas campestris* pv. *vesicatoria* showed elevated SOD, CAT, and POD activities compared to susceptible varieties. In another study, Yuan et al. (2025) reported that melatonin treatment in rice plants infected with *Magnaporthe oryzae*, the rice blast-causing microbe, significantly enhanced the activity of antioxidant enzymes. Specifically, SOD activity increased by up to 24.89%, CAT by up to 94.99%, and POD also showed a significant rise. These effects triggered a cascade of favorable physiological responses, including a significant reduction in hydrogen peroxide (H_2O_2) levels, a decrease in malondialdehyde (MDA) content by up to 18.65%, indicating reduced membrane damage, and an increase in soluble protein content, which functions as both an osmoregulator and cellular protector. Moreover, the data demonstrated that the treatment group with elevated antioxidant enzyme levels exhibited a 31.58% lower disease severity compared to the control group, in which antioxidant enzyme activity remained comparatively low. This evidence clearly demonstrates the critical role of antioxidant enzymes in mitigating pathogen-induced damage.

Beyond enzymatic defenses, non-enzymatic antioxidants such as flavonoids and phenolic compounds are critical in plant defense. These compounds function as ROS scavengers while also contributing to pathogen inhibition and cell wall strengthening. Jan et al. (2021) demonstrated that a mutant rice line with enhanced flavonoid biosynthesis exhibited reduced ROS accumulation and improved BLB resistance compared to its susceptible parental lines. Similarly, Kumar et al. (2013) reported that resistant rice varieties displayed a sharp increase in phenolic and flavonoid levels during the early stages of Xoo infection, indicating a protective role against pathogen invasion.

Central Java possesses diverse local rice germplasm, including Rojolele (Rezauji et al. 2023), Black Rice Jelitheng from Karanganyar, Red Rice from Karanganyar (Awanis et al. 2022), and Mentik Susu (Hanifah et al. 2020). These traditional varieties are recognized for tolerance to abiotic stresses and unique grain qualities, yet their resistance to BLB and related antioxidant responses remain underexplored. Their genetic diversity suggests potential as sources of resistance traits that can be harnessed in breeding programs.

Although antioxidant responses have been studied in modern and transgenic rice cultivars, little attention has been given to traditional varieties from Central Java under BLB stress. Moreover, the combined investigation of enzymatic antioxidants (SOD, CAT, POD) alongside non-enzymatic compounds (flavonoids, phenolics) has not been systematically undertaken.

This study addresses these gaps by evaluating the physiological responses of four traditional rice varieties from Central Java after Xoo infection, focusing on antioxidant enzyme activities and flavonoid and phenolic accumulation. By linking biochemical defense traits with resistance levels, this first integrated assessment provides new insights into the defensive capacity of local germplasm and identifies promising varieties for breeding BLB-resistant rice with strong antioxidant defenses.

MATERIALS AND METHODS

Study area

This study was conducted in a plastic greenhouse at the Agricultural Laboratory of Universitas Sebelas Maret, located in Jumantono, Karanganyar, Central Java, Indonesia. The research was carried out from November 1 to December 20, 2024. The local rice seeds used in this study were obtained from the Surakarta region. The experiment was arranged using a Completely Randomized Design (CRD) with four local rice varieties and three replications. The tested varieties were Mentik Susu (MS), Rojolele (RL), Hitam Mutiara (HM), and Merah Segreng (MSeg). All varieties were inoculated with the pathogenic bacterium Xoo. The observed variables included the activity levels of antioxidant enzymes, namely peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), as well as the concentrations of total flavonoids and phenolic compounds. Leaf samples for the analysis of antioxidant enzyme activities (POD, SOD, and CAT) were collected by selecting one infected leaf showing disease symptoms from each variety. Analyses were conducted in three biological replicates using leaves from the same inoculated varieties. For the analysis of total flavonoid and phenolic content, two infected leaves were collected from each variety.

Procedures

Inoculation of Xanthomonas oryzae *pv.* oryzae *on rice leaf*

The inoculation of Xoo followed the leaf clipping method, in which sterile scissors were dipped into the bacterial suspension for a few seconds before being used to cut approximately 2 cm of the leaf tip (Djatmiko et al. 2011). Biotic stress is confirmed when inoculated rice leaves exhibit symptoms of leaf blight, characterized by elongated necrotic lesions along the leaf veins. The length of these lesions is subsequently measured to assess disease severity and to determine the resistance status of each genotype against bacterial leaf blight.

Antioxidant enzymes assay (CAT, POD, SOD)

Leaf samples for antioxidant enzyme assays were collected twice, at 5 and 20 days after inoculation. Enzyme extracts were prepared by homogenizing fresh leaf biomass in an extraction buffer containing sodium phosphate buffer (pH 7.8), 0.1 mM EDTA, 1% (x/v) polyvinylpyrrolidone (PVP), and 0.5% (v/v) Triton X-100. The crude protein concentration in the supernatant was estimated using the Bradford method (Bradford 1976) with BSA as the standard.

Catalase (CAT) activity was determined following the method of Aebi (1974) with slight modifications. The enzyme extract was mixed with phosphate buffer and hydrogen peroxide (H_2O_2), and the decrease in absorbance was measured at 240 nm over a three-minute period. One unit of catalase activity was defined as the amount of enzyme required to oxidize 1 mM of H_2O_2 per minute. The enzyme activity was calculated using the following formula:

CAT activity (unit/mg protein) = $\Delta A_{240} \times (V/Vt) / (0.1 \times t) / Cp$

Where: ΔA_{240}: Change in absorbance at 240 nm, measured every 15 seconds, V: Total volume of the crude enzyme solution, Vt: Volume of crude enzyme extract used in the test tube, T: Reaction time in minutes, Cp: Crude protein concentration in mg/mL, 0.1: Constant representing the decrease in absorbance at 240 nm caused by one unit of catalase activity.

Superoxide dismutase (SOD) activity was determined using the method of Beauchamp and Fridovich (1971) with slight modifications. The enzyme extract was mixed with a reaction solution containing phosphate buffer (pH 7.8), L-methionine, $EDTA_2Na$, riboflavin, and nitro blue tetrazolium (NBT). Control samples were prepared under two conditions: Control I (dark conditions) and Control II (light conditions at 4000 lux). The absorbance change was measured at 560 nm, and one unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of NBT reduction. The enzyme activity was calculated using the following formula:

SOD total activity (unit/mg protein) = [(Ack - As) × V] / (0.5 × Ack × Vt) / Cp

Where: Ack: Absorbance at 560 nm from Control II (reaction exposed to light without crude enzyme), As: Absorbance at 560 nm from the sample test tube, V: Total volume of the crude enzyme solution, Vt: Volume of crude enzyme extract used in the test tube, Cp: Crude protein concentration in mg/mL, 0.5: Constant representing one unit of SOD activity, defined as the amount of enzyme required to inhibit 50% of nitro blue tetrazolium (NBT) photoreduction.

The peroxidase (POD) activity assay was performed using a 3.00 mL reaction mixture containing final reagent concentrations of 14 mM potassium phosphate, 0.5027% (v/v) hydrogen peroxide, 0.5% (w/v) pyrogallol, and 0.45-0.75 units of peroxidase. To initiate the reaction, the reagents were mixed and incubated at 20°C for approximately 10 minutes in a temperature-controlled spectrophotometer. Since the maximum reaction rate occurred within the first minute, absorbance measurements at 420 nm (A_{420}) were recorded for each cuvette one at a time. The reaction rate (DA_{420}/20 seconds) was determined using the maximum linear rate or 0.5 minute interval for all test and blank samples. The assay was deemed valid if the reaction rate fell within the range of 0.18-0.34; if the values were outside this range, adjustments to the enzyme concentration were made accordingly. It is important to note that while all cuvettes can be incubated simultaneously, only one cuvette should be measured at a time to ensure accuracy in the results. The enzyme activity was subsequently calculated using the following formula:

Units/mL enzyme = [(ΔA_{420} / 20 seconds test sample - ΔA_{420} / 20 seconds blank) × 3 × df] / (12 × 0.1)

Where: 3.00: Volume (in milliliters) of the assay reaction, Df: Dilution factor, 12.0: Extinction coefficient of 1 mg/mL Purpurogallin at 420 nm, as determined internally, 0.1: Volume (in milliliters) of the enzyme used in the reaction.

Total flavonoid content analysis procedure using spectrophotometry method

To determine the total flavonoid content, 5 g of the sample were weighed and placed into a 100 mL Erlenmeyer flask. The sample was then diluted with 96% ethanol using a volumetric flask until the solution reached the calibration mark. After preparation, the solution was filtered through filter paper to obtain a clear filtrate. From this filtrate, 1 mL was transferred into a test tube, followed by the addition of 2 mL of a 5% aluminum chloride ($AlCl_3$) solution and 7 mL of 96% ethanol. The mixture was vortexed until it became homogeneous. The absorbance of the resulting solution was then measured using a spectrophotometer at a wavelength of 415 nm. The absorbance data were recorded, and the total flavonoid content was calculated by referencing a quercetin standard curve, which had been prepared in advance for comparison (Suryanto 2007).

Phenol content analysis procedure using spectrophotometry method

To analyze the phenol content, 5 g of the ground sample were weighed and placed into a 100 mL Erlenmeyer flask. The sample was then diluted with distilled water (aquadest) to a final volume of 100 mL using a volumetric flask. After dilution, the solution was either filtered or centrifuged until a clear filtrate was obtained. From the clear filtrate, 1 mL was transferred into a test tube, to which 0.5 mL of Folin-Denis reagent (prepared at a 1:1 ratio) was added, followed by 1 mL of saturated sodium carbonate (Na_2CO_3) solution. The mixture was left to stand for 10 minutes to allow the reaction to occur. Subsequently, distilled water was added to bring the total volume to 10 mL, and the solution was vortexed until homogeneous. The absorbance of the resulting solution was measured using a spectrophotometer at a wavelength of 730 nm. The absorbance data were recorded, and the phenol content was calculated by referencing a gallic acid standard curve, which had been prepared in advance for comparison (Harborne 1987).

Lesion length measurement

The lesion lengths were measured 20 d after inoculation. Lesions were measured from each test plant. Lesion length measurements ≤ 5 cm were scored as resistant (R), 6-10 cm as moderately resistant (MR), 11-14 cm as moderately susceptible (MS) and ≥ 15 cm as susceptible (S) (Olivia et al. 2019). Three leaves were taken from each plant and then measured using a ruler, the measurement results were then averaged to determine the genotype categorization.

Data analysis

The data that has been collected is then analyzed using one way ANOVA then if the significance shows a value of < 0.05 then it is tested further using the Duncan Multiple Range Test (DMRT). Meanwhile, for the average phenol content, we analyzed the data descriptively.

RESULTS AND DISCUSSION

Disease severity

The disease severity variable is expressed in units of lesion length (cm) as a measure of symptoms caused by the pathogen. Based on the measurement of lesion length formed on the leaves, it was found that the four tested varieties exhibited resistance levels ranging from moderately resistant to resistant against bacterial leaf blight. In this study, the Merah Segreng and Hitam Mutiara varieties were classified as moderately resistant (MR) to Xoo infection. Meanwhile, the Mentik Susu and Rojolele varieties were categorized as resistant (R). Merah Segreng and Hitam Mutiara showed average lesion lengths of 9 and 9.33 cm, respectively, as shown in Table 1. While Mentik Susu and Rojolele had average lesion lengths of 5.66 and 5 cm, respectively.

The percentage comparison of lesion length between resistant and moderately resistant varieties showed that lesion lengths in Mentik Susu were 37.11% shorter than in Merah Segreng and 39.33% shorter than in Hitam Mutiara. Meanwhile, Rojolele showed lesion lengths that were 44.44% shorter than Merah Segreng and 46.39% shorter than Hitam Mutiara. These results support the classification of Mentik Susu and Rojolele as resistant (R) varieties. Figure 1 shows the phenotypic appearance of lesion length in each tested genotype.

Enzymatic antioxidant activity

ANOVA

The ANOVA results presented in Tables 2 and 3 show that, at both 5 and 20 days post-inoculation (dpi), all variables except for flavonoid content were not significantly different at the 5% significance level. This indicates that the four tested varieties did not exhibit significant differences in the levels of CAT, POD, and SOD enzymes at either sampling time. In contrast, a notable difference was observed among the varieties in terms of flavonoid content.

Catalase

Based on the catalase enzyme activity test conducted at 5 and 20 days after inoculation (dpi), a decrease in catalase enzyme concentration was observed between these two time points in all tested varieties. The highest catalase enzyme concentration at 5 dpi, as shown in Figure 2, was found in the Merah Segreng variety followed by the Rojolele variety which were categorized as moderately resistant and resistant varieties respectively in this study. It was observed that at 5 dpi, the catalase level in Rojolele (resistant) was higher than in Hitam Mutiara (moderately resistant), with Rojolele having 28.57% more catalase enzyme compared to Hitam Mutiara. While at 20 dpi the concentrations of all varieties showed similar values except Rojolele which was recorded as the lowest among the tested varieties. The DMRT test at a 95% confidence level showed no significant difference among the tested varieties in terms of catalase concentration at the two sampling times as indicated in Table 4.

Peroxidase (POD)

The peroxidase enzyme activity test on day 5 showed that the highest peroxidase level was recorded in the Rojolele variety (resistant), followed by Mentik Susu (resistant) as shown in Figure 3. By day 20 post-inoculation, dynamic changes in peroxidase concentration were observed, with the Hitam Mutiara (moderately resistant) and Merah Segreng (moderately resistant) varieties experiencing an increase in peroxidase concentration, while the Mentik Susu (resistant) and Rojolele (resistant) varieties showed a decline.

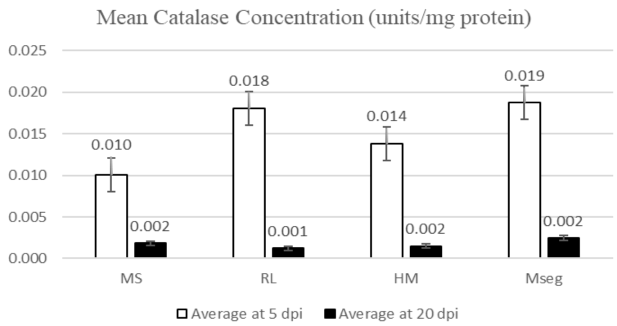

Figure 2. Graph of catalase concentration at 5 dpi and 20 dpi. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

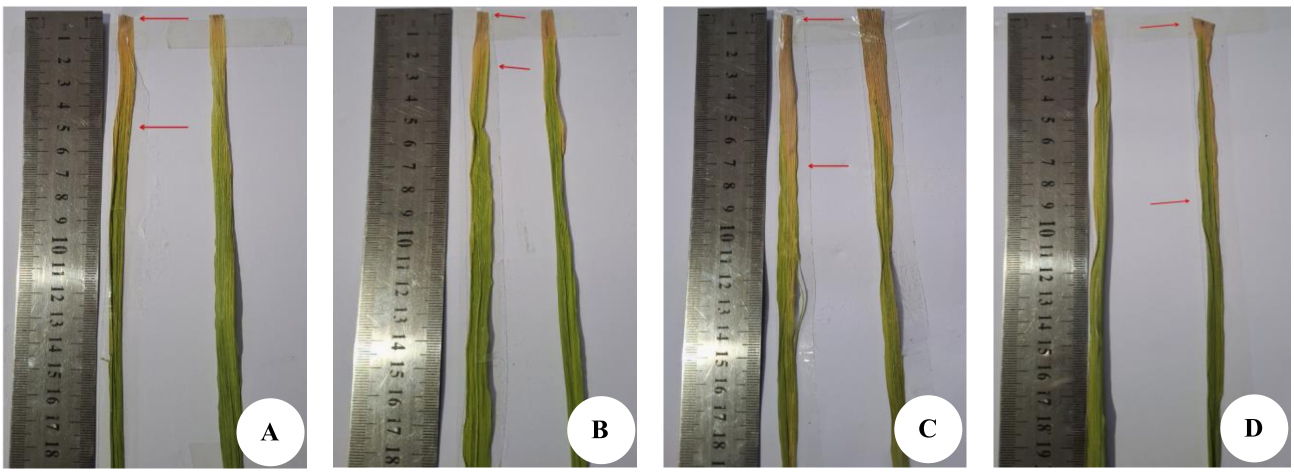

Figure 1. The effect of Xoo inoculation on the leaf blades of four rice genotypes. A. Mentik Susu, B. Rojolele, C. Hitam Mutiara, D. Merah Segreng on 15th day after inoculation. Rice plants were inoculated with Xoo isolate by leaf clipping method. The red arrows indicate the upper and lower ends of the lesion

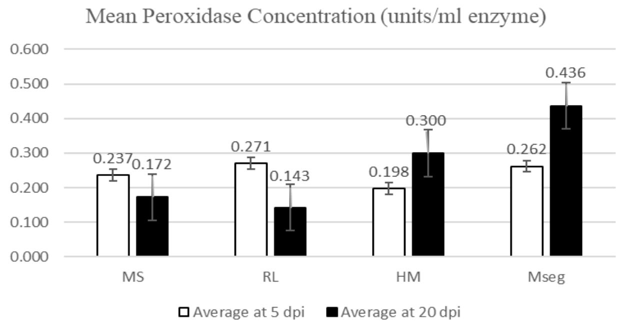

Figure 3. Graph of peroxidase concentration at 5 dpi and 20 dpi. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

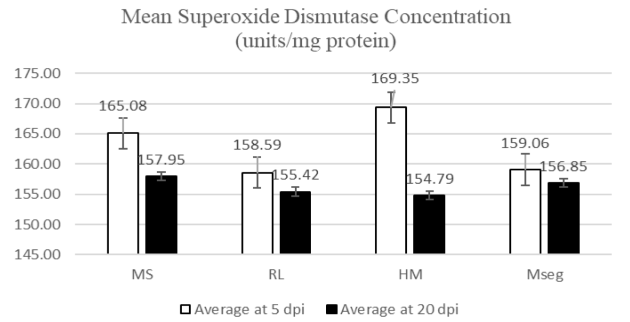

Figure 4. Graph of superoxide dismutase concentration at 5 dpi and 20 dpi. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 1. Length of leaf lesion formed

Varieties	Lesion length (cm)	Status
HM	9.33	Moderately resistant
RL	5	Resistant
MSeg	9	Moderately resistant
MS	5.6	Resistant

Note: HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 2. ANOVA test of quantitative variables at 5 dpi

Variabel	df1	df2	F Value	α = 5%	
				F Tabel	Signifikansi
CAT	3	8	0.586	4.07	ns
POD	4	10	0.222	4.07	ns
SOD	4	10	0.329	4.07	ns
FLA	4	10	4331.8	4.07	*

Note: CAT: Catalase concentration, POD: Peroxidase concentration, SOD: Superoxide dismutase concentration, FLA: Flavonoid concentration

Table 3. ANOVA test of quantitative variables at 20 dpi

Variabel	df1	df2	F Value	α = 5%	
				F Tabel	Signifikansi
CAT	4	10	1.233	4.07	ns
POD	4	10	0.736	4.07	ns
SOD	4	10	0.074	4.07	ns
FLA	4	10	4655.9	4.07	*

Note: CAT: Catalase concentration, POD: Peroxidase concentration, SOD: Superoxide dismutase concentration, FLA: Flavonoid concentration

Table 5. Standard deviation of catalase for each variety at 5 dpi and 20 dpi

Varieties	5 dpi	20 dpi
HM	0.011	0.007
RL	0.012	0.004
MSeg	0.006	0.012
MS	0.005	0.011

Note: HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 4. DMRT table of catalase concentrations at 5 and 20 days post-inoculation (dpi)

Varieties	5 dpi (units/mg protein)	20 dpi (units/mg protein)
HM	0.014a	0.002a
RL	0.018a	0.001a
MSeg	0.019a	0.002a
MS	0.010a	0.002a

Note: The average value of treatments followed by the same letter is stated as not significantly different according to the DMRT test at a significance level of $p<0.05$. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 6. DMRT table of peroxidase concentrations at 5 and 20 days post-inoculation (dpi)

Varieties	5 dpi (units/ml enzyme)	20 dpi (units/ml enzyme)
HM	0.198a	0.300a
RL	0.271a	0.143a
MSeg	0.262a	0.436a
MS	0.237a	0.172a

Note: The average value of treatments followed by the same letter is stated as not significantly different according to the DMRT test at a significance level of $p<0.05$. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 7. Standard deviation of peroxidase for each variety at 5 dpi and 20 dpi

Varieties	5 dpi	20 dpi
HM	0.11	0.1
RL	0.05	0.24
MSeg	0.15	0.38
MS	0.14	0.29

HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Observations at 5 dpi showed that resistant varieties such as Rojolele had higher peroxidase levels compared to Hitam Mutiara, with the resistant Rojolele variety exhibiting 36.87% more peroxidase. Meanwhile, according to the peroxidase enzyme activity test on day 20, the Merah Segreng variety had the highest peroxidase concentration, followed by Hitam Mutiara. However, the observed differences in peroxidase levels among the varieties were not statistically significant, as indicated by the DMRT test at the 95% confidence level in Table 6. This suggests that peroxidase concentrations among the tested varieties did not differ substantially at each sampling time.

Superoxide dismutase (SOD)

The superoxide dismutase (SOD) enzyme activity test on days 5 and 20 showed a decrease in SOD levels between these two time points. On day 5, the highest SOD concentration was found in the Hitam Mutiara variety (moderately resistant), while on day 20, the highest SOD concentration was recorded in the Mentik Susu variety (resistant), as shown in Figure 4. Based on the calculations, Mentik Susu (resistant) contained approximately 2.04% more superoxide dismutase enzyme than Hitam Mutiara (moderately resistant). A DMRT test, as shown in Table 8, indicated no significant differences in SOD concentrations among the tested varieties at either sampling time, with a 95% confidence level.

Non-enzymatic antioxidant activity

Total flavonoid

The total flavonoid content test showed a decrease in total flavonoid levels from day 5 to day 20. The highest total flavonoid content on both days was recorded in the Mentik Susu variety (resistant). Figure 5 shows that on day 5, the second highest flavonoid content was found in the Merah Segreng variety (moderately resistant), while on day 20, it was in the Rojolele variety (resistant).

Sampling at 5 days post-inoculation (dpi) showed that the Mentik Susu (resistant) and Merah Segreng (moderately resistant) varieties ranked first and second in total flavonoid concentration, with significantly higher levels than the other varieties. On day 20, the Mentik Susu (resistant) and Rojolele (resistant) varieties held the top two positions, with MS having a significantly higher flavonoid content than RL. At this observation point, Mentik Susu and Rojolele, both classified as resistant varieties, were found to contain 44.30 and 27.09% more flavonoids, respectively, compared to Merah Segreng (moderately resistant). Additionally, both Mentik Susu (resistant) and Rojolele (resistant) had significantly higher flavonoid concentrations than the other tested varieties, as determined by Duncan's test at the 95% confidence level in Table 10.

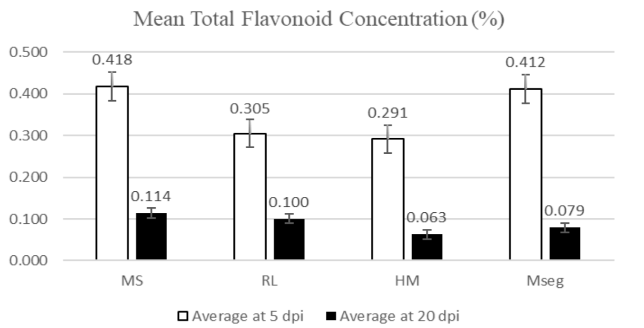

Figure 5. Graph of flavonoid concentration at 5 dpi and 20 dpi. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

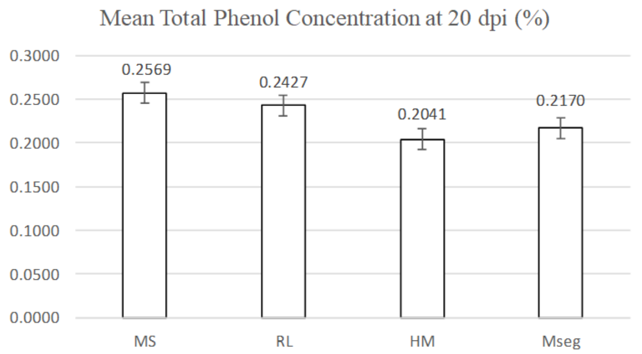

Figure 6. Graph of phenol concentration at 20 dpi. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 8. DMRT table of superoxide dismutase concentrations at 5 and 20 days post-inoculation (dpi)

Varieties	5 dpi (Units/mg protein)	20 dpi (Units/mg protein)
HM	169.35a	154.79a
RL	158.59a	155.42a
MSeg	159.06a	156.85a
MS	165.08a	157.95a

Note: The average value of treatments followed by the same letter is stated as not significantly different according to the DMRT test at a significance level of $p<0.05$. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 9. Standard deviation of superoxide dismutase for each variety at 5 dpi and 20 dpi

Varieties	5 dpi	20 dpi
HM	16.63	12.82
RL	23.03	5.4
MSeg	10.57	7.72
MS	7.001	8.8

Note: HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 10. DMRT table of flavonoid concentrations at 5 and 20 days post-inoculation

Varieties	5 days post-inoculation (%)	20 days post-inoculation (%)
HM	0.291a	0.0629a
RL	0.305b	0.1004c
MSeg	0.412c	0.0793b
MS	0.418d	0.1142d

Note: The average value of treatments followed by the same letter is stated as not significantly different according to the DMRT test at a significance level of $p<0.05$. HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Table 11. Standard deviation of flavonoid for each variety at 5 dpi and 20 dpi

Varieties	5 dpi	20 dpi
HM	0.0003	0.0006
RL	0.001	0.0006
MSeg	0.003	0.0006
MS	0.009	0.0006

Note: HM: Hitam Mutiara, RL: Rojolele, MSeg: Merah Segreng, MS: Mentik Susu

Total phenol

We also tested phenol levels on day 20, and the results in Figure 6 showed that, along with having the highest total flavonoid content, the Mentik Susu variety (resistant) also recorded the highest phenol concentration compared to the other varieties, followed by the Rojolele variety (resistant). These two varieties had total phenol levels that were 25.88 and 18.91% higher, respectively, than Hitam Mutiara (moderately resistant). This trend closely mirrored the total flavonoid content results observed on day 20. Additionally, both varieties exhibited a resistant phenotype against bacterial leaf blight.

Discussion

Observations of lesion lengths on the leaves of each variety revealed that two varieties, Rojolele (RL) and Mentik Susu (MS), were identified as resistant, with lesion lengths ranging from 0 to 5 cm. The remaining two varieties, Hitam Mutiara (HM) and Merah Segreng (MSeg), were classified as moderately resistant. All varieties examined in this study are local cultivars originating from Central Java. To further explore the underlying mechanisms of resistance, we correlated the resistance status of these genotypes with their antioxidant profiles, focusing on the activity of both enzymatic and non-enzymatic antioxidant compounds in resistant and moderately resistant genotypes.

The analysis of antioxidant enzyme activity across all tested varieties revealed a decreasing trend in concentration from day 5 to day 20 post-inoculation. This decline in antioxidant levels suggests that antioxidants actively scavenge oxidative radical molecules circulating throughout the plant, as stated by Kumar et al. (2013) in their study on antioxidant levels in biotically stressed rice plants. Their findings indicated a decrease in flavonoid levels from the early infection phase to the later stage, demonstrating the protective effect of antioxidants during periods of stress. This study also revealed that in the early stages of infection, antioxidant enzyme concentrations tend to be higher and subsequently decline as the plant progresses into the later stages of infection.

Superoxide dismutase (SOD) is the primary scavenger and the most effective intracellular antioxidant enzyme in aerobic organisms. Its main function is to catalyze the dismutation of the superoxide anion radical ($-O_2$) into oxygen (O_2) and hydrogen peroxide (H_2O_2) (Syman et al. 2024). This study revealed that the highest SOD levels at 5 days post-inoculation (dpi) were found in the moderately resistant Hitam Mutiara variety, while at 20 dpi, the highest SOD levels were observed in the resistant Mentik Susu variety.

Catalase (CAT) and peroxidase (POD) are antioxidant enzymes that scavenge superoxide radicals and H_2O_2, which are byproducts of SOD metabolism (Kumar et al. 2013). In this study, the resistant variety Rojolele and the moderately resistant Merah Segreng exhibited the highest catalase concentrations among all tested varieties at 5 days post-inoculation (dpi). According to Debona et al. (2012), leaves of resistant wheat varieties infected with *Pyricularia oryzae* Cavara tend to produce higher levels of CAT compared to more susceptible varieties. Similarly, Gujjar et al. (2025) investigated gene expression dynamics and catalase enzyme levels in sugarcane infected with *Colletotrichum falcatum* and *Fusarium sacchari.* Their study demonstrated that resistant varieties showed increased expression of catalase-encoding genes, resulting in higher catalase levels and a greater diversity of catalase isoforms compared to susceptible varieties. Catalase plays a key role in neutralizing hydrogen peroxide (H_2O_2), a major form of reactive oxygen species (ROS) produced during pathogen infection, thereby maintaining cellular homeostasis, preventing oxidative damage, and modulating stress signaling pathways that activate plant defense mechanisms.

However, there was a slight discrepancy in our study, where leaves of the moderately resistant Merah Segreng variety produced more catalase than the resistant Rojolele variety. Nonetheless, the difference in catalase levels between these two genotypes was not statistically significant, as indicated by the DMRT test. Meanwhile, at 5 dpi, the highest peroxidase concentration was recorded in the resistant variety Rojolele. However, by 20 dpi, the moderately resistant variety Merah Segreng exhibited the highest peroxidase levels, although the difference was not significant compared to other resistant varieties such as Rojolele. This study shares similarities with Ramzan et al. (2021), who observed comparable antioxidant enzyme behavior across different chili pepper varieties with varying resistance levels during temporal inoculation phases.

The absence of significant differences in antioxidant enzyme levels between resistant and moderately resistant varieties suggests that genotype resistance status is not solely determined by the concentration of antioxidant enzymes. Multiple factors contribute to genotype resistance against biotic stress, including hypersensitive responses (De Gara et al. 2003), phytohormones such as salicylic acid, jasmonic acid, ethylene, and abscisic acid (Yu et al. 2018; Zechmann 2020), melatonin and related metabolites (Yu et al. 2018), interactions with rhizobacteria (Hashem et al. 2019), ascorbate and redox interactions (Singh et al. 2024), as well as the role of flavonoids and phenolics (Chowdhary et al. 2022; Ramaroson et al. 2022).

The flavonoid content analysis at 5 days post-inoculation (dpi) showed that the resistant variety Mentik Susu had the highest total accumulated flavonoid levels compared to other varieties. Similarly, at 20 dpi, although flavonoid levels had drastically decreased, the two resistant varieties, Mentik Susu and Rojolele, consistently exhibited higher total flavonoid levels than the other varieties. The decrease in flavonoid antioxidant levels observed across the four varieties at two different sampling points suggests that flavonoids are actively metabolized as the disease progresses. This metabolic activity contributes to the plant's protective response against the harmful effects of pathogen attacks, particularly by detoxifying excess reactive oxygen species (ROS). This finding is consistent with the study by Ming et al. (2021), who inoculated banana plants with the pathogenic fungus *Fusarium oxysporum* f. sp. *cubense* to examine the dynamics of antioxidant compound levels. Their results showed that compounds such as quercetin, kaempferol-rhamnose-hexose, and rutin initially increased during the early stages of infection but declined as the disease progressed. This indicates that these flavonoid compounds were utilized in scavenging ROS generated by biotic stress due to fungal infection.

Although flavonoid levels declined overall, the decrease in resistant varieties was less pronounced than in moderately resistant ones, suggesting that resistant genotypes are better able to maintain more stable antioxidant levels. This observation aligns with findings by Toan et al. (2017), who reported that phenolic antioxidant levels in rice plants infected with the rice blast fungus (*Pyricularia grisea* Cooke ex Sacc.) decreased in all tested varieties. However, the reduction was significantly less severe in resistant varieties, which maintained the highest phenolic content. These results demonstrate a clear correlation between the stability of antioxidant levels following infection and the genotype's resistance status to pathogen attacks. Flavonoids play important protective roles in plants experiencing biotic stress. These compounds serve as essential antioxidants that help control the buildup of reactive oxygen species (ROS) during pathogen attacks. Their hydroxyl functional groups are capable of donating electrons via resonance, allowing them to neutralize free radicals and participate in redox processes. In addition to their antioxidant activity, many flavonoids, along with resveratrol and other phenolic substances, also act as phytoalexins, which are antimicrobial agents that exhibit toxicity against various pathogenic fungi and bacteria (Dias et al. 2021).

Statistical analysis confirmed that the flavonoid content differences in these two resistant varieties were significant when compared to other varieties, suggesting that flavonoid levels may be a contributing factor in determining their resistance to bacterial leaf blight. This corresponds with the total phenolic content at 20 dpi, which also showed that the two resistant varieties, Mentik Susu and Rojolele, had the highest total phenolic accumulation compared to other varieties. These findings align with the study by Jan et al. (2021), which stated that mutant genotypes producing higher levels of flavonoids, particularly kaempferol and quercetin, tended to mitigate pathogen-induced stress better and exhibited smaller lesion lengths compared to wild-type genotypes.

Moving forward, further confirmation of these findings is necessary to confirm the validity of the resistance status of the tested genotypes. This is because the factors influencing genotype resistance to biotic stress may differ between greenhouse and field conditions. Field studies and the collection of infected leaf samples from uncontrolled environments are crucial, as rice cultivation typically occurs in open fields. Conducting field research will provide more informative and validating data that closely reflect real-world cultivation environments to accurately determine genotype resistance status against Xoo, the causal agent of bacterial leaf blight.

The study identified two local rice varieties, Mentik Susu (MS) and Rojolele (RL), as resistant to bacterial leaf blight caused by Xoo, while Merah Segreng (MSeg) and Hitam Mutiara (HM) were classified as moderately resistant based on lesion length observations. Although the enzymatic antioxidant activity (CAT, POD, and SOD) showed no significant differences between resistant and moderately resistant varieties, other factors, such as non-enzymatic antioxidants, may contribute to resistance. Notably, resistant varieties, particularly Mentik Susu, exhibited the highest total flavonoid and phenolic compound levels at 20 dpi, demonstrating its ability to maintain stable flavonoid and phenolic levels under the stress of BLB infection. This reinforces the statement regarding the role of these compounds in plant defense against biotic stress, as previously reported in related studies.

These findings further emphasize that Mentik Susu (MS) and Rojolele (RL) possess strong potential as resistant cultivars that can be recommended for cultivation in areas endemic to bacterial leaf blight. Additionally, the elevated antioxidant activity, particularly of flavonoids and phenolic compounds, supports the plant's defense mechanisms through effective detoxification of reactive oxygen species (ROS), providing a strong foundation for sustainable disease management strategies. This research holds practical significance for the selection of environmentally friendly rice varieties that can reduce reliance on chemical pesticides, while also offering valuable prospects for future breeding programs focused on enhancing natural plant resistance.

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REFERENCES

Awanis S, Purwanto E, Rahayu M. 2022. The potential of Central Java local black rice and red rice as drought tolerant cultivars. E3S Web Conf 361: 04007. DOI: 10.1051/e3sconf/202236104007.

Aebi H. 1974. Catalase in Methods of Enzymatic Analysis, Second Eng, 2. Verlag Chemie Weinheim Academic Press, Inc New York and London A Subsidiary of Harcourt Brace Jovanovich, Publishers, pp. 673-679.

Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44: 276-287.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254. DOI: 10.1016/0003-2697(76)90527-3.

Choudhury FK, Rivero RM, Blumwald E, Mittler R. 2017. Reactive oxygen species, abiotic stress and stress combination. Plant J 90 (5): 856-867. DOI: 10.1111/tpj.13299.

Chowdhary V, Alooparampil S, Pandya RV, Tank JG. 2022. Physiological Function of Phenolic Compounds in Plant Defense System. Phenolic Compounds: Chemistry, Synthesis, Diversity, Non-Conventional Industrial, Pharmaceutical and Therapeutic Applications. IntechOpen. DOI: 10.5772/intechopen.101131.

De Gara L, de Pinto MC, Tommasi F. 2003. The antioxidant systems vis-à-vis reactive oxygen species during plant-pathogen interaction. Plant Physiol Biochem 41 (10): 863-870. DOI: 10.1016/S0981-9428(03)00135-9.

Debona D, Rodrigues FÁ, Rios JA, Nascimento KJ. 2012. Biochemical changes in the leaves of wheat plants infected by *Pyricularia oryzae*. Phytopathology 102 (12): 1121-1129. DOI: 10.1094/PHYTO-06-12-0125-R.

Dias MC, Pinto DCGA, Silva AMS. 2021. Plant flavonoids: Chemical characteristics and biological activity. Molecules 26 (17): 5377. DOI: 10.3390/molecules26175377.

Djatmiko HA, Prakoso B, Prihatiningsih N. 2011. Penentuan patotipe dan keragaman genetik *Xanthomonas oryzae* pv. *oryzae* pada tanaman padi di wilayah Keresidenan Banyumas. J HPT Tropika 11: 35-46. [Indonesian]

Gujjar RS, Kumar R, Goswami SK, Kumar S, Chinnaswamy A, Manimekalai R, Ramanathan V. 2025. Catalases are induced during fungal infections in sugarcane to instigate the defense responses during red rot and wilt disease. J Crop Health 77 (2). DOI: 10.1007/s10343-024-01084-7.

Hanifah WN, Parjanto, Hartati S, Yunus A. 2020. The performances of M4 generation of Mentik Susu rice mutants irradiated with gamma-ray. Biodiversitas 21 (9): 4041-4046. DOI: 10.13057/biodiv/d210915.

Harborne JB. 1987. Metode Fitokimia [Phytochemical Methods]. Penerbit ITB, Bandung. [Indonesian]

Hashem A, Tabassum B, Fathi Abd Allah E. 2019. *Bacillus subtilis*: A plant-growth-promoting rhizobacterium that also impacts biotic stress. Saudi J Biol Sci 26 (6): 1291-1297. DOI: 10.1016/j.sjbs.2019.05.004.

Huihui Z, Xin L, Zisong X, Yue W, Zhiyuan T, Meijun A, Yuehui Z, Wenxu Z, Nan X, Guangyu S. 2020. Toxic effects of heavy metals Pb and Cd on mulberry (*Morus alba* L.) seedling leaves: Photosynthetic function and reactive oxygen species (ROS) metabolism responses. Ecotoxicol Environ Saf 195: 110505. DOI: 10.1016/j.ecoenv.2020.110469.

Jan R, Aaqil Khan M, Asaf S, Lubna, Park JR, Lee IJ, Kim KM. 2021. Flavonone 3-hydroxylase relieves bacterial leaf blight stress in rice via overaccumulation of antioxidant flavonoids and induction of defense genes and hormones. Intl J Mol Sci 22 (11): 6152. DOI: 10.3390/ijms22116152.

Kumar A, Gul MZ, Zeeshan A, Bimolata W, Qureshi IA, Ghazi IA. 2013. Differential antioxidative responses of three different rice genotypes during bacterial blight infection. Aust J Crop Sci 7 (12): 1893-1900. DOI: 10.3316/informit.669093920925580.

Maurya A. 2020. Oxidative Stress in Crop Plants. In: Hasanuzzaman M (eds.). Agronomic Crops (pp. 349-380). Springer.

Ming FS, Razali Z, Somasundram C. 2021. Involvement of phenolic compounds and their composition in the defense response of *Fusarium oxysporum*-infected Berangan banana plants. Sains Malaysiana 50 (1): 23-33. DOI: 10.17576/jsm-2021-5001-03.

Mistiyah M, Julipprijanto W, Septiani Y. 2018. Analyze of rice production determination in Central Java 1998-2018. DINAMIC: Directory J Econ 2 (3): 821-833. DOI: 10.31002/dinamic.v2i3.1426. [Indonesian]

Oliva R, Ji C, Atienza-Grande G, Huguet-Tapia JC, Perez-Quintero A, Li T, Eom JS, Li C, Nguyen H, Liu B, Auguy F, Sciallano C, Luu VT, Dossa GS, Cunnac S, Schmidt SM, Slamet-Loedin IH, Cruz CV, Szurek B, Frommer WB, White FF, Yang B. 2019. Broad-spectrum resistance to bacterial blight in rice using genome editing. Nat Biotechnol 37: 1344-1350. DOI: 10.1038/s41587-019-0267-z.

Ramaroson ML, Koutouan C, Helesbeux JJ, Le Clerc V, Hamama L, Geoffriau E, Briard M. 2022. Role of phenylpropanoids and flavonoids in plant resistance to pests and diseases. Molecules 27 (23): 8371. DOI: 10.3390/molecules27238371.

Ramzan M, Sana S, Javaid N, Shah AA, Ejaz S, Malik WN, Yasin NA, Alamri S, Siddiqui MH, Datta R, Fahad S, Tahir N, Mubeen S, Ahmed N, Ali MA, El Sabagh A, Danish S. 2021. Mitigation of bacterial spot disease induced biotic stress in *Capsicum annuum* L. cultivars via antioxidant enzymes and isoforms. Sci Rep 11 (1): 9445. DOI: 10.1038/s41598-021-88797-1.

Rezauji NA, Suminah, Widiyanti E. 2023. Minat generasi muda pertanian dalam budidaya padi Rojolele Srinuk di Desa Delanggu Kecamatan Delanggu Kabupaten Klaten. Jurnal Triton 14 (1): 202-215. DOI: 10.47687/jt.v14i1.362. [Indonesian]

Sahu PK, Jayalakshmi K, Tilgam J, Gupta A, Nagaraju Y, Kumar A, Hamid S, Singh HV, Minkina T, Rajput VD, Rajawat MVS. 2022. ROS generated from biotic stress: Effects on plants and alleviation by endophytic microbes. Front Plant Sci 13: 1042936. DOI: 10.3389/fpls.2022.1042936.

Singh K, Gupta R, Shokat S, Iqbal N, Kocsy G, Pérez-Pérez JM, Riyazuddin R. 2024. Ascorbate, plant hormones and their interactions during plant responses to biotic stress. Physiol Plant 176 (4): e14388. DOI: 10.1111/ppl.14388.

Suparyono, Sudir, Suprihanto. 2004. Pathotype profile of *Xanthomonas oryzae* pv. *oryzae* isolates from the rice ecosystem in Java. Indones J Agric Sci 5 (2): 63-69. DOI: 10.21082/ijas.v5n2.2004.p63-69.

Suryanto E. 2007. Aktivitas antioksidan ekstrak flavonoid dari buah Andaliman (*Zanthoxylum acanthopodium* DC) pada ikan mas (*Cyprinus carpio* L.). Jurnal Sains. UNSRAT. Manado. [Indonesian]

Syman K, Turpanova R, Nazarova GA, Berdenkulova AZ, Tulindinova GK, Korogod NP, Izbassarova ZZ, Aliyeva ZG, Utegaliyeva R. 2024. Role of oxidative stress enzymes in abiotic and biotic stress. Casp J Environ Sci 22 (2): 521-528. DOI: 10.22124/CJES.2024.7744.

Toan NP, Ha PTT, Xuan TD. 2017. Effects of rice blast fungus (*Pyricularia grisea*) on phenolics, flavonoids, antioxidant capacity in rice (*Oryza sativa* L.). Intl Lett Nat Sci 61: 1-7. DOI: 10.56431/p-g791ge

Waszczak C, Carmody M, Kangasjärvi J. 2018. Reactive oxygen species in plant signalling. Annu Rev Plant Biol 69: 209-236. DOI: 10.1146/annurev-arplant-042817-040322.

Yu Y, Lv Y, Shi Y, Li T, Chen Y, Zhao D, Zhao Z. 2018. The role of phyto-melatonin and related metabolites in response to stress. Molecules 23 (8): 1887. DOI: 10.3390/molecules23081887.

Yuan H, Qian J, Wang C, Shi W, Chang H, Yin H, Xiao Y, Wang Y, Li Q. 2025. Exogenous melatonin enhances rice blast disease resistance by promoting seedling growth and antioxidant defense in rice. Intl J Mol Sci 26 (3): 1171. DOI: 10.3390/ijms26031171.

Zechmann B. 2020. Subcellular roles of glutathione in mediating plant defense during biotic stress. Plants (Basel) 9 (9): 1067. DOI: 10.3390/plants9091067.