J. Trop. Plant Pests Dis.
Vol. 25, No. 2, September 2025
Pages: 255–261

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.225255-261

RESEARCH PAPER

Maize induced resistance against downy mildew mediated by benzoic acid increased photosynthesis rate and chlorophyl content

Muhammad Habibullah, Tri Joko, Arif Wibowo, & Ani Widiastuti

Manuscript received: 15 September 2024. Revision accepted: 5 March 2025. Available online: 16 July 2025.

ABSTRACT

Maize downy mildew, caused by *Peronosclerospora* spp., is a major disease affecting maize cultivation in tropical regions, particularly in Indonesia. One potential control strategy is the induction of plant resistance. Previous studies have demonstrated that benzoic acid (BA), a non-fungicidal chemical compound, can induce maize resistance against downy mildew. Induced resistance mechanisms do not directly inhibit pathogen development but enhance the plant's defensive response to infection. This study utilized various maize lines, ranging from susceptible to resistant, to evaluate the impact of BA-induced resistance on photosynthesis rate and chlorophyll content as the key agronomic parameters associated with the plant growth. The results showed that BA treatment did not significantly increase the photosynthetic rate and chlorophyll content in moderately resistant and resistant maize lines. However, in susceptible and moderately susceptible lines, BA treatment enhanced both parameters, indicating its role in strengthening plant resistance. These findings suggest that resistant maize lines do not respond to BA induction, whereas susceptible and moderately susceptible lines benefit significantly from its application. This provides new insight that BA can potentially induce resistance in susceptible maize plants against downy mildew.

Key words: chlorophyll, induced resistance, non-fungicide chemical, *Peronosclerospora* spp., photosynthetic rate

INTRODUCTION

Maize downy mildew is a disease caused by *Peronosclerospora* spp. and is a significant disease threat to maize production in tropical regions, particularly in Indonesia. Rashid et al. (2013) reported that global yield losses due to this disease can reach up to 30%, with some Asian countries experiencing total losses of up to 100%. Downy mildew remains a major biotic constraint to maize production worldwide. Sumardiyono (2008) noted that excessive pesticide use to control this disease has led to resistance in *Peronosclerospora* spp., the causal agents of maize downy mildew.

To manage the disease, breeders have developed various maize varieties with improved resistance, in addition to discovering fungicide active ingredients. However, in Indonesia, maize downy mildew has become an endemic disease, consistently appearing at varying levels of severity. Some of the maize lines used in this research (50B; 50CF; 50CG) were also developed by breeders to produce moderately resistant and resistant varieties against downy mildew.

Peronoschlerospora spp. is oomycetes that have recently gained global attention due to developments in their taxonomy. *P. neglecta* was identified in maize samples from East Java, Central Java, South Sulawesi, and Thailand (Muis et al., 2023). Advances in molecular techniques for plant disease diagnosis and management have highlighted the need for alternative control strategies. To date, seed treatment has been the most widely used method to control maize downy mildew. Habibullah et al. (2018) developed agents to induce maize resistance through seed treatment. Many researchers have explored the use of substances as resistance inducers to enhance plant defense and reduce disease severity. Ashraf et al. (2011) explained that the application of induction compounds, such as organic or mineral solutions, can boost plant resistance. Habibullah et al. (2018) tested several chemical compounds and found that they reduced disease severity and increased maize resistance. One such compound is benzoic acid (BA) (Habibullah et al., 2018; Habibullah et al., 2020).

The use of benzoic acid (BA) as a resistance inducer has been applied in various plant species (Senaratna et al., 2003; Surendran et al., 2018; Habibullah et al., 2020). Several studies have reported that BA can reduce disease incidence, enhance

Corresponding author:
Ani Widiastuti (aniwidiastuti@ugm.ac.id)

Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Jl. Flora No. 1 Bulaksumur, Yogyakarta, 55281

salicylic acid accumulation, and activate the plant defense system in response to pathogen attacks and other stress factors. However, the precise mechanism by which benzoic acid reduces disease incidence and increases maize resistance to downy mildew is not yet fully understood.

Previous research reported that BA induced maize resistance by promoting the production of reactive oxygen species (ROS) and enhancing cell wall lignification (Habibullah et al., 2018; Habibullah et al., 2020). However, there are no reports explaining how BA affects primary plant metabolism, particularly in relation to photosynthesis rate and chlorophyll content. High chlorophyll content and photosynthetic activity contribute significantly to plant growth and development (Agustamia et al., 2016). Loss of chlorophyll due to disease can result in stunted growth and reduced crop yield. Therefore, observing chlorophyll content is essential to assess the impact of downy mildew, particularly its chlorosis symptoms, which contribute to yield losses.

In plants, chlorophyll content is closely related to their ability to carry out photosynthesis. Latifa et al. (2019) and Widyawati et al. (2023) emphasized that leaf chlorophyll is vital for plant biochemical processes, especially photosynthesis and energy production. Chlorophyll is a key pigment located in the thylakoid membranes within chloroplasts, where it functions to absorb sunlight. Plant growth and development depend on cell division, enlargement, and differentiation in meristematic tissue, processes that require energy and organic compounds generated through photosynthesis. Chlorophyll and solar energy are essential for this process.

Agustamia et al. (2016) reported that the higher severity of maize downy mildew was associated with decreased chlorophyll content, and that resistant plants tend to have higher chlorophyll content and greater dry weight. Therefore, this study aims to explore the relationship between BA-induced resistance and photosynthesis rate and chlorophyll content. The research was conducted using maize lines with varying levels of resistance (from susceptible to resistant) to determine whether genotypic differences affect plant responses to BA-induced resistance against downy mildew in terms of photosynthesis and chlorophyll content.

MATERIALS AND METHODS

Research Site. The experiment was conducted in the experimental field of the Faculty of Agriculture, Universitas Gadjah Mada, located in Banguntapan, Bantul, Special Province of Yogyakarta.

Plants Preparation and Inoculation. The maize used in this study was sweet corn (*Zea mays saccharata* L.), consisting of the following lines: 50B (resistant), NUSA 1 (moderately resistant), 50CF (moderately susceptible), and 50CG (susceptible). A completely randomized design was used with the following treatments: 50B without treatment; 50B inoculated; NUSA 1 inoculated; NUSA 1 induced + inoculated; 50CF inoculated; 50CF induced + inoculated; 50CG inoculated; and 50CG induced + inoculated. Each treatment had three replicates, and each replicate consisted of three experimental units.

Benzoic acid (Merck 1.00136.0250 Benzoic Acid Pro Analysis) was used as the resistance inducer at a concentration of 2 g/L in distilled water. Seeds were soaked in this solution for 1 hour prior to planting. Seeds in the non-induced treatment group were soaked in distilled water for the same duration.

Inoculation was performed 10 days after planting following the method of Habibullah et al. (2018), by inserting leaves showing downy mildew symptoms into the shoot region of healthy maize plants.

Disease severity was measured at five weeks post-inoculation based on the method by Habibullah et al. (2020), using the following formula:

$$DSI = \frac{\sum(n \times v)}{N \times V} \times 100\%$$

DSI = Disease Severity Index;
n = Number of plants observed at a specific score;
v = Score scale value;
N = Total number of observed plants;
V = Highest score value (4) (Table 1).

Changes in resistance categories were evaluated

Table 1. Downy mildew disease severity score

Percentage of disease severity	Score scale
> 0–10	1
> 11–30	2
> 31–50	3
> 51	4

relative to the initial resistance classification, based on Table 2.

Plant Growth Parameters. Plant growth parameters observed included plant height, root length, and dry weight. Plant height was measured weekly until the seventh week (growth stagnation stage), from the base of the stem to the highest point. Root length was measured from the root base to the tip after cleaning with water, using a ruler. Dry weight was measured by drying the entire plant sample (placed in paper bags) in an oven at 60 ºC for three days, then weighing the mass using a digital scale.

Total Chlorophyl Content and Photosynthesis Rate. Chlorophyll content was observed five weeks after pathogen inoculation. Three representative samples per treatment were taken from the upper, young leaves. The method used followed Arnon (1949).

Photosynthesis rate was measured in the fourth week post-inoculation using the CID Bio Science Handheld Photosynthesis System CI-340 (USA). Measurements were taken from the third or fourth leaf from the top, which are actively involved in photosynthesis. Leaves were clamped into the chamber sensor and sealed without applying excessive pressure. The instrument automatically recorded the photosynthesis values.

Correlation Analysis between Disease Severity and Chlorophyl Content and Photosynthesis Rate. Correlation analysis between disease severity, plant dry weight, chlorophyll content, and photosynthetic rate was conducted using Microsoft Excel in the Microsoft Office 2016. Correlation calculation (r) were calculated, ranging from -1 to 1:

r > 0 indicates a positive correlation (both variables increase together),

r < 0 indicates a negative correlation (as one increases, the other decreases),

r = 0 indicates no linear relationship between variables.

A comparison of treatments was carried out using the Fisher’s test (P ≤ 0.05) to evaluate whether relationships between variables showed statistically significant differences. The t-test for correlation significance was calculated using the formula:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

r = Correlation coefficient;

n = Number of data pairs (sample size).

The calculated t-value (tcalculatedt_{calculated} tcalculated) was then compared to the critical t-value from the t-distribution table at a significance level of P ≤ 0.05P \leq 0.05 P≤ 0.05 (5%).

If tcalculated>ttablet_{calculated} > t_{table} t calculated > t table, the relationship between variables is considered statistically significant.

If t calculated ≤ t tablet, the relationship is considered not significant.

This statistical analysis was performed using SAS® software version 9.4 for Windows.

RESULTS AND DISCUSSION

The result shown in Table 3 indicate that maize lines with resistant or moderately resistant genotypic trait (50B and NUSA 1) remained in their respective resistance categories and did not exhibit any change before or after benzoic acid (BA) treatment followed by pathogen inoculation. This suggests that resistant plants do not require induction to overcome pathogen infection, as they inherently possess the ability to combat the pathogen.

In contrast, the susceptible and moderately susceptible maize lines responded positively to the BA seed treatment, demonstrating increased resistance to downy mildew five weeks after inoculation. Both 50CG (susceptible line) and 50CF (moderately susceptible line) remained in their original categories when they did not receive BA treatment. However, when treated with BA, both lines shifted to the moderately resistant category.

Habibullah et al. (2020) screened several non-fungicidal chemicals to induce maize resistance to downy mildew via seed treatment and found that soaking seeds in 2000 ppm benzoic acid reduced disease incidence and severity by up to 80%. This effect is attributed to the production of reactive oxygen

Table 2. Plant resistance categories based on disease severity

Percentage of disease severity	Resistance category
> 0–10	Resistance
> 10–30	Moderate resistance
> 30–50	Moderately susceptible
> 50	Susceptible

species (ROS), which are part of the plant's early defense responses and serve as signals to activate further resistance mechanisms. Such responses are also commonly observed under both biotic and abiotic stress conditions (Sharma et al., 2012). This study confirms previous findings by showing that BA-induced resistance can effectively enhance disease resistance in susceptible maize genotypes.

Further observation on plant height, dry weight, chlorophyll content, and photosynthesis rate (Table 4) showed genotype-specific responses to BA-induced resistance. NUSA 1 (moderately resistant) exhibited significantly higher plant height, dry weight, and chlorophyll content following BA treatment and inoculation. The 50CF line (moderately susceptible) showed a notable increase in plant height, while the 50CG line (susceptible) had a significant increase in dry weight. Both 50CF and 50CG lines also showed significant increases in chlorophyll content and photosynthesis rate, consistent with their improved resistance categories.

A major symptom of maize downy mildew is leaf chlorosis along the veins, caused by damage to chloroplasts, which leads to yellowing leaves (Agustamia et al., 2016). Chlorophyll plays a central role in photosynthesis, capturing solar energy and converting it into carbohydrates, which serve as the energy source for plant growth and ecosystem productivity (Mandal & Dutta, 2020). The application of benzoic acid, which functions as a resistance-inducing signal and modulator of oxidative stress, can enhance chlorophyll content and increase the photosynthesis rate, thereby promoting plant growth (Hussain et al., 2021; Gao et al., 2024).

The increases in chlorophyll content in the susceptible and moderately susceptible lines help sustain photosynthesis, enabling the plants to better compensate for pathogen attack. In untreated susceptible plants, inoculation with *Peronoschlerospora* sp. likely caused chlorophyll degradation, lowering chlorophyll content and, consequently, the photosynthesis rate. BA treatment helped prevent chlorophyll degradation, resulting in significantly higher chlorophyll levels, which supported improved photosynthesis and resistance.

Figure 1 showed that the disease severity

Table 3. Resistance categories of maize plants against downy mildew, prior to and after BA induced resistance

Treatment	Disease severity (%)	Initial resistance category	Resistance category at 5 weeks post-inoculation
50B Control	0.00	Resistance	Resistance
50B Inoculated	7.41	Resistance	Resistance
NUSA 1 Inoculated	24.69	Moderately Resistance	Moderately Resistance
NUSA 1 Induced and Inoculated	14.81	Moderately Resistance	Moderately Resistance
50CF Inoculated	44.44	Moderately Susceptible	Moderately Susceptible
50CF Induced and Inoculated	14.82	Moderately Susceptible	Moderately Resistance
50CG Inoculated	60.18	Susceptible	Susceptible
50CG Induced & Inoculated	22.22	Susceptible	Moderately Resistance

Table 4. Plant growth, chlorophyll content, and photosynthesis rate of maize plants induced by benzoic acid against downy mildew

Plant	Plant height (cm)	Root length (cm)	Dry weight (g)	Chlorophyll content (mg/g)	Photosynthesis rate (μmol/m^2/s)
50 B Control	117.22 a	34.47 a	9.17 a	63.61 a	13.37 a
50 B Inoculated	118.18 a	33.93 a	8.93 a	63.95 a	13.36 a
NUSA 1 Inoculated	96.30 b	32.53 a	4.51 b	38.18 b	8.42 a
NUSA 1 Induced & Inoculated	116.55 a	33.80 a	6.77 a	51.18 a	11.02 a
50 CF Inoculated	83.91 b	32.27 a	5.80 a	37.83 b	7.05 b
50 CF Induced & Inoculated	115.35 a	34.20 a	4.83 a	48.83 a	10.38 a
50 CG Inoculated	77.83 a	32.80 a	3.94 b	32.27 b	5.67 b
50 CG Induced & Inoculated	74.08 a	33.77 a	6.58 a	51.61 a	11.12 a

Numbers followed by the same letters are not significantly different from Fisher's test at the 95% confidence level.

negatively correlated with chlorophyll content, photosynthesis rate, and dry weight. Disease severity was inversely proportional to chlorophyl content, photosynthetic rate, and dry weight, while chlorophyl content was directly proportional to the dry weight and photosynthetic rate. Conversely, chlorophyll content showed a positive correlation with both dry weight and photosynthesis rate, indicating that higher chlorophyll levels contribute to plant health and resistance. This suggests that improved photosynthetic capacity, promoted by higher chlorophyll content, may lead to enhanced resistance.

Previous studies have shown that benzoic acid acts as a signaling molecule that triggers antioxidant responses, reduces oxidative stress, and protects chloroplasts from pathogen-induced damage, thereby enhancing chlorophyll biosynthesis (Hussain et al., 2021; Gao et al., 2024). As an elicitor, BA activates pathways involved in chlorophyll production and helps plants maintain optimal photosynthetic efficiency, supporting both energy production and growth during stress (Enciso et al., 2018; Cham et al., 2022).

Increased chlorophyll levels enhance light absorption, generate more ATP, and promote the synthesis of secondary metabolites that strengthen plant defense mechanisms and accelerate recovery post-infection (Riddhipratim & Gorachand, 2020). Moreover, BA induces the production of phenolic compounds and lignin, which reinforce cell walls, inhibit pathogen penetration, and reduce disease severity (Rakib et al., 2019). In infections caused by *Peronosclerospora* spp., lead to chlorosis through chlorophyll degradation, BA helps maintain chlorophyll stability and preserve the plant's photosynthetic function (Agustamia et al., 2016).

Taken together, the results in Table 3 and Figure 1 demonstrate that disease severity has a direct impact on plant growth, mainly through disruption of

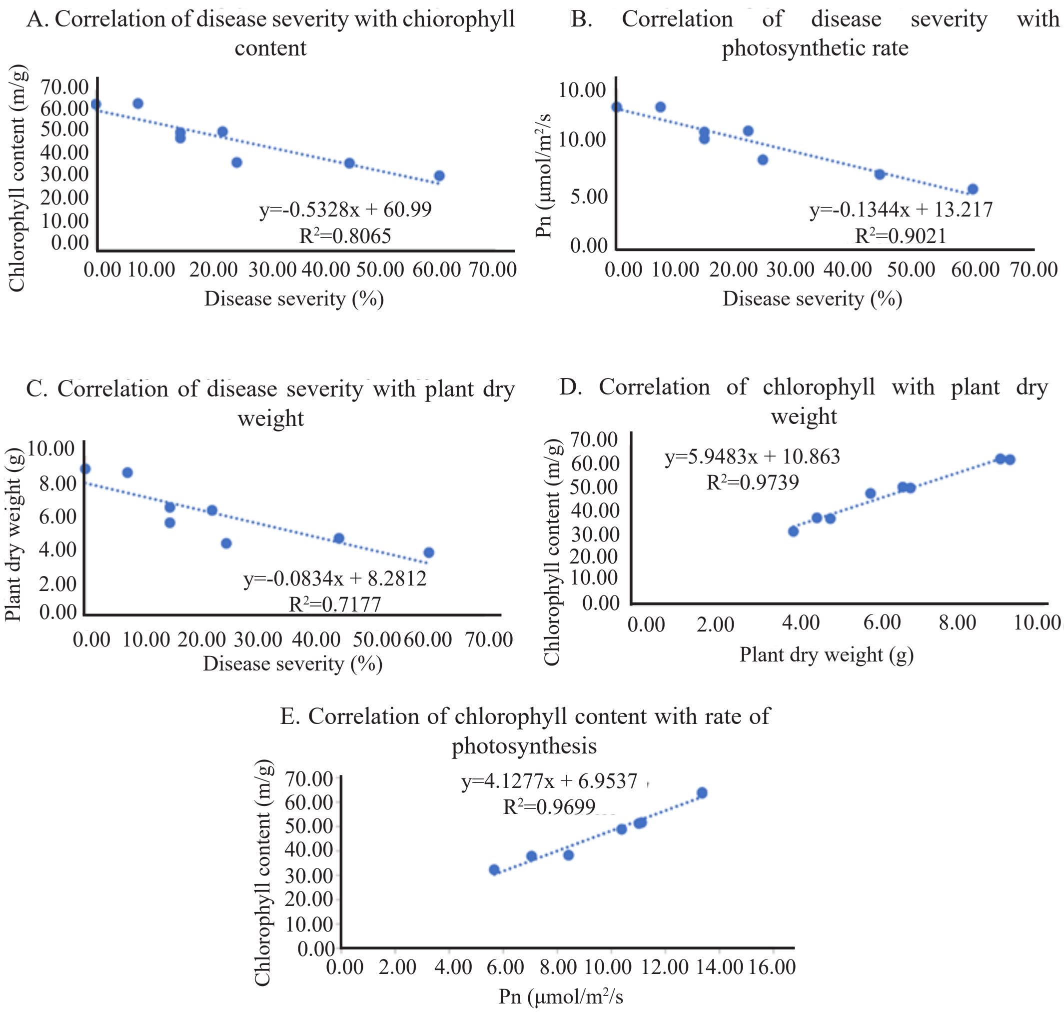

Figure 1. Correlation analysis of disease severity and chlorophyll content. A. Disease severity and the photosynthetic rate; B. Disease severity and plant dry weight; C. Chlorophyll and plant dry weight. D. Chlorophyll content; E. The photosynthesis rate.

chlorophyll production, which subsequently affects the photosynthesis rate. This study reveals that BA treatment enhances the resilience and survival of susceptible maize plants under pathogen stress. The findings provide valuable insight into the potential application of BA-induced resistance as an effective strategy to improve maize resistance to downy mildew in field conditions.

CONCLUSION

Benzoic Acid (BA) induced resistance of the susceptible maize lines against downy mildew by increasing the chlorophyl content and photosynthesis rate, therefore changed the resistance category from susceptible or moderately susceptible to moderately resistance. Chlorophyl content has direct proportional to the dry weight and photosynthetic rate which contributed to the better plant growth and higher disease resistance. This study brings a new understanding that BA treatment is potentially applicable to improve plant health in field and give opportunity for the susceptible plants to survive against plant disease.

ACKNOWLEDGMENTS

Authors expressed gratitude to Ministry of Education, Culture and Research, and Technology Indonesia for financially supporting the research by Doctoral Dissertation Research Grant 2023 (Hibah Penelitian Disertasi Doktor 2023) with contract number 122/E5/PG.02.00.PL/2023; 3113/UN1/DITLIT/Dit-Lit/PT.01.03/2023.

FUNDING

Source of funding for this research work was provided by Doctoral Dissertation Research Grant 2023 (Hibah Penelitian Disertasi Doktor 2023), Ministry of Education, Culture and Research, and Technology Indonesia with contract number 122/E5/PG.02.00.PL/2023; 3113/UN1/DITLIT/Dit-Lit/PT.01.03/2023.

AUTHORS' CONTRIBUTIONS

MH prepared the method, carried out the research operational and experiments, analysis and prepared the data for manuscript and manuscript writing; TJ, Awib and Awid conducted research supervising, checking methods. AWib checked the *Peronoschlerospora* sp. specimen and field treatment, TJ checked analysis data and Awid contributed in the manuscript writing. The authors provided response and comments on the research flow, data analysis and interpretation as well as shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

Authors declare that there is no potential conflict of interests.

REFERENCES

Agustamia C, Widiastuti A, & Sumardiyono C. 2016. Pengaruh stomata dan klorofil pada ketahanan beberapa varietas jagung terhadap penyakit bulai [Stomata and chlorophyl's influence on the resistance of several maize varieties against downy mildew]. *Indones. J. Plant Prot.* 20(2): 89–94. https://doi.org/10.22146/jpti.17703

Arnon, D.I.1949. Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. *Plant Physiology*. 24, 1–15. https://doi.org/10.1104/pp.24.1.1

Ashraf M, Akram NA, Al-Qurainy F, & Foolad MR. 2011. Chapter five - Drought Tolerance: Roles of Organic Osmolytes, Growth Regulators, and Mineral Nutrients. In: Sparks DL (Ed). *Adv Agron.* 111: 249–296. https://doi.org/10.1016/B978-0-12-387689-8.00002-3

Cham AK, Ojeda-Zacarías MdC, Lozoya-Saldaña H, Olivares-Sáenz E, Alvarado-Gómez OG, & Vázquez-Alvarado RE. 2022. Effects of elicitors on the growth, productivity and health of tomato (*Solanum lycopersicum* L.) under greenhouse conditions. *J. Agr. Sci. Tech.* 24(5): 1129–1142.

Gao X, Xin D, Zhao Y, Li J, Cao Y, Zhang S, & Guo J. 2024. Potential molecular mechanism of photosynthesis regulation by *PeMPK7* in poplar under *para*-hydroxybenzoic acid stress, *Ecotoxicol. Environ. Saf.* 276: 116329. https://doi.org/10.1016/j.ecoenv.2024.116329

Enciso ELG, Olivo AR, Mendoza AB, Gaona SS, & Morales SG. 2018. Effect of elicitors of natural origin on tomato plants subjected to biotic stress. *Rev. Mexicana Cienc. Agríc*. 20: 4211–4221. https://doi.org/10.29312/remexca.v0i20.991

Habibullah M, Widiastuti A, & Sumardiyono C. 2018. Respons awal ketahanan jagung terhadap *Peronosclerospora maydis* dan induksi bahan kimia [Early response of maize resistance to *Peronosclerospora maydis* and chemical induction]. *Indones. J. Plant Prot.* 22(1): 27–32. https://doi.org/10.22146/jpti.26877

Habibullah M, Sumardiyono C, & Widiastuti A. 2020. Potency of non-fungicide chemicals for maize inducing resistance against downy mildew. *Indones. J. Plant Prot.* 24(2): 154–160. https://doi.org/10.22146/jpti.55057

Hussain A, Noureen A, Shoukat W, Hussain S, Ali F, Ujjan JA, Memon FS, & Bapar NA. 2021. Mitigating the effect of salinity stress through foliar application of benzoic acid in spring maize (*Zea mays*. L). *Elementary Education Online*. 20(5): 7707–7712. https://doi.org/ 10.17051/ilkonline.2021.05.874

Latifa R, Hadi S, & Nurrohman E. 2019. The exploration of chlorophyll content of various plants in city forest of Malabar Malang. *Bioedukasi.* 17(2): 50–61. https://doi.org/10.19184/bioedu.v17i2.14091

Muis A, Ryley MJ, Tan YP, Suharjo R, Nonci N, Danaatmaja Y, Hidayat I, Widiastuti A, Widinugraheni S, Shivas RG, & Thines M. 2023. *Peronosclerospora neglecta* sp. nov.—a widespread and overlooked threat to corn (maize) production in the tropics. *Mycol. Progress.* 22: 12. https://doi.org/10.1007/s11557-022-01862-5

Rakib MRM, Borhan AH, & Jawahir AN. 2019. The relationship between SPAD chlorophyll and disease severity index in Ganoderma-infected oil palm seedlings. *J. Bangladesh Agril. Univ.* 17(3): 355–358. https://doi.org/10.3329/jbau.v17i3.43211

Rashid Z, Zaidi PH, Vinayan MT, Sharma SS, & Setty TAS. 2013. Downy mildew resistance in maize (*Zea mays* L.) across *Peronosclerospora* species in lowland tropical asia. *Crop Prot.* 43: 183–191. https://doi.org/10.1016/j.cropro.2012.08.007

Mandal R & Dutta G. 2020. From photosynthesis to biosensing: Chlorophyll proves to be a versatile molecule. *Sens. Int.* (1): 100058. https://doi.org/10.1016/j.sintl.2020.100058

Senaratna T, Merritt D, Dixon K, Bunn E, Touchell D, & Sivasithamparam K. 2003. Benzoic acid may act as the functional group in salicylic acid and derivatives in the induction of multiple stress tolerance in plants. *Plant Growth Regul.* 39(1): 77–81. https://doi.org/10.1023/A:1021865029762

Sharma P, Jha AB, Dubey RS, & Pessarakli M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* 2012(1): 217037. https://doi.org/10.1155/2012/217037

Sumardiyono C. 2008. Ketahanan jamur terhadap fungisida di Indonesia [Resistance of fungi against fungicide in Indonesia]. *Indones. J. Plant Prot.* 14(1): 1–5.

Surendran A, Siddiqui Y, Manickam S, & Ali A. 2018. Role of benzoic and salicylic acids in the immunization of oil palm seedlings-challenged by *Ganoderma boninense. Ind. Crop. Prod.* 122: 358–365. https://doi.org/10.1016/j.indcrop.2018.06.005

Widyawati N, Herawati MM, Kurnia TD, Murdono D, Simanjuntak BH, & Setiawan AW. 2023. Kandungan klorofil, pertumbuhan dan hasil vertikultur padi (*Oryza sativa* L.) varietas Situ Bagendit [Chlorophyll content, growth, and yield of verticulture rice plants (*Oryza sativa* L.) variety of Situ Bagendit]. *Vegetalika.* 12(3): 256–271. https://doi.org/10.22146/veg.83196