AGRIVITA Journal of Agricultural Science. 2024. 46(2): 276-288

AGRIVITA
Journal of Agricultural Science
www.agrivita.ub.ac.id

Early Detection of Fungicide Resistance Through Sensitivity Testing of Various Fungicide Active Ingredients and Genetic Variation of Downy Mildew-Causing *Peronosclerospora maydis* from Maize (Corn) Production Centers in Java, Indonesia

Satriyo Restu Adhi[1)], Fitri Widiantini[2*)] and Endah Yulia[2)]

[1)] Universitas Singaperbangsa Karawang, Karawang, West Java, Indonesia
[2)] Universitas Padjadjaran, Bandung, West Java, Indonesia

ARTICLE INFO

Keywords:
Dimetomorph
Fenamidone
Metalaxyl
Mutation
Oxathiapiproline

Article History:
Received: August 16, 2023
Accepted: March 29, 2024

*) Corresponding author:
E-mail: fitri.widiantini@unpad.ac.id

ABSTRACT

Maize downy mildew disease in Java, caused by *Peronosclerospora maydis*, can cause yield losses of up to 100%. Disease management of downy mildew using synthetic fungicides has been reported to cause resistance to *P. maydis*. This study identified early fungicide resistance in *P. maydis* from several maize production centers in Java (Blitar, Kediri, Klaten, Cianjur, Garut, Jatinangor, Rancakalong, and Sukabumi) by examining fungicide sensitivity levels and detecting genetic variation. The study was conducted at the Laboratory of Biotechnology of Plant Protection, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran from November 2018 to August 2019. The results showed that isolates from Blitar and Kediri (East Java) indicated resistance to metalaxyl and fenamidone. While in general, *P. maydis* isolates from West Java and Central Java still have sensitivity to metalaxyl, dimethomorph, fenamidone, and oxathiapiproline. Oxathiapiproline was the most effective fungicide in damaging *P. maydis* conidia in all locations. Based on the results of molecular identification, there is intraspecies genetic variation based on phylogenetic analysis.

INTRODUCTION

One of the important diseases in maize is downy mildew caused by *Peronosclerospora* spp. (Crouch et al., 2022). The disease can cause a loss of up to 30% of world maize production (Rashid et al ., 2013). Downy mildew in Indonesia causes 50-100% yield losses in susceptible plants. The high loss of maize yields is caused by downy mildew, which can affect plant development at every growth stage.

Symptoms of maize downy mildew are generally striped chlorosis on the leaves, and the plants become stunted (León, 1984). Other symptoms will cause inhibition of vegetative and generative growth that can cause crop failure (Muis et al., 2018). Symptoms shown in the vegetative stage are the appearance of chlorosis in the leaf venation, the leaves being shaped like a fan, and the plant becoming stunted. Meanwhile, in the generative stage, downy mildew disease causes the cobs not to be covered, and the seeds will not be filled. Signs of disease can be seen on the upper or lower surface of the leaves in the morning. There will be a powdery white color, which is the propagule of the conidia mass.

Downy mildew disease in maize in South-East Asia is caused by six different species, namely *Peronosclerospora maydis*, *Peronosclerospora philippinensis*, *Peronosclerospora sacchari*, *Peronosclerospora sorghi*, *Peronosclerospora neglecta,* and *Sclerospora rayssiae* var. *zeae* (Muis et al., 2023; Rashid et al., 2013). Meanwhile in Indonesia, downy mildew is reported to be caused by four species, namely *P. maydis, P. philippinensis, P. sorghi,* and *P. neglecta* (Muis et al., 2023). *P. maydis* infects maize plants in West Java, Central Java, Lampung, and Kalimantan; *P. sorghi* infects maize plants in the highlands of the Brastagi area,

ISSN: 0126-0537

Cite this as: Adhi, S. R., Widiantini, F. & Yulia, E. (2024). Early detection of fungicide resistance through sensitivity testing of various fungicide active ingredients and genetic variation of downy mildew-causing *Peronosclerospora maydis* from maize (corn) production centers in Java, Indonesia. *AGRIVITA Journal of Agricultural Science, 46*(2), 276-288. http://doi.org/10.17503/agrivita.v46i2.4276

North Sumatra; *P. philippinensis* found on Sulawesi Island, such as Gorontalo, North Sulawesi, and South Sulawesi; *P. neglecta* infects maize plants in several regions in Sulawesi, East Java, and Kalimantan (Muis et al., 2023; Widiantini et al., 2015).

One of the maize production centers is Java, which accounts for a total national contribution of 40.17%. The contribution details are divided into 25.26% from East Java Province, 11.61% from Central Java Province, and 3.30% from West Java Province (Pusdatin, 2020a). The spread of downy mildew in several maize production centers in Java has been reported. According to a report from the Pusdatin (2020b), East Java Province has the highest infection rate of downy mildew, followed by Central Java and West Java Provinces.

Control efforts to minimize yield loss due to downy mildew can be carried out through cultural control practices by adjusting planting times, eradication, using resistant varieties, biological control, and chemical control (Frederiksen, 1980; Singh et al., 1987). Chemical control using fungicides has been reported to cause many cases of resistance to plant pests and diseases. The possible risk of emergence of resistance will be high if the fungicide is applied frequently in one growing season in the same area (Ishii, 2006). According to Gisi & Sierotzki (2008), there are at least four active fungicidal ingredients that can be used to control downy mildew, namely phenylamides (e.g. metalaxyl or mefenoxam), carboxylic acid amides (e.g. dimetomorph), cyanoacetamidoximes (e.g. cyumoxanil), and quinone outside inhibitors (e.g. azoxystrobin, famoxadone, and fenamidone). Examples of fungicides from the phenylamide group include metalaxyl, which has been used frequently since 1983 (Hamilton, 2002; Department of Environmental Conservation, 2015). Even in Indonesia itself, metalaxyl has become a control package recommended by the government in maize cultivation (Zubachtirodin et al., 2016; Eliestya et al., 2014).

Pathogenic resistance to fungicides can occur because resistant strains of pathogens generally exist in nature due to natural mutations. Still, the application of fungicides can act as a selector for mutated strains (Bradley et al*.,* 2012). According to Hobbelen et al. (2014), the existence of pathogenic strains that are resistant to fungicides is passed through several stages, namely: (1) resistant strains are present due to natural mutations, the number is low and random, and (2) the application of fungicides can increase the number of resistant strains in the pathogen population because it acts as an agents selectors so that the existence of these stages will leave a population of pathogenic strains that are already resistant.

The method to determine the resistance status of pathogenic strains from certain regions can be done by testing their sensitivity to fungicides and looking at the various responses of conidial germination morphological characteristics, then linked to their genetic profile (Beckerman, 2013). Bock et al. (2000) reported differences in morphological characteristics of *P. sorghi* isolates from several locations in Africa and showed different levels of pathogenicity. Morphological variations were also found in *P. maydis* isolates from various maize planting locations in Java, especially from the dimensions of the conidia and conidiophores (Widiantini et al*.,* 2015). Differences in conidia and conidiophores sizes indicate genetic variation among *P. maydis* isolates. Therefore, determining the presence of resistant strains from certain regions is an important step for an appropriate control management strategy.

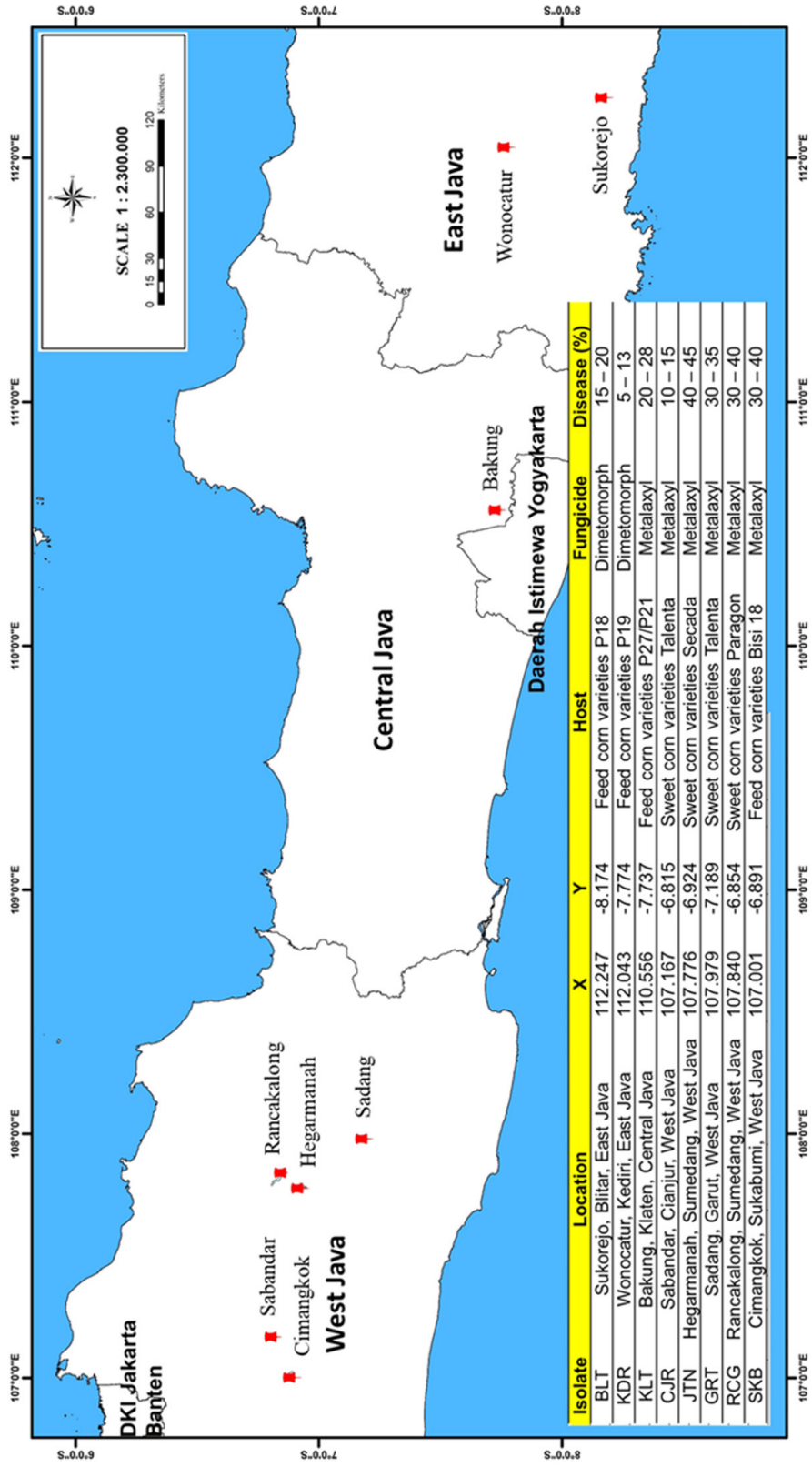
MATERIALS AND METHODS

Sampling Collection of *P. maydis*

The research began with purposive sampling of *P. maydis* isolates in East Java, Central Java, and West Java (Fig. 1), then testing fungicide sensitivity and genetic variation of *P. maydis* was carried out using experimental methods from November 2018 to August 2019. The experiment was conducted at the Laboratory of Biotechnology of Plant Protection Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran.

Detection of Fungicide Resistance by Sensitivity Test in *P. maydis*

This experiment uses a linear model Completely Randomized Design Factorial pattern. The first factor used was the type of fungicides commonly used to control maize downy mildew in which containing different active ingredient which consisted of 4 levels, namely dimethomorph (Demorf™ 60 WP), fenamidone (Target™ 500 SC), metalaxyl (Saromyl™ 35 SD), and oxathiapiprolin (Plenaris™ 200 FS). The second factor was the dose/concentration of the fungicide, the level consisted of the recommended dose/concentration (X), 1/2X, 1/4X, and 1/8X for dimetomorph, fenamidone, metalaxyl, and oxathiapiprolin fungicides and 1 control level (aquadest). The third factor was isolates of *Peronosclerospora maydis*. origin of the sampling location in Java Island, which consists of 8 levels namely BLT (Blitar), KDR (Kediri), KLT (Klaten), CJR (Cianjur), GRT (Garut), JTN (Jatinangor Sumedang), RCG (Rancakalong Sumedang), and SKB (Sukabumi).



Isolate	Location	X	Y	Host	Fungicide	Disease (%)
BLT	Sukorejo, Blitar, East Java	112.247	-8.174	Feed corn varieties P18	Dimetomorph	15 – 20
KDR	Wonocatur, Kediri, East Java	112.043	-7.774	Feed corn varieties P19	Dimetomorph	5 – 13
KLT	Bakung, Klaten, Central Java	110.556	-7.737	Feed corn varieties P27/P21	Metalaxyl	20 – 28
CJR	Sabandar, Cianjur, West Java	107.167	-6.815	Sweet corn varieties Talenta	Metalaxyl	10 – 15
JTN	Hegarmanah, Sumedang, West Java	107.776	-6.924	Sweet corn varieties Secada	Metalaxyl	40 – 45
GRT	Sadang, Garut, West Java	107.979	-7.189	Sweet corn varieties Talenta	Metalaxyl	30 – 35
RCG	Rancakalong, Sumedang, West Java	107.840	-6.854	Sweet corn varieties Paragon	Metalaxyl	30 – 40
SKB	Cimangkok, Sukabumi, West Java	107.001	-6.891	Feed corn varieties Bisi 18	Metalaxyl	30 – 40

Fig. 1. Map of *P. maydis* sampling location

Each recommended dose/concentration for each fungicide active ingredient used in this test was dissolved in 10 ml of sterile distilled water. The test was carried out using a modified method, namely mixing the conidia of *Peronosclerospora* isolates in each fungicide solution. Conidia were harvested slowly from the leaves of corn infected with downy mildew using a brush. Then, 50 µl of fungicide + conidia suspension samples were transferred to a concave *glass object* and covered using *a cover glass*. Furthermore, the samples were incubated in a plastic box with a damp tissue paper cov*er* for 24 hours in a dark room.

Observations were made by looking at the number of damaged conidia. Each treatment sample observed 50-55 conidia repeated 4 times so that the total conidia observed were 200-220 conidia in each treatment. Damaged conidia are characterized by changes in conidial morphology, such as lysis, incomplete shape, and damage to the cell wall. The number of damaged conidia is entered into the formula for the percentage of damaged conidia.

$$\text{Conidia damage (\%)} \frac{\text{Number conidia damaged}}{\text{Total number conida damaged}} \text{ x } 100\% \text{..1)}$$

Statistical data on the percentage of conidia damage to fungicide in each area was analyzed by analysis of variance (ANOVA). The analysis program used is SPSS 21 and SASM-Agri. If the results of the F test on the fungicide and the area show a significantly different interaction, then proceed with the Scott-Knott follow-up test, which has a significance level of 5%.

Identification and Detection of *P. maydis* Genetic Variation

DNA extraction was carried out using the modified method of Mathiyazhagan et al. (2008), starting with mixing 100 - 200 µl of conidial suspension harvested using a brush from downy mildew symptomatic plants in 500 µl of CTAB buffer solution (50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 0.7 M NaCl; 1% cetyltrimethylammonium bromide (w/v); and 1% 2-mercaptoethanol) in a 2 ml Eppendorf tube. The solution was then vortexed for 30 seconds. They were then incubated using a dry bath for 60 minutes at 60°C. After incubation, the CTAB-buffered conidial mixture was centrifuged at 13,000 g for 10 minutes and 600 µl of chloroform: isoamyl alcohol (CIA) solution and then incubated for 60 minutes on a shaker machine with a speed of 100 rpm and a temperature of 27-28ºC. After that, it was centrifuged at 13,000 g for 10 minutes.

Then the supernatant was transferred to a new Eppendorf tube and re-extracted using chloroform:isoamylalcohol (CIA). Then, the supernatant was transferred back to a new 1.5 ml Eppendorf tube, and the DNA was precipitated by adding 600 µl of cold isopropanol, then centrifuged again at 13,000 g for 10 minutes. Then, the DNA pellet was washed using 70% cold ethanol. The pellets were dried for 10-15 minutes and redissolved in TRIS-EDTA buffer solution (10 mM Tris-HCl and 1 mM EDTA, pH 8.0).

Quantification of the extracted DNA was carried out using the Nanodrop® Spectrophotometer ND-2000. The DNA template was taken 1 µl and read at 260 and 280 nm wavelengths. The level of DNA purity is obtained from the absorbance ratio of 260/280 nm. Pure DNA is in the range of 1.8–2.0. The quality test method for extracted DNA was carried out using an agarose gel electrophoresis method with a concentration of 0.8%. A 0.8% agarose gel was prepared by dissolving 0.8 g in 100 ml of 0.5x TBE solution. Then, it is heated using a magnetic hot plate stirrer until it boils. After that, 10 µl/100 ml of agarose was used for staining. The agarose gel is poured into a mold and placed on a comb to make a well. A 2.5 µl of DNA sample was added with 0.5 µl of loading dye, put into the agarose well, and then electrophoresed at 70 Volts for 45 minutes (Mupid). DNA visualization was performed under a UV Transilluminator (BluPAD).

DNA samples showing genomic DNA bands based on the results of the DNA quality test by electrophoresis were then subjected to the PCR process using a specific primer for *P. maydis* (Rustiani et al., 2015), namely PmUF (5'- TCGTTATAGAAGCTAT(T/C)CATTAG -3') and PmUR (5'- GCCATCGAGTAATCCATTGTT -3'). A total of 5 µl DNA sample (concentration < 100 ng/µl) was added with 12.5 µl KAPA2G Fast ReadyMix PCR Kit+Dye, 5 µl PCR -grade water, and primers PmUF and PmUR each 1.25 µl (what are the concentrations). The amplification program for the primer is an initial synthesis of 95°C for one minute, followed by 35 cycles, which are divided into denaturation stages of 95°C for one minute, annealing temperature of 57°C for one minute, extension temperature of 72°C for one 30 seconds. The final synthesis is carried out at 72ºC for five minutes. Amplification was carried out using a PCR machine (SelectCycler II Thermal Cycler).

The PCR product is then purified to clean and purify the DNA from impurities and reagents. Purification using Geneaid Gel/PCR DNA

Fragments Extraction Kit. The procedure follows the manual instructions, which begin by mixing the PCR product with DF buffer solution into a 1.5 ml Eppendorf tube with a volume of 1:5. Then the PCR product and DF buffer solution are mixed using a vortex. After mixing the suspension, put it in two DF column tubes and 2 ml tubes. They were then centrifuged at 16,000 g for 30 seconds.

The solution in the 2 ml tube was discarded, and the 2 ml tube was reassembled with the DF column tube. Then 600 µl wash buffer was added through the DF column tube, allowed to stand for one minute, and centrifuged at 16,000 g for 30 seconds. After that, the solution in the 2 ml tube was discarded and then reassembled with the DF column tube. The DF column tube was dried by centrifuging at 16,000 g for five minutes. After drying, the DF column tube was replaced with a new 1.5 ml Eppendorf tube. Then, 50 µl of elution buffer solution (Tris-HCl 10 mM, pH 8.5) was added to it via the DF column and allowed to stand for ten minutes. After that, it was centrifuged at 16,000 g for ten minutes. The purified DNA is in a 1.5 ml Eppendorf tube.

Visualization of purified DNA was carried out using the agarose gel electrophoresis method. Agarose gel concentration of 1.7% and 100bp markers (Geneaid) were used at 70 Volts for 45 minutes (Mupid). Furthermore, DNA visualization was carried out under UV Transilluminator (BluPAD). The purified DNA obtained was analyzed for sequencing at Macrogen Korea using the ABI PRISM 377 DNA Sequencher to obtain the nucleotide sequences.

Analysis of genetic variation begins with carrying out DNA barcoding procedures. The nucleotide sequences obtained from the sequencing results were subjected to homology analysis on the Basic Local Alignment Search Tool Nucleotide (BLASTN) – National Center of Biological Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Homology analysis aims to match the data available in the NCBI and GenBank databases.

The genetic diversity of *P. maydis* isolates was identified by comparing the nucleotide sequence alignment between isolates and accessions from Genbank. The alignment of the nucleotide sequences of *P. maydis* isolates from several locations in Java Island was initially edited/contiguous using the ClustalW method in the BioEdit software. In addition, genetic diversity and kinship between isolates were further analyzed using MEGA-X software through the Neighbor Joining (NJ) method with a bootstrap value of 1000x repetitions in the phylogenetic dendrogram construction. Analysis of diversity based on amino acids was carried out by translating nucleotide sequences into amino acids using the MEGA-X software.

RESULTS AND DISCUSSION

Detection of Fungicide Resistance by Sensitivity Test in *P. maydis*

Treatment of the four fungicide active ingredients at four concentration levels significantly affected the conidia damage of *P. maydis* tested. This influence shows varying values. This is listed in Table 1. The use of metalaxyl at the recommended concentration (packaging label) produced a different effect on each *P. maydis*. Referring to the percentage value of damaged conidia (Table 1, column MX) metalaxyl is generally still effectively used in West Java (Cianjur, Garut, Jatinangor Sumedang, Rancakalong Sumedang, and Sukabumi), but not in Central Java (Klaten) and East Java (Blitar dan Kediri). Metalaxyl treatment caused a significantly higher percentage of conidia damage in several *P. maydis* isolates taken from five areas in West Java (Cianjur, Garut, Jatinangor Sumedang, Rancakalong Sumedang, and Sukabumi) and Central Java (Klaten) compared to isolates from East Java (Blitar and Kediri). The percentage of damaged conidia in Blitar and Kediri showed the lowest values. In contrast to the five isolates from West Java which still showed sensitivity to metalaxyl and the isolate from Central Java, although at concentrations lower than recommended.

Furthermore, damage to the conidia of *Peronoscleropsora* spp. generally tend to be lower in isolates from Blitar and Kediri when compared to isolates from other areas. Not only for metalaxyl, but for other fungicide-active ingredients, except for oxathiapiprolin. This shows that the isolates from Blitar and Kediri are indicated to be resistant to fungicides with certain active ingredients. For example, isolates from Blitar and Kediri were suspected of showing resistance to the fenamidone. Indications of fenamidone resistance can be seen in the percentage value of conidia damage from Blitar and Kediri isolates (Table 1 column FX), which is low and significantly different from isolates from other regions.

Kediri and Blitar are maize (corn)-growing centers in Java. The harvested area in 2017 was around 51,273 ha for Kediri and 52,098 ha for Blitar

(Badan Pusat Statistik, 2018). Intensive maize (corn) cultivation for several years in the area is thought to be one of the causes of metalaxyl and fenamidone fungicides becoming resistant to downy mildew. Intensive crop cultivation will increase agricultural external inputs such as inorganic fertilizers, pesticides, monoculture cultivation, and mechanization (Kughur et al., 2015). This will have an impact on several things such as the environment, biodiversity, and human life. The relationship between intensive cultivation and plant disease will continue, causing an increase in the number of inoculums and the intensity of attacks (Waceke & Kimenju, 2007).

Table 1. Damage to conidia (%) of *P. maydis* from several locations in Java Island which were treated with dimethomorph, fenamidone, metalaxyl, and oxathiapiprolin

Fungicide	BLT	KDR	KLT	CJR	GRT	JTN	RCG	SKB
K	0 a A	0 a A	0 a A	0 a A	0 a A	0 a A	0 a A	0 a A
DX	11.5 a E	18.9 b G	28.9 c G	19.5 b D	28.2 c D	39 d E	26 c B	21.5 b B
D1/2	7 a D	15.8 b F	20.5 b E	17.5 b C	21.2 b C	35 d D	26 c B	19.5 b B
D1/4	4 a C	11.5 b E	15 b D	16 b C	20.5 c C	24 c B	26 d B	19 c B
D1/8	2.5 a B	10,1 b E	11.8 b D	14 b C	14.3 b B	25.5 d B	22 c B	18.5 c B
FX	5.9 a D	7.4 a D	23.2 b F	28 c E	31 c D	43.5 d E	29.5 c C	33 c C
F1/2	2.5 a B	5.9 a C	16.4 b D	20 b D	19.8 b C	38.5 d E	28 c B	30.5 c C
F1/4	1 a A	5.5 a C	13.9 b D	14 b C	18.5 b C	35.5 d D	26 c B	29.5 c C
F1/8	2 a B	4.5 a C	9.7 b C	8.5 b B	13.6 c B	31.5 f C	26 e B	19 d B
MX	5.4 a D	9.1 a E	18.4 b E	51.5 d G	22.3 b C	30 c C	31 c C	44.5 d D
M1/2	4 a C	5 a C	14 b D	33 e F	16.2 b D	29.5 d C	30 d C	33.5 e C
M1/4	2 a B	3.9 a C	13.9 b D	22 c D	15.3 b B	29.5 d C	26.5 d B	32.5 d C
M1/8	0.5 a A	2.5 a B	6.5 b B	13 c C	14.4 c B	23 d B	23.5 d B	28 f C
OX	14.2 a F	20.7 b G	47.8 d J	34 c F	37.3 c E	44 d E	47.5 d D	31 c C
O1/2	7 a D	17.1 b F	35.3 e I	28.5 d E	34.5 e E	39 f E	35 e C	24.5 c B
O1/4	4.5 a C	9.6 a E	31.6 d H	27.5 c E	33.5 d E	36 d D	34 d C	23.5 c B
O1/8	3.5 a C	7.5 a D	28 d G	22 c D	22.2 c C	22 c B	24.5 c B	21 c B

Remarks: The same letter in one line shows results that are not significantly different based on the Scott-Knott test at the 5% level; The same letter in one column shows results that are not significantly different based on the Scott-Knott test at the 5% level; D (Dimetomorph), F (Fenamidone), M (Metalaxyl), O (Oxathiapiprolin), K (Aquadest), BLT (Blitar), KDR (Kediri), KLT (Klaten), CJR (Cianjur), JTN (Jatinangor Sumedang), RCG (Rancakalong Sumedang), SKB (Sukabumi)

The decrease in metalaxyl and fenamidone sensitivity is thought to be due to mutations in the species *P. maydis* from Kediri and Blitar. Naturally, in nature, there are sensitive pathogenic strains and pathogens resistant to fungicides. Continuous fungicide application will reduce the number of sensitive strains and increase the number of resistant pathogenic strains. In addition, sensitive pathogenic strains can change properties due to the accumulation of mutated genes (Damicone, 2008). According to Sierotzki et al. (2019), phenylamide fungicides such as metalaxyl cause single gene mutations (monogenic) in target organisms. Metalaxyl has a mechanism of action inhibiting the activity of nucleic acid biosynthesis in RNA polymerase 1 and exerts a concurrent effect on mitosis (Fisher & Hayes, 1984, 1982; Yang et al., 2011).

Randall et al. (2014) stated that an amino acid (AA) mutation in the RNApoll gene (RPA190), which encodes the RNA polymerase I subunit in oomycete *P. infestans* will be associated with a decrease in the sensitivity of metalaxyl and mefenoxam fungicides. Chen et al. (2018) generally found two pathways for changes in the mechanism of metalaxyl resistance in *P. infestans.* The first pathway is that the RPA190 gene in a metalaxyl-sensitive isolate undergoes a single amino acid mutation that changes from valine to glycine at the amino acid position 1476 (V1476G). At the same time, the second pathway will involve mutations at several amino acid positions, such as R296H, F382Y, P980S, E1174A, D1228E, V1476G, D1546Y, and P1600S. Changes in amino acids are thought to affect metabolic processes in target organisms.

A decrease in the sensitivity of QoI (quinone outside inhibitors) fungicides such as fenamidone has been reported. According to Gisi & Sierotzki (2015) QoI fungicides, such as fenamidone, have a high risk of causing oomycete resistance. Toffolatti et al. (2011) reported that QoI fungicide has caused resistance in *P. viticola* due to mutations of the G143A amino acid in the mitochondrial cytochrome b gene region. Fenamidone is a QoI fungicide with a mechanism of action that inhibits mitochondrial respiration by inhibiting electron transfer in cytochrome b (complex III) (Gisi & Sierotzki, 2008).

The fungicide with the active ingredient oxathiapiprolin at four concentration levels (X, 1/2X, 1/4X, and 1/8X) in this experiment resulted in the highest percentage of conidia damage in almost all sampling locations (Table 1). This is because oxathiapiprolin is an active ingredient that was only sold to the market in 2015 and is an active ingredient specifically made to control oomycetes (oomisides) (Cohen, 2015). The mechanism of action of oxathiapiprolin is by inhibiting homologous *oxysterol binding protein* (OSBP), which plays a role in the movement of lipid compounds between membranes which interferes with signaling processes, maintains cell membranes, and forms complex lipid compounds that are important for oomycete cells to stay alive (Fungicide Resistance Action Comitee, 2017, 2018; Pasteris et al., 2015).

The active ingredients of dimetomorph and fenamidone fungicides also have different sensitivity values. *P. maydis* isolates from Kediri and Blitar each have a tendency to be insensitive to dimetomorphs and fenamidone. While some isolates from West Java have the opposite tendency. This difference is thought to be due to the influence of the fungicide's range, frequency, and rotation in the area, which affects the response of the target organism.

Molecular Identification Result of *P. maydis*

The use of specific primers for *P. maydis* i.e. PmUF and PmUR succeeded in amplifying the DNA of *Peronosclerospora* spp. extraction results from eight areas in Java Island. Based on visualization (Fig. 2) all *Peronosclerospora* spp. isolates were confirmed as *P. maydis.* The PmUF and PmUR primers have a target size of about 304 bp.

Based on the molecular identification and homology analysis (Table 2), all isolates are identified as *P. maydis*. Isolates from Blitar, Kediri, Klaten, Cianjur, Garut, Jatinangor Sumedang and Sukabumi have similarities with accession HM988978.1 from Malang, which was deposited at GenBank. Meanwhile, the isolate from Rancakalong Sumedang had similarities with the accession HM988976.1 from Malang. It shows that the distribution of *P. maydis* species from all sampling locations is related. According to Bonde (1982), *P. maydis* was first reported as a cause of downy mildew in Java in 1897 by Raciborski.

All isolates that were identified had similarities to the species *P. maydis* isolates 123062 and 310106 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial. The use of the COX region to identify the oomycete

class has been reported. Robideau et al. (2011) reported that the *DNA* barcoding method in the cytochrome oxidase subunit I (COXI) gene region is a new step in identifying the oomycete group. In addition, Hudspeth et al. (2000) also reported that the COXII region can be used to determine the genetic relationships of 15 *Peronosporomycete* species. According to Kammarnjesadakul et al. (2011), the COXII gene region was able to separate species to the intraspecific level.

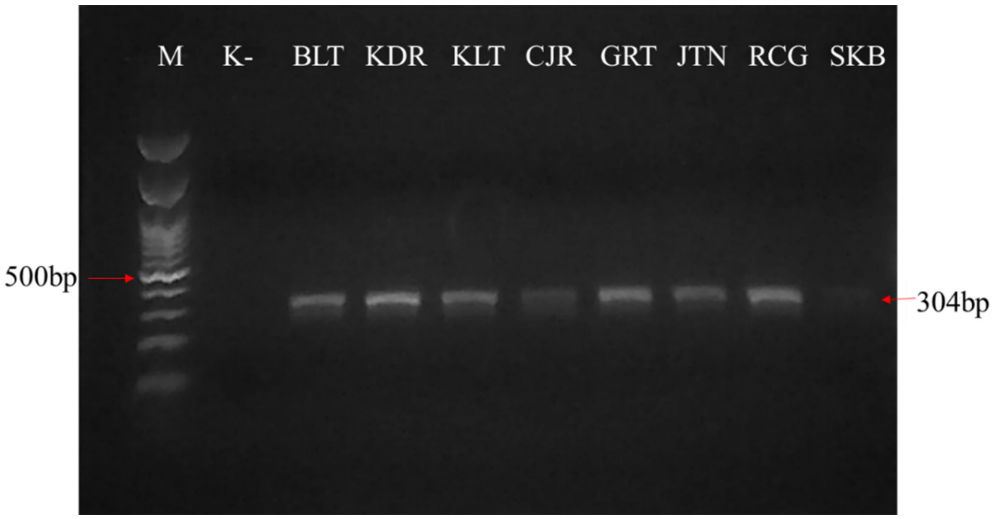
Using the COX gene region can also play a role in identifying species and their kinship in a location. Studholme et al. (2019) conducted a phylogenetic analysis of *P. kerinoviae* that causes stem canker disease in COXI-based forest plants from Chile, which turned out to have a close relationship with *P. kerinoviae* from New Zealand. Rustiani (2015) also reported that *P. sorghi* from East Nusa Tenggara is similar to *P. sorghi* from Texas. Analysis based on a combination of COX and ITS was also able to find a new species of *P. subutonaiense* from China, closely related to *P. utonaiense* from Japan (Chen & Zheng, 2019). Identification of this species kinship is also valuable for supporting quarantine activities in a location. According to Gao & Zhang (2013) DNA-based identification barcoding will play an essential role in ensuring the security of a location from the Quarantined Plant Pest Organisms. Therefore, the spread of species that may have never been found and are more pathogenic can be prevented.

Table 2. Identification results, similarity (%), specimen/origin, and GenBank accession numbers with nucleotide sequences available in the NCBI database

Isolate	Identification Results	Similarity (%)	Specimen/ Origin	GenBank Accession Number
BLT	*P. maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacer, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	97.86	*P. maydis* Malang	HM988978.1
KDR	*P. maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	97.50	*P. maydis* Malang	HM988978.1
KLT	*P. maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	96.43	*P. maydis* Malang	HM988978.1
CJR	*P. maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	98.21	*P. maydis* Malang	HM988978.1
GRT	*P. maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacers, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	98.89	*P. maydis* Malang	HM988978.1
JTN	*P. maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacer, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	96.79	*P. maydis* Malang	HM988978.1
RCG	*P. maydis* isolate 310106 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII- COXI intergenic spacer, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	97.19	*P. maydis* Malang	HM988976.1
SKB	*P. maydis* isolate 123062 cytochrome oxidase subunit II (COXII) gene, partial cds; COXII-COXI intergenic spacer, complete sequence; and cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	98.60	*P. maydis* Malang	HM988978.1

Genetic Variation *of P. maydis*

The Neighbor Joining (NJ) method, with a bootstrap value of 1000 repetitions, resulted in a dendrogram divided into five clusters (Fig. 3). The first cluster consists of isolates from Blitar, Garut, and Klaten. The second cluster consists of isolates from Rancakalong, Kediri, and Jatinangor. The third cluster consists of isolates from Cianjur. Sukabumi Isolate is in cluster four. Meanwhile, GenBank accessions HM988976.1 and HM988978.1 from Malang formed their group in cluster five.



Remarks: M = 100bp DNA marker (Geneaid), K- = Negative control (aquades), BLT = *P. maydis* isolate from Blitar, KDR = Kediri isolate, KLT = Klaten isolate, CJR = Cianjur isolate, GRT = Garut isolate, JTN = Jatinangor isolate, RCG = Rancakalong isolate, SKB = Sukabumi isolate

Fig. 2. Visualization of DNA fragments using *P. maydis* specific primers PmUF and PmUR on 1.7% agarose gel

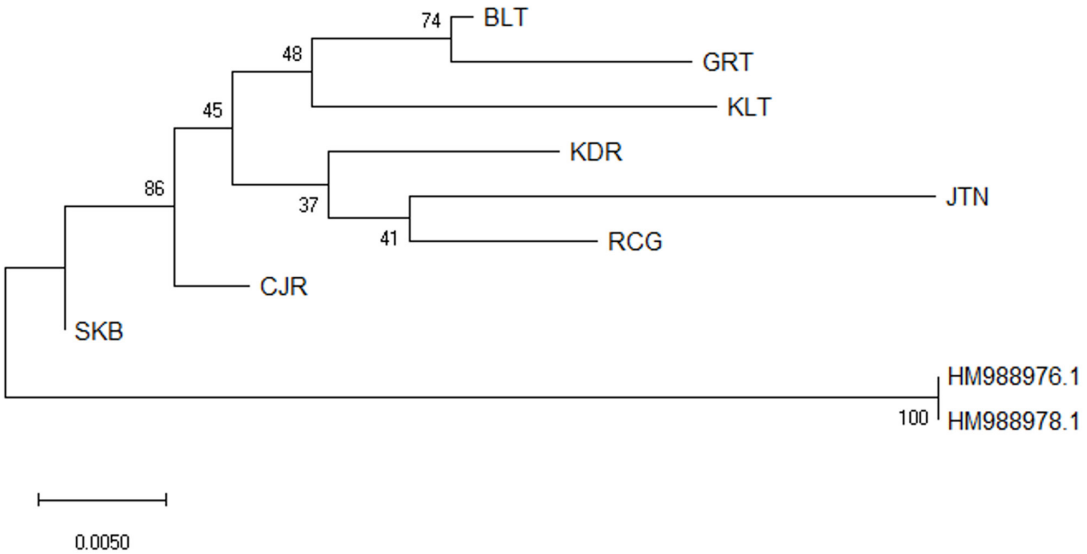


Fig. 3. Relationship dendrogram of *P. maydis* isolates from several locations on Java Island and GenBank (HM) accession. The dendrogram is generated from the Neighbor Joining (NJ) method with a bootstrap value of 1000x repetitions

P. maydis isolate from Java Island, even though it is in the same species, has differences at the intraspecific level (Fig. 3). The combination of isolates in one cluster is suspected due to the similarity factor based on the characteristics of the sampling location (Fig. 1). For example, in the second cluster, the sampling locations, namely in Rancakalong Sumedang, Kediri, and Jatinangor Sumedang, based on the author's observations during the research and interviews with local farmers (unpublished) are endemic areas of downy mildew where disease incidence occurs every season, although at a low percentage.

Muis et al. (2016) reported that seven isolates identified as *P. maydis* from Kediri were grouped into the same cluster based on the closest genetic kinship. Kediri Regency is one of the areas where downy mildew is endemic (Burhanuddin, 2015). Adhi et al. (2021) also reported that *Peronosclerospora* spp. in Java Island has diverse variations based on the morphology and morphometry of each isolate. Another example of Thai strain *P. insidiosum* taken from relatively the same source, namely water, tends to join in the same cluster after Neighbor Joining (NJ) analysis (Kammarnjesadakul et al., 2011). Thus, the kinship of each isolate in the same cluster is thought to be influenced by the similarity of the characteristics of the sampling location or the environment.

According to Lukman et al. (2013) variations among *P. maydis species* from several regions on Java Island are suspected to be due to high genetic variation or the presence of other species that infect maize plantations. This is supported by Muis et al. (2016), which states that if there is reasonableness in an area, there is more than one type of *Peronosclerospora* spp. due to environmental factors such as wind, water, soil, and circulation of seeds from another area, it supports the distribution of *Peronosclerospora* spp.

The limitation of this study is the small number of maize production centers sampled, namely 8 maize centers on the island of Java, so additional samples are needed to see wider variations in resistance and genetics. However, from the existing literature, the detection of resistance and genetics of *P. maydis* in the locations studied along the island of Java has not been reported, a challenge that we will solve in the next study.

CONCLUSION

P. maydis have different sensitivities according to their location to the four active ingredients of the fungicide. There have been indications of resistance to metalaxyl and fenamidone in East Java, but not in part in West Java and Central Java. In contrast, *P. maydis* isolates from West Java and Central Java generally still have sensitivity to metalaxyl, dimetomorph, fenamidone, and oxathiapiproline. All *P. maydis* isolates were sensitive to oxathiapiproline even at 1/8 of the recommended concentration. There is intraspecific variation in *P. maydis* as evidenced by the formation of different groups (clusters) based on phylogenetic analysis.

ACKNOWLEDGEMENT

The authors acknowledge and thank Ida Nurhelawati, Ardi Zulfikar Muchlis, and PT. Bayer Indonesia for making much of the field and laboratory work possible. To the Direktorat Riset, Teknologi, dan Pengabdian kepada Masyarakat (DRTPM) DIKTI Kemendikbudristek for providing training in writing scientific papers to Satriyo Restu Adhi and providing direction to make this article better.

REFERENCES

Adhi, S. R., Widiantini, F., & Yulia, E. (2021). Variasi Morfometri dan Patogenisitas *Peronosclerospora* spp. Penyebab Penyakit Bulai Jagung di Pulau Jawa, Indonesia. *Jurnal Fitopatologi Indonesia*, *17*(5), 173–182. https://doi.org/10.14692/jfi.17.5.173-182

Badan Pusat Statistik. (2018). *Provinsi Jawa Timur Dalam Angka 2018*. Badan Pusat Statistik Provinsi Jawa Timur. https://jatim.bps.go.id/publication/2018/08/16/9999b727d316c006ee2fd7e7/provinsi-jawa-timur-dalam-angka-2018.html

Beckerman, J. L. (2013). Detection of Fungicide Resistance. In M. Nita (Ed.), *Fungicides - Showcases of Integrated Plant Disease Management from Around the World* (pp. 281–310). InTech. https://doi.org/10.5772/55981

Bock, C. H., Jeger, M. J., Mughogho, L. K., Cardwell, K. F., Mtisi, E., Kaula, G., & Mukansabimana, D. (2000). Variability of *Peronosclerospora sorghi* isolates from different geographic locations and hosts in Africa. *Mycological Research*, *104*(1), 61–68. https://doi.org/10.1017/S0953756299008965

Bonde, M. R. (1982). Epidemiology of downy mildew diseases of maize, sorghum and pearl millet. *Tropical Pest Management*, *28*(1), 49–60. https://doi.org/10.1080/09670878209370674

Bradley, C. A., Hollier, C., & Kelly, H. (2016). *Principles of Fungicide Resistance* (pp. 1–5). Fungicide Resistance Action Comitee (FRAC). https://www.plantmanagementnetwork.org/hub/SoyFungicideResistance/files/FungicideResistance.pdf

Burhanuddin. (2015). Masalah penyakit bulai dan alternatif pemecahannya di Kecamatan Pagu Kabupaten Kediri Propinsi Jawa Timur. *Prosiding Seminar Nasional Serealia*, 375–380. https://docplayer.info/111562191-Masalah-penyakit-bulai-dan-alternatif-pemecahannya-di-kecamatan-pagu-kabupaten-kediri-propinsi-jawa-timur.html

Chen, F., Zhou, Q., Xi, J., Li, D., Schnabel, G., & Zhan, J. (2018). Analysis of RPA190 revealed multiple positively selected mutations associated with metalaxyl resistance in *Phytophthora infestans*. *Pest Management Science*, *74*(8), 1916–1924. https://doi.org/10.1002/ps.4893

Chen, J.-J., & Zheng, X.-B. (2019). *Pythium subutonaiense*, a new aquatic oomycete from Southern China based on morphological and molecular Characters. *Mycobiology*, *47*(3), 1–7. https://doi.org/10.1080/12298093.2019.1642700

Cohen, Y. (2015). The novel oomycide oxathiapiprolin inhibits all stages in the asexual life cycle of *Pseudoperonospora cubensis* - causal agent of cucurbit downy mildew. *PLoS ONE*, *10*(10), 1–22. https://doi.org/10.1371/journal.pone.0140015

Crouch, J. A., Davis, W. J., Shishkoff, N., Castroagudín, V. L., Martin, F., Michelmore, R., & Thines, M. (2022). *Peronosporaceae* species causing downy mildew diseases of Poaceae, including nomenclature revisions and diagnostic resources. *Fungal Systematics and Evolution*, *9*(June), 43–86. https://doi.org/10.3114/fuse.2022.09.05

Damicone, J. (2008). Fungicide resistance management. In *Oklahoma Cooperative Extension Fact Sheet* (Vol. 1, pp. 1–8). Oklahoma State University. https://extension.okstate.edu/fact-sheets/print-publications/epp-entomology-and-plant-pathologhy/fungicide-resistance-management-epp-7663.pdf

Department of Environmental Conservation. (2015). *Active Ingredient Data Package Metalaxyl and Mefenoxam* (Vol. 4). https://www.dec.ny.gov/docs/materials_minerals_pdf/mefenoxamdata.pdf

Eliestya, S., Anto, A., & Suriansyah. (2014). *Teknologi Budidaya Jagung dengan Pendekatan PTT* (R. Massinai (ed.); 1st ed.). Balai Pengkajian Teknologi Pertanian (BPTP). http://kalteng.litbang.pertanian.go.id/ind/images/data/buku-jagung-2014.pdf

Fisher, D. J., & Hayes, A. L. (1982). Mode of action of the systemic fungicides furalaxyl, metalaxyl and ofurace. *Pesticide Science*, *13*(3), 330–339. https://doi.org/10.1002/ps.2780130316

Fisher, D. J., & Hayes, A. L. (1984). Studies of mechanisms of metalaxyl fungitoxicity and resistance to metalaxyl. *Crop Protection*, *3*(2), 177–185. https://doi.org/10.1016/0261-2194(84)90052-8

Frederiksen, R. A. (1980). Sorghum downy mildew in the United States: Overview and Outlook. *Plant Disease*, *64*(10), 903–908. https://doi.org/https://doi.org/10.1094/PD-64-903

Fungicide Resistance Action Comitee. (2017). *First meeting of the FRAC OSBPI working group*. http://www.frac.info/docs/default-source/osbpi-wg/minutes-of-the-2016-osbpi-wg-meeting-recommendations-for-2017---4apr17.pdf?sfvrsn=b8824a9a_2

Fungicide Resistance Action Comitee. (2018). FRAC Code List 2018: fungicide sorted by mode of action (including FRAC Code numbering). In *Frac Code List*. http://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2015-finalC2AD7AA36764.pdf?sfvrsn=4

Gao, R., & Zhang, G. (2013). Potential of DNA barcoding for detecting quarantine fungi. *Phytopathology*, *103*(11), 1103–1107. https://doi.org/10.1094/PHYTO-12-12-0321-R

Gisi, U., & Sierotzki, H. (2015). Oomycete Fungicides: Phenylamides, Quinone Outside Inhibitors, and Carboxylic Acid Amides. In H. Ishii & D. Hollomon (Eds.), *Fungicide Resistance in Plant Pathogens* (pp. 145–174). Springer Japan. https://doi.org/10.1007/978-4-431-55642-8

Gisi, Ulrich, & Sierotzki, H. (2008). *Fungicide modes of action and resistance in downy mildews* (A. Lebeda, P. T. N. Spencer-Phillips, & B. M. Cooke (eds.); pp. 157–167). Springer Netherlands. https://doi.org/10.1007/978-1-4020-8973-2_12

Hamilton, D. (2002). *Metalaxyl-m (212)* (Issue 212). http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Evaluation04/MetalaxylM.pdf

Hobbelen, P. H. F., Peveley, N. D., & Bosch, F. (2014). The Emergence of Resistance to Fungicides. *PLoS ONE*, *9*(3), 1–14. https://doi.org/10.1371/journal.pone.0091910

Hudspeth, D. S. S., Nadler, S. A., & Hudspeth, M. E. S. (2000). A COX2 molecular phylogeny of the Peronosporomycetes. *Mycologia*, *92*(4), 674–684. https://doi.org/10.2307/3761425

Ishii, H. (2006). Impact of fungicide resistance in plant pathogens on crop disease control and agricultural environment. *Japan Agricultural Research Quarterly*, *40*(3), 205–211. https://doi.org/10.6090/jarq.40.205

Kammarnjesadakul, P., Palaga, T., Sritunyalucksana, K., Mendoza, L., Krajaejun, T., Vanittanakom, N., Tongchusak, S., Denduangboripant, J., & Chindamporn, A. (2011). Phylogenetic analysis of *Pythium insidiosum* Thai strains using cytochrome oxidase II (COX II) DNA coding sequences and internal transcribed spacer regions (ITS). *Medical Mycology*, *49*(3), 289–295. https://doi.org/10.3109/13693786.2010.511282

Kughur, P. G., & Audu, O. (2015). Effects of intensive agricultural production on the environment in Benue State, Nigeria. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, *8*(8), 7–11. https://doi.org/10.9790/2380-08810711

León, C. De. (1984). *Maize Diseases: a guide for field identification* (1st ed.). CIMMYT. https://repository.cimmyt.org/bitstream/handle/10883/3707/13180.pdf?sequence=1&isAllowed=y

Lukman, R., Afifuddin, A., & Lubberstedt, T. (2013). Unraveling the genetic diversity of maize downy mildew in Indonesia. *Journal of Plant Pathology & Microbiology*, *4*(2), 2–9. https://doi.org/10.4172/2157-7471.1000162

Mathiyazhagan, S., Karthikeyan, M., Sandosskumar, R., & Velazhahan, R. (2008). Analysis of variability among the isolates of *Peronosclerospora sorghi* from sorghum and cornbased on restriction fragment length polymorphismof ITS region of ribosomal DNA. *Archives of Phytopathology and Plant Protection*, *41*(1), 31–37. https://doi.org/10.1080/03235400600628062

Muis, A., Nonci, N., & Pabendon, M. B. (2016). Geographical distribution of *Peronosclerospora* spp., the causal organism of maize downy mildew, in Indonesia. *AAB Bioflux*, *8*(3), 143–155. http://www.aab.bioflux.com.ro/docs/2016.143-155.pdf

Muis, A., Ryley, M. J., Tan, Y. P., Suharjo, R., Nonci, N., Danaatmadja, Y., Hidayat, I., Widiastuti, A., Widinugraheni, S., Shivas, R. G., & Thines, M. (2023). *Peronosclerospora neglecta* sp. nov.—a widespread and overlooked threat to corn (maize) production in the tropics. *Mycological Progress*, *22*(2), 1–7. https://doi.org/10.1007/s11557-022-01862-5

Muis, A., Suriani, Kalqunty, S. H., & Nonci, N. (2018). *Penyakit Bulai pada Tanaman Jagung dan Upaya Pengendaliannya* (1st ed.). Penerbit Deepublish.

Pasteris, R. J., Hanagan, M. A., Bisaha, J. J., Finkelstein, B. L., Hoffman, L. E., Gregory, V., Shepherd, C. P., Andreassi, J. L., Sweigard, J. A., Klyashchitsky, B. A., Henry, Y. T., & Berger, R. A. (2015). The Discovery of Oxathiapiprolin: A New, Highly-Active Oomycete Fungicide with a Novel Site of Action. In P. Maienfisch & T. M. Stevenson (Eds.), *Discovery and Synthesis of Crop Protection Products* (Vol. 1204, pp. 11–149). American Chemical Society. https://doi.org/doi:10.1021/bk-2015-1204.ch011

Pusdatin. (2020a). Outlook Jagung 2020: Komoditas Pertanian Subsektor Tanaman Pangan. In *Pusat Data dan Sistem Informasi Pertanian Kementrian Pertanian*. http://epublikasi.setjen.pertanian.go.id

Pusdatin. (2020b). *Statistik Iklim, Organisme Pengganggu Tanaman dan Dampak Perubahan Iklim 2017-2020*. https://satudata.pertanian.go.id/assets/docs/publikasi/Statistik_Iklim,_OPT_dan_DPI_2017-2020.pdf

Randall, E., Young, V., Sierotzki, H., Scalliet, G., Birch, P. R. J., Cooke, D. E. L., Csukai, M., & Whisson, S. . (2014). Sequence diversity in the large subunit of RNA polymerase I contributes to Mefenoxam insensitivity in *Phytophthora infestans*. *Molecular Plant Pathology*, *15*(7), 664–676. https://doi.org/10.1111/mpp.12124

Rashid, Z., Zaidi, P. H., Vinayan, M. T., Sharma, S. S., & Setty, T. A. S. (2013). Downy mildew resistance in maize (*Zea mays* L.) across *Peronosclerospora* species in lowland tropical Asia. *Crop Protection*, *43*, 183–191. https://doi.org/10.1016/j.cropro.2012.08.007

Robideau, G. P., Cock, A. W. A. M., Coffey, M. D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D. W., Desaulniers, N., Eggerston, Q. A., Gachon, C. M. M., Hu, C. H., Kupper, F. C., Rintoul, T. L., Sarhan, E., Verstappen, E. C. P., Zhang, Y., Bonants, P. J. M., Ristaino, J. B., & Levesque,

C. A. (2011). DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Molecular Ecology Resources*, *11*(6), 1002–1011. https://doi.org/10.1111/j.1755-0998.2011.03041.x

Rustiani, U. S. (2015). Keragaman dan Pemetaan Penyebab Penyakit Bulai Jagung di 13 Provinsi Indonesia [Dissertation Institut Pertanian Bogor]. In *Insitut Pertanian Bogor Repository*. https://repository.ipb.ac.id/handle/123456789/77406

Rustiani, U. S., Sinaga, M. S., Hidayat, S. H., & Wiyono, S. (2015). Tiga spesies *Peronosclerospora* penyebab penyakit bulai jagung di Indonesia. *Berita Biologi*, *14*(1), 29–37. https://e-journal.biologi.lipi.go.id/index.php/berita_biologi/article/view/1860/1745

Sierotzki, H., Quaranta, L., Müller, U., & Gisi, U. (2019). Nucleic Acid Synthesis Inhibitors: Metalaxyl-M. In P. Jeschke, M. Witschel, W. Kramer, & U. Schrimer (Eds.), *Modern Crop Protection Compounds* (1st ed., pp. 949–958). Wiley. https://doi.org/10.1002/9783527699261.ch25

Singh, S. D., Bal, S., & Thakur, D. P. (1996). Problems and strategies in the control of downy mildew. *Proceedings of the International Pearl Millet Workshop*, 1–12. https://oar.icrisat.org/4436/1/CP_377.pdf

Studholme, D. J., Panda, P., Stowasser, E. S. V., Gomzalez, M., Hill, R., Sambles, C., Grant, M., Williams, N. M., & McDougal, R. L. (2019). Genome sequencing of oomycete isolates from Chile supports the New Zealand origin of *Phytophthora kernoviae* and makes available the first Nothophytophthora sp. genome. *Molecular Plant Pathology*, *20*(3), 423–431. https://doi.org/10.1111/mpp.12765

Toffolatti, S. L., Prandato, M., Serrati, L., Sierotzki, H., Gisi, U., & Vercessi, A. (2011). Evolution of QoI resistance in *Plasmopara viticola* oospores. *European Journal of Plant Pathology*, *129*(2), 331–338. https://doi.org/10.1007/s10658-010-9677-y

Waceke, J. W., & Kimenju, J. . W. (2007). Intensive subsistence agriculture: impacts, challenges and possible interventions. *Dynamic Soil, Dynamic Plant*, *1*(1), 43–53. http://www.globalsciencebooks.info/Online/GSBOnline/images/0706/DSDP_1(1)/DSDP_1(1)43-53o.pdf

Widiantini, F., Yulia, E., & Purnama, T. (2015). Morphological variation of *Peronosclerospora maydis*, the causal agent of maize downy mildew from different locations in Java-Indonesia. *Journal of Agricultural Engineering and Biotechnology*, *3*(2), 23–27. https://doi.org/10.18005/JAEB0302002

Yang, C., Hamel, C., Vujanovic, V., & Gan, Y. (2011). Fungicide: modes of action and possible impact on nontarget microorganisms. *ISRN Ecology*, *2011*, 1–8. https://doi.org/10.5402/2011/130289

Zubachtirodin, Saenong, S., Pabbage, M. S., Azrai, M., Setyorini, D., Kartaatmadja, S., & Kasim, F. (2016). *Pedoman Umum PTT Jagung* (3rd ed.). Pusat Penelitian dan Pengembangan Tanaman Pangan. https://repository.pertanian.go.id/server/api/core/bitstreams/a55952f8-4a13-4284-94da-a7da207331c9/content