RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
55 (2) 541-549, 2023
http://doi.org/10.54910/sabrao2023.55.2.26
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

POSSIBLE MORPHOLOGICAL AND CHEMICAL RESISTANCE MECHANISM OF SHALLOTS (*ALLIUM CEPA* VAR *ASCALONICUM*) TO *COLLETOTRICHUM GLOEOSPORIOIDES* PENZ

A. MAHARIJAYA[1,3*], D. KURNIANINGTYAS[1], SOBIR[1,3], S. WIYONO[2,3], and A. PURWITO[3]

[1]Department of Agriculture and Horticulture, Bogor Agricultural University, Indonesia
[2]Department of Plant Protection, Bogor Agricultural University, Indonesia
[3]Center for Tropical Horticulture Studies, Indonesia
*Corresponding author's email: awangmaharijaya@apps.ipb.ac.id
Email addresses of co-authors: dyah.kurnianingtiyas@gmail.com, rsobir@yahoo.com, suryowi@apps.ipb.ac.id, apurwito@ipb.ac.id

SUMMARY

Shallot production has many challenges, including the anthracnose disease caused by the pathogen *Colletotrichum gloeosporioides* Penz. Disease characteristics include severe twisting of leaves, neck elongation, and necrosis of leaves. This disease can cause yield loss of up to 100 percent. In addition to chemical control practices, resistant shallot varieties will be very useful in decreasing losses. However, information about existing shallot varieties resistant to *C. gloeosporioides* is limited. The study objectives are to identify the shallot varieties resistant to *C. gloeosporioides* and identify morphological characters and secondary metabolites of the leaves that may associate with the defense mechanism in shallot. Fourteen shallot varieties underwent a single factor use in a randomized block design in the field and laboratory experiments. The experiment transpired from January to June 2021. Planting healthy shallot bulbs of all genotypes in a polybag containing sterile media continued by artificial inoculation of *C. gloeosporioides* after the plant had 3–5 leaves. The field test showed that the Sumenep variety has the best resistant level to *C. gloeosporioides,* with a disease severity score of about 30.19%. On the other hand, the Blue Lancor variety is most susceptible, with a disease severity score reaching 95.05%. The study also found an indication that a possible resistance of shallots to *C. gloeosporioides* relates to the thickness of the palisade tissue (r =-0.8, $P < 0.001$), with the induced mechanism associated with the detection of the presence and increase of carbamic acid concentrations.

Keywords: Carbamic acid, defense mechanism, palisade tissue, secondary metabolite, Sumenep variety

Key findings: Identifying the resistance of leaf cellular morphology showed that palisade tissue is a character associated with shallot resistance to *C. gloeosporioides*.

Communicating Editor: Dr. Aris Hairmansis

Manuscript received: November 10, 2022; Accepted: February 14, 2023.

Citation: Maharijaya A, Kurnianingtya D, Sobir, Wiyono S, Purwito A (2023). Possible morphological and chemical resistance mechanism of shallots (*Allium cepa* var *Ascalonicum*) to *Colletotrichum gloeosporioides* penz. *SABRAO J. Breed. Genet.* 55(2): 541-549. http://doi.org/10.54910/sabrao2023.55.2.26.

INTRODUCTION

Shallot (*Allium cepa* var *Ascalonicum*) is grown on a large scale in many Asian countries, such as, Indonesia, the Philippines, Thailand, Sri Lanka, and India. Shallot consumption can be in both fresh and processed forms. The use of shallot has been widely known as a flavoring in food or an ingredient in herbal medicines (Grubben and Denton, 2004; Marlin *et al*., 2018; Billa *et al*., 2022). Demand for shallots in Indonesia continues to increase yearly (Pane *et al*., 2019; Fairuzia *et al*., 2022). However, shallot production in Indonesia has several problems, including anthracnose caused by the pathogen *Colletotrichum gloeosporioides* Penz. The disease typically shows severe twisting of leaves, neck elongation, and necrosis of leaves. The causal organisms showed as *C. gloeosporioides* causing the anthracnose symptom. This disease can cause yield loss of up to 100 percent (Herlina *et al*., 2019). Overcoming these problems can result from using varieties resistant to the attack of *C. gloeosporioides*. The use of better disease-resistant types can contribute to an increase in crop production and also help to decrease the use of fungicides (De Marchi *et al*., 2019). Unfortunately, the information about shallot varieties resistant to *C. gloeosporioides* is limited. Although several studies stated that the Sumenep variety was moderately resistant to anthracnose (Galvan *et al*, 1997; Hidayat and Sulastrini, 2016), more studies need pursuing indicated by the absence of newly developed resistant varieties with high yields in Indonesia. A cause of this may be the inability of the Sumenep variety to flower (Galvan *et al*., 1997; Dutta *et al*., 2022). Therefore, screening for resistance to *C. gloeosporioides* is highly required to find good resistance sources in shallot.

In general, plants can defend themselves against pathogen attacks. Pathogens will enter the plant through the stomata or by first destroying plant cell walls. The defense abilities in the plant can be constitutive or inducible. Constitutive means that the defense mechanisms in plants are always expressed, even before pathogen attacks. Inducible refers to defense mechanisms displayed as responses to pathogens' attacks. Classifying the defense mechanism in plants against a pathogen can also be physical or biochemical defense mechanisms. Plants show some physical responses in the wax layer, cuticle tissue, stomata, cell wall, and hypersensitive cell death. Their biochemical reactions that appear include phytoalexin. Phytoalexin is a small molecular weight, antifungal, and antimicrobial compound. For example, in the Brassicaceae plant group, plants will release volatile aldehyde chemical compounds, C_6 alcohol, and acetate and synthesize specific compounds, glucosinolates, when herbivorous pests are present to eat plant leaves. Reports said strawberry plants correlate disease resistance with epidermal tissue thickness and the cutin content of the cuticle (Lara *et al*., 2019). Sadly, there is not much information about the defense mechanisms of shallots against pathogens.

The existing sources of resistance, especially from farmer-planted varieties or near commercial genotypes, are an opportunity to develop new superior varieties faster than using sources of resistance from relatives or wild species. In addition, information on resistance mechanisms will serve breeding activities and plant protection strategies against diseases. This information needs build-up in shallot, although some reports regarding the identification of resistance in the Sumenep variety exist (Galvan *et al.*, 1997; Hidayat and Sulastrini, 2016). Inducible resistance is preferred because plants do not need to produce substrates or form unrequired structures if there is no pathogen attack. Inducible resistance can increase tolerance only when plants experience stress symptoms so that productivity will not decrease and can reduce the use of chemical pesticides (Gupta *et al*., 2016). The study objective sought to obtain information on the resistance and possible resistance mechanism of shallots to the pathogen *C. gloeosporioides* using morphological and metabolomics approaches that could be useful for future breeding programs.

MATERIALS AND METHODS

Screening test

The experiment, conducted from January to June 2021, performed a screening test using 14 shallot varieties as a single factor in randomized block design in the field. Varieties of shallots in this study consisted of Bima Brebes, Biru Lancor, Manjung, Superphilip, Trisula, Tajuk, Kramat-2, Bauji, Bali Karet, Maja Cipanas, Mentes, Pikatan, Pancasona, and Sumenep. The *C. gloeosporioides* isolate came from the Indonesian Vegetable Research Institute and was maintained and prepared in a concentration of $1.0 - 1.5 \times 10^6$ spores/mL.

Healthy shallot bulbs of all genotypes got planted in a polybag containing sterile compost and soil (1:1). Research using pathogens prefer using polybags because it minimizes contamination of the surrounding soil. Previous reports stated that similar results of the anthracnose resistance levels of plant genotypes showed under laboratory, pilot, and field conditions (Kunkeaw *et al*., 2010). Inoculating the pathogen *C. gloeosporioides* started when the plant was two weeks old or the shallot had 4–5 leaves (Galván *et al*., 1997; Hidayat and Sulastrini, 2016). The inoculation of *C. gloeosporioides* isolates proceeded by spraying the *C. gloeosporioides* suspension on the shallot leaves. Before inoculation, the plant leaves are moistened with water and after inoculation, a wet cloth covered the leaves for 48 h to maintain humidity > 90% (Rodríguez-Salamanca *et al*., 2018). The experiment setup also included a set of genotypes sprayed without isolates of *C. gloeosporioides* (water only) as a control in the same greenhouse using a distance of 15 m and a net as a barrier. Observations ensued for seven days after inoculation when significant differences occurred between susceptible and resistant plants to the *C. gloeosporioides* pathogen. The estimated severity of anthracnose symptoms in leaves was as follows: severity = (infected leaf area/total leaf area) × 100% (Miller-Butler *et al.,* 2019).

Leaf anatomical test

Conducting leaf anatomical test used three genotypes of selected shallot genotypes from the screening resistance test (Table 1). Sumenep represented the resistant level against *C. gloeosporioides*; Biru Lancor represented the susceptible level against *C. gloeosporioides*, and Superphilip represented a level between Sumenep and Biru Lancor. The observations under an electron stereo microscope used a 10 × 40 magnification level at 30 days after inoculation on the thickness of the following tissues: cuticle, epidermal, palisade, and sponge, and on the length and width of vascular vessels, stomata density, index, and length. Observations were on three genotypes' set with infestation and the other three without an infestation of *C. gloeosporioides.*

Table 1. Mean values of the severity of anthracnose disease in leaves of various shallot genotypes.

Varieties	Weeks after inoculation (%)				
	1	2	3	5	7
Sumenep	02.72 ± 1.01 d	5.28 ± 1.89 d	07.89 ± 3.86 e	16.68 ± 6.85 e	30.19 ± 5.34 c
Tajuk	07.08 ± 1.22 cd	21.73 ± 4.75 bcd	28.88 ± 3.56 d	55.27 ± 8.45 d	75.76 ± 4.94 b
Kramat-2	08.60 ± 1.02 bc	14.87 ± 2.64 cd	37.28 ± 13.43 abc	66.47 ± 11.07 cd	78.48 ± 3.34 b
Maja	08.65 ± 1.01 bc	25.92 ± 8.02 bc	49.26 ± 11.12 abc	71.78 ± 10.02 bc	82.51 ± 8.00 ab
Superphilip	12.90 ± 1.32 ab	27.24 ± 7.64 bc	45.47 ± 12.04 abcd	73.09 ± 2.23 b	83.95 ± 7.64 ab
Mentes	08.31 ± 1.01 bc	17.48 ± 6.95 cd	37.21 ± 11.45 bcd	69.42 ± 8.89 c	84.73 ± 8.24 ab
Bali Karet	11.11 ± 1.68 abc	20.91 ± 9.82 cd	48.60 ± 12.02 abcd	72.14 ± 6.54 bc	85.48 ± 6.84 ab
Pikatan	07.17 ± 1.21 cd	14.80 ± 6.12 cd	38.86 ± 12.44 bcd	72.60 ± 7.12 bc	85.37 ± 7.01 ab
Trisula	10.72 ± 2.54 abc	17.71 ± 10.01 cd	33.83 ± 5.78 cd	72.58 ± 6.88 bc	85.82 ± 6.21 ab
Pancasona	10.86 ± 2.18 abc	33.73 ± 8.90 bc	47.94 ± 9.54 abcd	74.98 ± 12.34 abc	85.07 ± 6.11 ab
Manjung	09.74 ± 1.08 bc	26.49 ± 4.56 bc	47.05 ± 13.42 abcd	77.48 ± 9.45 abc	87.20 ± 5.43 ab
Bima Brebes	11.86 ± 1.44 abc	24.22 ± 4.32 bc	44.26 ± 12.98 abcd	78.95 ± 8.34 abc	88.76 ± 6.14 ab
Bauji	10.89 ± 2.02 abc	39.68 ± 8.14 ab	56.05 ± 7.34 ab	85.82 ± 5.65 ab	93.18 ± 4.12 a
Biru Lancor	15.44 ± 0.89 a	53.91 ± 3.60 a	61.87 ± 4.40 a	89.29 ± 4.21 a	95.05 ± 2.11 a

Numbers followed by the same letters in the same column are not significantly different based on Duncan's advanced test with a level of 5%.

Secondary metabolite test

The metabolomic test used two selected shallot varieties, resistant and susceptible to *C. gloeosporioides,* in a nested design, using the same set of leaves from the leaf anatomical test, taken ± 30 days after inoculation. Dipping the selected leaves in liquid nitrogen and putting them in a centrifuge tube, their analysis used the GCMS Pyrolysis tool Shimadzu GCMS QP 2010. The freshly picked leaves were immediately dried using an oven at 40 °C for three days. All parts of the harvested leaves in each plant underwent mixing and pooling to make a representative leaf sample. Later, placing the leaf samples in 99.9% pure ethanol gained maceration for three days, with 10 mL of the macerated sample transferred to a new tube and evaporated for 1 h at 40 °C.

Employing an Agilent Technologies 7890 A Gas Chromatograph (GC) coupled with a 5975 C Mass Spectrometer (MS) system analyzed the metabolite contents of the shallot leaves. The GC equipment comprised HP Ultra 2 capillary columns (30 m, 0.25 mm i.d., 0.25 mm film thickness; Agilent, Santa Clara, CA, USA). In the study, the injection volume was 5 µL with an 8:1 split ratio and 250 °C injection port temperature. The initial oven temperature was at 70 °C held for 0 min, increased at 3 °C/min to 150 °C, held for 1 min, and finally raised at 20 °C/min to 250 °C and held for 26 min. Using carrier gas (helium) has a constant flow rate of 1.2 mL/min was used. The MS system setting was at 70 eV electron impact ionization, EM voltage of 2318 V, source 230 °C, quadrupole 150 °C, solvent delay: 2.5 min, and full scan (40–650 a.m.u) at a scan rate of 2.42 scan s-1.

Applying an untargeted metabolomics approach processed the raw GC-MS using Masslynx 4.0 software package. For each metabolite, comparing the resistance scores of the variety to *C. gloeosporioides* observed from the screening test versus the relative abundance and concentration of metabolites transpired to correlate the metabolites data with resistance data. The data acquisition of GC-MS analysis used MS-Chemstation G1701-DA with Wiley and NIST spectral libraries. Metabolites that showed mass spectra with match factors quality of $\geq$ 90 were considered as putatively identified substances (de Vos *et al*., 2007).

Statistical analysis

Data underwent ANOVA analyses, followed by the Duncan Multiple Range Test for mean separation using SAS software. Calculating Spearman rank correlations determined the association between disease severity and various cellular variables in shallot.

RESULTS AND DISCUSSION

Screening test

Signs of damage from the leaves, like the yellowing of the leaf tip, the presence of black and white patches on the leaves, bent leaves, and dried leaves with many black spots, appeared one week after inoculation with *C. gloeosporioides* isolate. All genotypes that served as a control without *C. gloeosporioides* were free of damage symptoms.

Noted different responses to *C. gloeosporioides* of the genotypes started one week after inoculation (Table 1). Sumenep showed a significantly low severity value compared with the other genotypes. Contrarily, Biru Lancor and Bauji were consistently the most severe genotypes during the experiment. No genotype was able to recover from the damage caused by the pathogen. The findings support previous reports about the tolerance of the Sumenep variety to *C. gloeosporioides* (Hidayat and Sulastrini, 2016). However, the two previously reported tolerant varieties, i.e., Bali Karet and Maja, exhibited susceptibility in the study. However, the study identified another alternative, Tajuk, which showed lower severity, compared with the other genotypes, up to five weeks after inoculation. As reports on Sumenep not producing a flower that might cause a problem in the utilization as a source of resistance for breeding (Dutta *et al*., 2022), the partial resistance found in Tajuk could be useful and noteworthy, in addition to fungicide application to control *C. gloeosporioides*.

Leaf anatomical test

C. gloeosporioides is an airborne disease that damages leaves first in the field. Later, the infection moves to the neck region or above the basal plate. Further, the symptoms superficially will appear on bulbs, spread to inner tissue, causing deformation and rotting of

the bulb, and in the worst case, continue to complete death of the plant (Dutta *et al.*, 2022). Since the leaf infection could result in yield reduction and rotting of bulbs during and after storage, the resistance properties in the shallot leaves are most relevant, determining the success of shallot production.

The results of identifying resistance of cellular levels of shallots (Table 2) show a highly significant effect on the thickness of the cuticle, epidermal, and palisade tissues and the vascular length, vascular vessel width, and stomatal index. The infection pathogen of *C. gloeosporioides* in this study greatly impacted the thickness of the cuticle, epidermal, and palisade tissues, stomata density, and stomatal width. The thickness of the cuticle, epidermal, and palisade tissues, the vascular vessel length and width, and stomata density, index, length, and width appear in Tables 3, 4, and 5. In the cuticle tissue, the extreme thickness increase resulted in the variety Superphilip at 13.16 µm with shallot inoculation by *the C. gloeosporioides* pathogen. In the palisade tissue, the Sumenep variety provided the highest increase among other varieties at 63.14 µm with shallot inoculation of the pathogens. Figure 1 shows distinct differences in the cuticle, epidermal, and palisade structure of the shallot leaves, non-infected versus infected with *C. gloeosporioides* in susceptible variety (Biru Lancor) and resistant variety (Sumenep), with associated findings displayed in Table 3.

Table 2. Recapitulation of variance in various cellular variables in shallots and related with resistance against *C. gloeosporioides.*

SK	Thickness of cuticle	Thickness of epidermal	Thickness of palisade	Vascular vessel length	Vascular vessel width	Stomata density	Stomata index	Stomata length	Stomata width
G	<0.0001**	<0.0001**	0.0049**	0.0032**	0.0003**	0.0243*	0.0012**	0.7416^{ns}	0.0699^{ns}
DS	<0.0001**	<0.0001**	<0.0001**	0.4157^{ns}	0.0505^{ns}	0.0043**	0.0007**	0.5557^{ns}	0.0020**
G × DS	0.2273^{ns}	00.0019**	0.0009**	0.0033**	0.0005**	<0.0001**	0.0005**	<0.0001**	0.0071**

**, * = Significant effect at the 1% and 5% levels, respectively, ^{ns} = not significant. G = genotype, DS = disease severity, G × DS = interaction of genotype and disease severity.

Table 3. The thickness of the cuticle, the thickness of the epidermal, and the thickness of the palisade in three shallot genotypes with different resistance to *C. gloeosporioides.*

Genotype	The thickness of cuticle (µm)		The thickness of epidermal (µm)		The thickness of the palisade (µm)	
	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated
Sumenep	9.16±2.11ab	12.91±1.93aa	25.96±1.00b	30.53±1.12a	64.59±2.91d	127.73±9.01a
Superphilip	3.58±2.43cd	8.69±3.13ca	18.83±1.86c	31.99±0.98a	3.93±6.44cd	106.79±4.21b
Biru Lancor	6.58±2.03bb	9.88±1.98ba	14.80±2.68c	16.62±2.64c	9.87±5.98cd	81.73±6.86c

Numbers followed by the same capital letters on the same line are not significantly different based on Duncan's test with a 5% level. [a] = Numbers followed by the same letters in the same column is not significantly different based on Duncan's test with a 5% level.

Table 4. Vascular vessel length, vascular vessel width, and stomata density in three shallot genotypes with different resistance to *C. gloeosporioides.*

Genotype	Vascular vessel length (µm)		Vascular vessel width (µm)		Stomata density (stomata/mm^2)	
	Non inoculated	Inoculated	Non inoculated	Inoculated	Non inoculated	Inoculated
Sumenep	73.97±3.11b	87.79±5.12a	62.42±1.12b	55.87±5.32c	20.48±3.34b	15.34±0.98c
Superphilip	90.38±6.77a	100.92±11.05a	62.23±1.08b	70.42±2.43a	15.00±1.00c	28.75±1.48a
Biru Lancor	89.98±9.87a	73.77±4.12b	61.41±2.89bc	48.87±2.82d	16.87±1.31bc	19.79±4.21b

Numbers followed by the same letters in the same column are not significantly different based on Duncan's test with a 5% level.

Table 5. Stomata index and stomata width in three shallot genotypes with different resistance to *C. gloeosporioides.*

Genotype	Stomata index (mm)		Stomata width (µm)	
	Non inoculated	Inoculated	Non inoculated	Inoculated
Sumenep	15.57±1.15c	14.02±1.17c	6.12±1.43c	13.45±0.68a
Superphilip	15.20±1.08c	22.09±1.20a	12.11±1.02b	12.51±0.70b
Biru Lancor	15.40±0.80c	18.64±2.24b	10.31±1.18b	11.73±0.88b

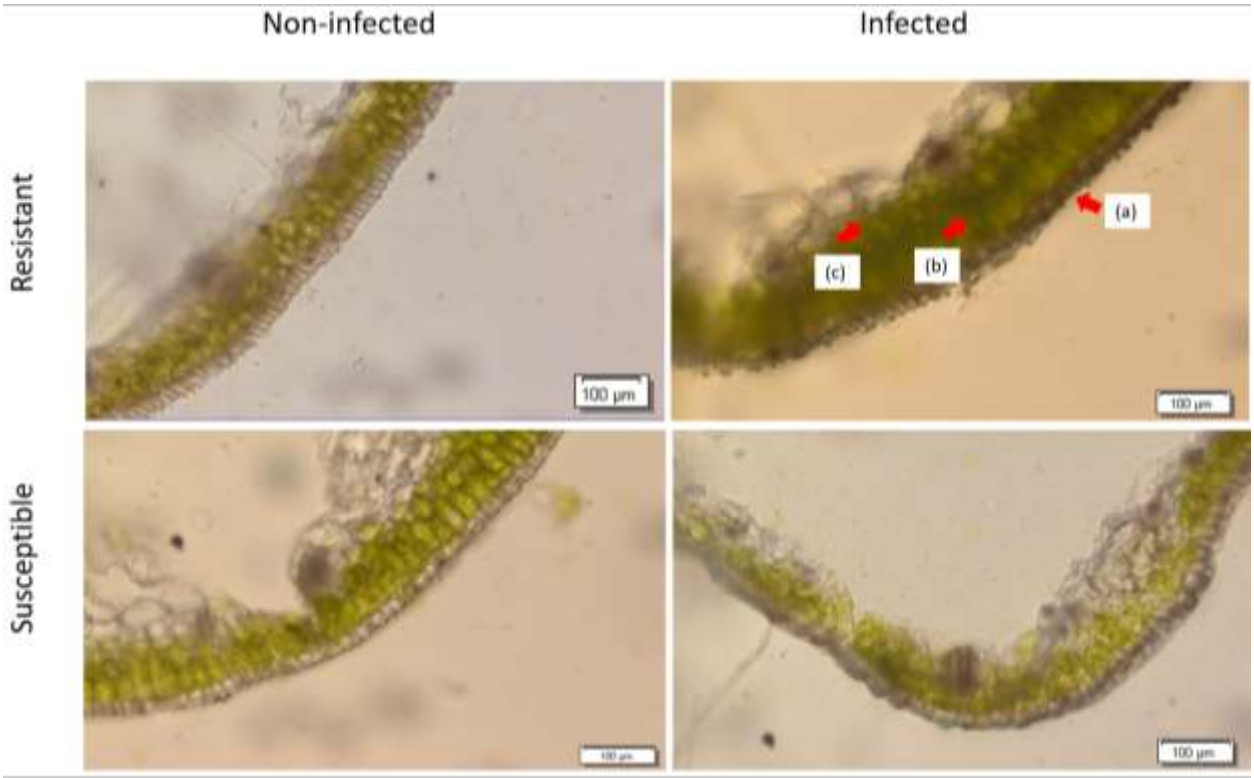

Figure 1. Cuticle (a), epidermal (b), and palisade (c) structure of leaves of shallot, non-infected and infected with *Colletotrichum gloeosporioides* in susceptible variety (Biru Lancor) and resistant variety (Sumenep) under electron stereo microscope using 10 × 40 magnification level.

The Sumenep and Superphilip varieties have increased the thickness of the vascular tissue, but the Biru Lancor variety has decreased thickness (Table 4). The decreasing thickness of vascular tissue width occurred in Sumenep and Biru Lancor varieties after pathogen inoculation. The pathogen has two methods for infecting plants. The first is by directly damaging the cell wall of plants, and the second is by penetrating through water on the stomata (Freeman and Gwyn, 2008).

Based on the correlation analysis, the thickness of epidermal and palisade tissues showed connectivity with the resistance level (Table 6). It means that the more resistant to anthracnose the shallot variety, the palisade and epidermal tissues will be thicker than the susceptible shallot variety to anthracnose. The epidermal and palisade tissues have different thickness increases. Palisade tissue has the most notable response to the other variables. Thickening of the palisade tissue in all three levels of resistance occurs when inoculated by *C. gloeosporioides*. The Sumenep variety has the highest significant increase in thickness of the palisade tissue. In contrast, the Biru Lancor variety has the smallest increase in thickness of the palisade tissue.

The palisade tissue is between the upper and lower tissue of the epidermal tissue. Pathogens will break down the epidermal cell wall to enter the palisade tissue. In this tissue, pathogens will grow and segregate to form a thin layer structure used in attacking vascular

Table 6. Correlation between disease severity and various cellular variables in shallot and related with resistance against *C. gloeosporioides*.

Variables	Disease severity	Thickness of epidermal	Thickness of palisade	Vascular vessel length	Stomata density	Stomata index	Stomata width
Disease severity	1.000						
Thickness of epidermal	- 0.803**	1.000					
Thickness of palisade	- 0.803**	0.475^{ns}	1.000				
Vascular vessel length	0.289^{ns}	- 0.671*	- 0.140^{ns}	1.000			
Stomata density	0.683*	- 0.699*	- 0.587*	0.426^{ns}	1.000		
Stomata index	0.567^{ns}	- 0.783**	- 0.350^{ns}	0.727**	0.811**	1.000	
Stomata width	0.662*	- 0.706*	- 0.643*	0.608*	0.657*	0.573^{ns}	1.000

**, * = significant effect at the 1% and 5% levels, respectively, ^{ns}= not significant.

vessels (xylem) (Rott *et al*., 2017). Previous studies reported disease resistance response in palisade tissue in coffee and tomato leaves when attacked by pathogens *P. syringae* pv *garcae*. Hypertrophy and hyperplasia occur in the palisade tissue of coffee leaves after 20 days from inoculation, causing an increase in palisade tissue thickness. Hypertrophy and hyperplasia occur due to hormone induction (Rodrigues *et al*., 2015). Increasing the palisade tissue thickness on leeks is thought to be an inducible defense mechanism because the response (thickening tissue) appears when the shallot is inoculated with the *C. gloeosporioides* pathogen.

Secondary metabolite test

Plants can release chemical compounds, such as, phytoalexin when experiencing biotic or abiotic stress (Setiawati *et al*., 2016). Plants can produce secondary metabolites directly or produce some proteins which are toxic to prevent attacks (War *et al*., 2012). Study results show that shallot resistant to *C. gloeosporioides* (Sumenep) have different profiles of metabolites compared with the susceptible variety (Biru Lancor). The Sumenep variety has 18 secondary metabolites, and the Biru Lancor variety has 12. Table 7 shows the concentration values in secondary metabolites of Sumenep and Biru Lancor varieties when inoculated and non-inoculated by *C. gloeosporioides* pathogens. The carbamic acid compound is the only compound with the highest concentration in the resistance variety (Sumenep) and has not appeared in the susceptible variety (Biru Lancor). The other compound appears with high concentration when plants are healthy, but when the pathogen has inoculated the plants, the compound decreases rapidly until almost zero concentration. It happens because the plants produce secondary metabolites only for defense mechanisms (Iriti and Faoro, 2009).

The carbamic acid compound in the resistant variety was 1.03% in controlled plants, and the concentration increased to 54.57% in inoculated plants (Table 7). Carbamic acid is a natural compound in plants commonly found as carbamate, which works on pesticides (Senol *et al*., 2019). Carbamic acid serves as an elicitor in the jasmonic acid (JA) transduction pathway. Jasmonic acid is a phytohormone in the defense mechanism of plants associated with physical damage caused by herbivorous pests and pathogens in plants (Bruinsma *et al*., 2010). There are three phytohormone transduction pathways related to disease resistance, namely, jasmonic acid, salicylic acid, and ethylene. Jasmonic acid plays a role in inducing volatile emissions, increasing toxic levels, and increasing resistance genes' expressions (Scala *et al*., 2013). Elicitor is an active component in the chemical defense response in plants. Elicitor compounds will appear after the detection of pathogen in plants, recognized by protein receptors that induce the expression of resistance genes (Garcia-Brugger *et al*., 2006). The expression forms of resistance genes include phytoalexin biosynthesis, strengthening of plant cell walls associated with phenylpropanoid compounds, synthesizing endurance enzymes, and accumulating pathogenesis-related proteins (Thakur and Sohal, 2013; Mejía-Teniente *et al*., 2015).

Table 7. The concentrate of secondary metabolites produced by resistant and susceptible varieties in inoculated and non-inoculated plants by *C. gloeosporioides.*

No.	Metabolites	Susceptible variety (Biru Lancor)		Resistant variety (Sumenep)	
		Not inoculated (%)	Inoculated (%)	Not inoculated (%)	Inoculated (%)
1	Acetic acid, anhydride (CAS) Acetic oxide	n.d.	n.d.	n.d.	2.80
2	11-Tetradecen-1-ol, acetate, (Z)-(CAS) cis-11-Tetradecenyl acetate	n.d.	8.24	n.d.	4.12
3	cis-1,3-Dideuterio-1,3-cyclohexandiamine	n.d.	n.d.	n.d.	5.95
4	Carbamic acid, monoammonium salt (CAS) Ammonium carbamate	n.d.	n.d.	1.03	54.57
5	2-propanone, 1-hydroxy-(CAS) Acetol	n.d.	n.d.	4.72	n.d.
6	Phenol, 2-methoxy- (CAS) Guaiacol	n.d.	n.d.	2.26	n.d.
7	Cyclopropyl carbinol	5.48	n.d.	5.02	n.d.
8	1-Butanol, 3-methyl-, acetate (CAS) Isoamyl acetate	n.d.	n.d.	10.07	n.d.
9	Neophytadiene	19.53	n.d.	12.28	n.d.
10	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, (CAS) Phytol	5.64	n.d.	3.60	n.d.
11	Hexadecanoic acid (CAS) Palmitic acid	3.39	n.d.	1.60	n.d.
12	16-Hentriacontanone (CAS) Palmitone	26.10	n.d.	10.33	n.d.
13	Acetic acid (CAS) Ethylic acid	n.d.	n.d.	12.32	n.d.
14	thiophene, 2,4-dimethyl-(CAS) 2,4-Dimethylthiphene	n.d.	n.d.	1.10	n.d.
15	Phenol, 4-ethenyl-2-methoxy	1.08	n.d.	0.70	n.d.
16	Hexadecanamide (CAS) Amide 16	1.40	n.d.	1.23	n.d.
17	Nonacosane (CAS) n-Nonacosane	n.d.	n.d.	0.64	n.d.
18	Tetratetracontane (CAS) n-Tetratetracontane	5.59	n.d.	1.98	n.d.
19	Acetic acid, pentyl ester (CAS) n-Amyl acetate	n.d.	6.61	n.d.	n.d.
20	formamide (CAS) Methanamide	12.85	n.d.	n.d.	n.d.
21	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate	1.04	n.d.	n.d.	n.d.
22	9,12-Octadecadienoyl chloride	0.85	n.d.	n.d.	n.d.

n.d. = not detected

CONCLUSIONS

The observation of the severity of anthracnose in shallot showed that the Sumenep was the most resistant variety to *C. gloeosporioides,* and the Biru Lancor variety was the most susceptible. Identification of the resistance of leaf cellular morphology indicated that palisade tissue is a character associated with shallot resistance to *C. gloeosporioides*. The Sumenep variety has the highest increase in thickness when attacked by *C. gloeosporioides*. Identification of secondary metabolites on shallots revealed that carbamic acid is an elicitor compound in the jasmonic acid transduction pathway and induced the response of shallot resistance to C*. gloeosporioides*.

ACKNOWLEDGMENTS

This research received funding from the Center for Tropical Horticulture Studies, Bogor Agricultural University, Indonesia, and a World Class Research Grant from the Ministry of Research, Indonesia.

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