



Research Article

Determination of anthracnose (*Colletotrichum gloeosporioides*) resistance group in shallot (*Allium cepa* var. *aggregatum*)

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ABSTRACT

Shallot anthracnose, caused by *Colletotrichum gloeosporioides*, is a devastating disease in a tropical country with high humidity and rainfall. Chemical control of anthracnose is neither economical nor eco-friendly, and genetic resistance is considered an efficient management method. This study aimed to determine the resistance groups of several shallot varieties and predict resistance heritability characteristics. In this study, a total of 13 Indonesian shallot varieties were evaluated for anthracnose resistance and separated into two groups, resistance and susceptible, based on K-means clustering developed by using disease resistance/susceptibility characteristics such as incubation period, disease incidence, disease severity, and spot diameter. The results indicate that the Agrihorti, Maja Cipanas, Batu Ijo, and Rubaru varieties were identified as resistant groups based on lower disease severity and incidence, smaller spot diameter, and longer incubation period. Maja Cipanas and Rubaru were more consistent in all variables, which is recommended as a source of genetic resistance genotypes. On the other hand, the Biru Lancor, Bima Brebes, Srikayang, Violetta, Slupu Merah, Pancasona, Sakato, Katumi, and Kuning varieties were identified as susceptible groups based on high disease severity and incidence, large spot diameter, and short incubation period.

Keywords: susceptibility characteristics, genetic resistance, k-means clustering, disease severity

INTRODUCTION

Shallots (*Allium cepa* var. *aggregatum*) hold a significant role as agricultural plants and indispensable vegetable spices in numerous countries. They serve as fundamental ingredients in culinary traditions and play a crucial role in traditional medicine, rendering their presence irreplaceable. The average global yield is 19.25 tons ha⁻¹, it places second after tomatoes and is estimated to contribute 9% to the total share of vegetables in the world (FAO, 2021; Muhie, 2022).

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Shallots are primarily grown in Asia, where the total area worldwide is 3991.51 thousand hectares, and as much as 55% is grown in Asia (Miassi et al., 2018; Muhie, 2022). China is the country with the highest yield of shallot (38.55 tons ha⁻¹). However, shallot yield in several countries is still below the global average yield such as Indonesia 10.23 tons ha⁻¹, Ethiopia 10.02 tons ha⁻¹, Pakistan 12.80 tons ha⁻¹ (Etana et al., 2019; Khan et al., 2020; Saptana et al., 2021). The main causes of low shallot productivity may due to shallot varieties/seeds, improper agronomic practices, and pest and disease disturbances (Alberto, 2014).

Anthracoze disease is an important disease in shallot cultivation. Anthracnose disease caused by *Colletotrichum gloeosporioides* is a severe disease that can reduce yield by up to 90% (Basuki, 2014; Mishra et al., 2014), damage nearly 100% of crops, and decrease sugar and protein content (Alberto, 2014). Disease control is commonly by applying intensive fungicides consequently farming costs increase markedly and increasing health issues (Sahara et al., 2018). Therefore, it is important to implement cost-effective methods such as anthracnose-resistant shallot varieties that can be obtained through breeding efforts.

The development of shallot varieties resistant to anthracnose commences by characterizing disease-resistant genetic resources. Genetic resources with the character of resistance to anthracnose are essential for developing resistant varieties. The assessment of shallot resistance can be conducted through phenotypic or molecular characterization methods. Molecular characterization is reported to be conducted using molecular markers, such as ISSR, which differentiate three groups of shallot resistance to Fusarium wilt (Aprilia et al., 2020). Molecular characterization to evaluate resistance to anthracnose has also been carried out with RAPD markers (Panday et al., 2002). Another characterization technique easily used is phenotypic testing through artificial inoculation.

Phenotypic characterization by estimating several genetic parameters related to plant resistance can establish a base population for developing shallots resistant to anthracnose. Many activities have been carried out to characterize shallot genetic resources related to disease resistance, such as against Fusarium wilt (Aprilia et al., 2020), and Purple blotch (Nur, 2017). Information regarding genetic resources that are resistant to anthracnose is still rarely reported. Several previous studies have identified the resistance of shallot genetic resources to anthracnose, such as the Sumenep variety (Hekmawati et al., 2018; Hidayat & Sulastrini, 2016). However, the information and research methods that have been done previously need to be further improved to prove that the determination of anthracnose-resistant genotypes is more reliable and adds value to this kind of research. On the other hand, there are few test reports on different varieties with information on quantifying their resistance, such as disease incidence and severity. Sources of genetic material with resistance to anthracnose can be used as parents in developing shallot varieties (Alberto, 2014). Therefore, the first step to assembling shallot varieties resistant to anthracnose disease must be characterized to form the base population at the start of cultivated varieties. This study aimed to determine the resistance groups of several shallot varieties and predict resistance heritability characteristics.

MATERIALS AND METHODS

Plant material

A collection of shallots was tested in a greenhouse from April to June 2022 located at Sukamantri Experimental Farm, Bogor, Indonesia. The plant material used is collected from breeder farmers (Table 1). The plant material was then treated with hot water treatment at 50 °C for 15 minutes to ensure bulbs were clear from pathogens (Wibowo et al., 2016). The plant was grown in a polybag containing sterilized media of manure and soil mixture (1:1, v/v). After a three to four weeks growth period was used in a screening test.

Fungal inoculum

Isolates of *Colletotrichum gloeosporioides* used in the screening experiment were obtained from the Plant Health Services (Klinik Tanaman) collection at IPB University, Indonesia. The fungus was propagated in vitro using potato dextrose agar (PDA) media in the darkroom at 26 °C. Sporulation of the fungus occurred after approximately 12 days. The spore suspension was produced by rubbing the fungus, which was fully grown with acervulus, in sterilized water. It was then filtered using a syringe filter of 0.2 µm and diluted to a concentration of 1-1.5 x 10⁶ spores per milliliter.

Table 1. Varieties used in the experiment and their origin.

No	Variety name	Origin
1	Agrihorti I	Indonesia, Indonesian Vegetable Research Institute (IVEGRI)
2	Bima Brebes	Central Java, Indonesia
3	Batu Ijo	East Java, Indonesia
4	Biru Lancor	East Java, Indonesia
5	Kuning	Indonesia, Indonesian Vegetable Research Institute (IVEGRI)
6	Katumi	Indonesia, Indonesian Vegetable Research Institute (IVEGRI)
7	Maja Cipanas	West Java, Indonesia
8	Pancasona	Indonesia, Indonesian Vegetable Research Institute (IVEGRI)
9	Rubaru	East Java, Indonesia
10	Sakato	West Sumatra, Indonesia
11	Srikayang	Yogyakarta, Indonesia
12	Slupu Merah	Bengkulu, Indonesia
13	Violeta	Indonesia, Indonesian Vegetable Research Institute (IVEGRI)

Screening test

The plants with 3 to 5 fully expanded leaves were manually inoculated with *C. gloeosporioides*. The inoculation was performed using a hand sprayer which consisted of 1-1.5x10⁶ spores per milliliter. The inoculated plant was covered with a plastic bag to maintain the humidity near 100% for two days. After removing the plastic bags, water was sprayed over the plant using a sprayer twice a day in the morning and evening on a daily basis. A control treatment was sprayed using sterilized water and then covered with a plastic bag to maintain the humidity. Only plants that showed anthracnose symptoms like necrotic spots were scored. Yellowish and older necrotic leaves and tips were not considered to be scored. The incubation period (day after inoculation) was estimated by the observed first day the symptom appeared. Spot diameter (millimeter) was observed by calculating the average diameter from the minimum and maximum diameter. The disease was scored every week to know disease severity (%), and disease incidence (%) was scored every two days after inoculation, as described by Allen (1983). The formula used to calculate disease severity was

$$DS = \frac{\sum(n_i \times v_i)}{Z \times N} \times 100\%$$

DS= Disease severity, n_i= number of infected plants, v_i= infected plant score category, Z= the highest category score, N= number of observed plants. An ordinal scale was used to score the individual plant reaction to the fungus was adapted from Alberto (2014): 0 (No symptoms), 1 (Oval-shaped white spots on leaves, 1-5 spots present), 2 (Large oval-shaped white spots, 1-2 large spots present), 3 (Sunken necrotic white spots with the appearance of acervuli), 4 (Appearance of concentric orange acervuli spots, leaves start to turn yellow), and 5 (Dark concentric acervuli spots causing death in leaf tissue).

The experiment was conducted in a randomized complete block design with three blocks and thirteen genotypes for each block. The experiment unit contains fifteen individuals which used as sample for the test. The total population had 195 plants per

block and 585 plants for the entire unit experiment. The control treatment unit was planted in different areas to ensure the bulbs were used clear from other pathogens.

Data analysis

The analysis of variance (ANOVA) test was arranged on each resistance character to estimate the differences between shallot varieties and performed using RStudio 2022.07.2 Build 576 software. For any significant differences in the source of varietal diversity, further testing was done using the Tukey test at $\alpha = 5\%$.

Pearson's correlation analysis was employed to further assess resistance characters to estimate the correlation between characters. For those that had a strong correlation, further grouping varieties was obtained based on four resistance characters (incubation period, disease incidence, disease severity, spot diameter) using K-means cluster analysis. The results obtained were then grouped into varieties categorized into two groups, namely 'Resistant' and 'Susceptible'. To estimate the comparison of the contribution of environmental and genetic influences, the broad sense heritability value of the resistance character was estimated using a formula of Poehlman & Sleeper (1995).

RESULTS AND DISCUSSION

Resistance to anthracnose

The result of analysis of variance (ANOVA) at a 5% level indicated that the genotypes had a significant effect ($p < 0.01$) on the incubation period, disease incidence, disease severity, and spot diameter. Symptoms caused by anthracnose infection in shallot plants appear on the leaves with a distinctive appearance in the form of concentric spots. Spot symptoms can be found at the base of the leaf, the middle to the tip. Early spots appear as small white spots, which then enlarge to form concentric rings in which brownish-yellow spots are enlarged, this part contains acervuli (Figure 1a-b) and then causes the death of leaf tissue (Figure 1c).



Figure 1. Shallot genotype symptom appearance after anthracnose inoculation indicated by arrow. (A) spot on shallot distal leaf, (B) spot on leaf, (C) death of the leaf tissue.

Genotypic responses to anthracnose can vary depending on genetic background and environmental conditions. This study characterizes the variance of resistance/susceptibility traits among shallot varieties in Indonesia that have the potential to be used as parents in plant breeding programs. Based on the symptoms, the

resistance level of several shallot genotypes can be seen based on the values of the incubation period, disease incidence, disease severity, and spot diameter.

Table 2. Number of disease incidence, incubation period, disease severity, and spot diameter of shallot anthracnose.

Variety	Incubation period (DAI)*	Disease incidence (%)*	Disease severity (%)*	Spot diameter (mm) *
Agrihorti I	3.33abc	78.57ab	33.21a	5.83bcd
Bima Brebes	4.33abc	85.00bc	72.22cd	8.16abc
Batu Ijo	4.33abc	86.90bc	24.52a	5.50bcd
Biru Lancor	2.00c	100.00c	83.11d	10.83a
Kuning	2.67bc	100.00c	55.90b	6.67bc
Katumi	3.67abc	90.48bc	70.24cd	5.33cd
Maja Cipanas	5.00ab	63.69a	23.57a	6.33bcd
Pancasona	2.33c	95.24bc	70.83cd	8.33abc
Rubaru	5.33a	62.10	20.95a	2.67d
Sakato	3.00abc	95.83bc	75.56cd	7.67abc
Srikayang	2.67bc	95.83bc	81.27cd	9.17ab
Slupu Merah	3.67abc	95.24bc	73.61cd	7.50abc
Violeta	2.33c	83.33bc	68.44c	7.83abc

Note: *Value followed by the same letter in the same column are not significantly different based on Tukey Test at $\alpha = 5\%$.

One genotype, i.e., Rubaru with a value of 5.33 days after inoculation, had the most extended disease incubation period compared to the other genotypes (Table 2). For the disease incidence, two genotypes were found to have a lower incidence than the other genotypes, i.e., Rubaru, with a value of 62.10%, and Maja Cipanas, with a value of 63.69%. Based on the disease severity value, four genotypes had a lower value than others, i.e., Rubaru with a value of 20.95%, Maja Cipanas with a value of 23.57%, Batu Ijo with a value of 24.52% and Agrihorti Ambassador I with value 33.21%. In addition, three genotypes (Maja Cipanas with a value of 6.33 mm and Batu Ijo with a value of 5.50 mm, Agrihorti I with a value of 5.83 mm) had a high value of spot diameter otherwise the other characters had a lower value which means positive effect for the resistance character.

Findings in the present research are different from previous especially the response is characterized as having high incidence and low severity or low incidence and high severity (Hidayat & Sulastrini, 2016). In this study, most of the genotypes with a high mean disease severity were also having a high incidence value (Table 2). However, certain genotypes with high incidence and low disease severity were exhibited. The complexity of phenotyping resistance/susceptibility of shallots is even more complicated when considering other resistance properties and the correlation between resistance characters where the incubation period negatively correlates with disease severity and incidence. Previous research by Monteon-Ojeda et al. (2017) found that extended incubation periods were negatively associated with disease severity and low incidence. In contrast, incidence positively correlated with disease severity—a positive correlation between spot diameter and disease severity (Miller-Butler et al., 2019).

Several collections of shallot varieties that have been characterized for anthracnose by *C. gloeosporioides* in a controlled environment show a complex of responses. The difference in the tested shallot genotypes' response indicates that genetic resistance is carried in the test population and can be utilized in plant breeding programs. Identifying shallot genotypes with resistance to anthracnose offers future opportunities to produce resistant cultivars as an effective disease control measure (Alberto, 2014). Resistant germplasm to anthracnose has been found in several Indonesian local varieties such as 'Sumenep', 'Maja', and 'Bali Karet' (Hidayat & Sulastrini, 2016). However, statistically, the grouping of resistance status still needs to be clarified if it is based on just one character of resistance. The resistance of a plant variety can be assessed based on consideration of several resistance characteristics such as incubation period, disease incidence, and disease severity because several categories can assess resistance, such as the ability to

prevent infection, absence of factor which reacts with the product of pathogens, physical barriers that retard the growth of the pathogen could be estimated using these characteristics (De Silva et al., 2017).

Correlation between resistance characters

The incubation period, disease incidence, and severity are closely related to determining the resistance character of the shallot varieties tested based on Pearson's correlation analysis (Figure 2). Correlation analysis showed a significant correlation value between each resistant character. The correlation diagram shows a significant positive correlation (69%) between disease incidence and disease severity. The other characteristic that had a significant positive correlation to disease severity was the spot diameter (69%). The disease severity possessed a highly significant negative correlation to the incubation period (-64%). All of the characters had negative significant correlation values to the incubation period. On the other hand, the spot diameter was positively significant and correlated with disease severity (69%) and incidence (53%).

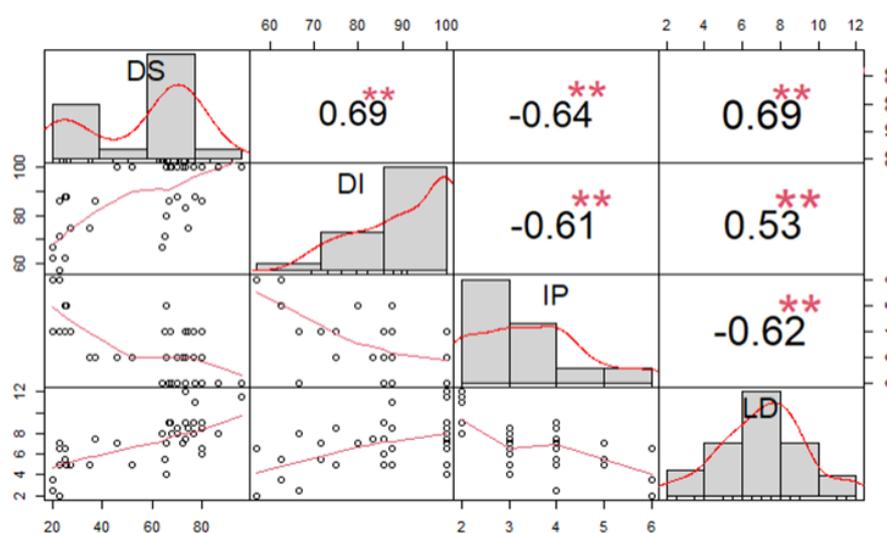


Figure 2. Pearson correlation of shallot resistance characters against anthracnose disease. DS=disease severity, DI=disease incidence, IP=incubation period, LD=spot diameter. Value followed by (**) means a significant correlation at 0.01 level

Determination of susceptibility group

The complexity of phenotyping anthracnose symptoms in shallot varieties would contribute to explaining the causal factors of resistance/susceptibility. Resistance traits such as incubation period, disease incidence, disease severity, and spot diameter can determine the susceptibility of the shallot cultivar. The incubation period of anthracnose in shallot can vary significantly among different varieties, with some varieties showing symptoms as early as the second day after inoculation. The fastest incubation period of anthracnose in shallot appears on the second day after inoculation (Alberto 2014). Different resistance mechanisms between varieties can cause differences in incubation periods between varieties. The incubation period of anthracnose was reported by Hekmawati et al. (2018) which has an incubation period of up to five to ten days after artificial inoculation. Disease prevention based on the incubation period data is related to the time it takes for symptoms to appear (Rimbaud et al., 2015).

However, disease incidence, representing the sum of plants infected by anthracnose, may not accurately represent the susceptibility status of the plants. It is essential to acknowledge that the susceptibility status of the plants may not be accurately represented by disease incidence, which is defined as the total number of plants infected by anthracnose. Disease incidence tends to increase as the plant grows and develops. In some

cases, a low disease incidence value indicate a higher level of plant susceptibility (Kone et al., 2017). This means that a low disease incidence doesn't necessarily signify a healthier or less susceptible plant; instead, it can be indicative of a delayed or more extended incubation period before symptoms manifest.

Disease severity usually represents the plant's susceptibility status to disease. Elaborating disease severity with other resistance characters to make a susceptibility group could represent whole aspects of plant susceptibility. The relationship between resistance characters based on correlation values shows that one character can already explain the resistance of the shallot varieties tested. Combining the four resistance characteristics to determine the final status of resistance for each variety would be more convincing through K-means cluster analysis.

Cluster analysis using the K-means method (Figure 3) classifies shallot varieties into two groups. The grouping of resilience status is based on four susceptibility characteristics using the K-means method. The model explains a total variance of 89.7% and classified thirteen genotypes into two groups, susceptible and resistant group. The first group (red) is a susceptible group consisting of Bima Brebes, Slupu Merah, Katumi, Sakato, Srikayang, Biru Lancor, Pancasona, Kuning, and Violeta. The second group (green) consisted of Batu Ijo, Maja Cipanas, Rubaru, and Agrihorti I. The K-means method to classify varieties based on disease resistance characteristics has been widely used, such as banana plants, for grouping resistance to fusarium wilt (Ribeiro et al., 2017), cacao (Chang et al., 2020), and almond (Moral et al., 2021). K-means was used to group resistance status because it can determine different resistance groups based on the resistance characteristics of the tested genotypes (Lisiecki et al., 2022).

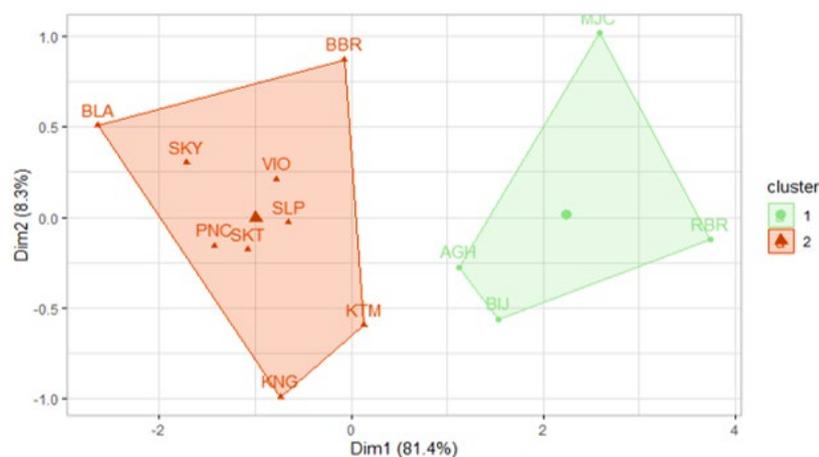


Figure 3. Grouping of susceptibility status of shallot based on four resistance characters. Cluster 1 is the resistance group, Cluster 2 is the susceptible group. BLA=Biru Lancor, BBR=Bima Brebes, SKY=Srikayang, VIO=Violeta, SLP=Slupu Merah, SKT=Sakato, PNC=Pancasona, KTM=Katumi, KNG=Kuning, AGH=Agrihorti I, BIJ=Batu Ijo, RBR=Rubaru, MJC=Maja Cipanas.

Variance and heritability of resistance character of shallot

Disease severity has the highest value of heritability (i.e., 0.76), followed by spot diameter (i.e., 0.70), disease incidence (i.e., 0.60), and incubation period (i.e., 0.56). With a value of 0.76, disease severity was categorized as high heritability, as did the spot diameter, incidence, and incubation period (Table 3). Genetic variance contributes greatly to a classification method that can objectively identify the degree of similarity and differences in genotypes tested in breeding programs. The high contribution of genetics in this study is indicated by the high heritability value of each character evaluated, indicating that the assessment also involves a genetic contribution in the grouping of resistance status (Chang et al., 2020; De Oliveira et al., 2016; Moral et al., 2021). The

resistance groups formed can represent the resistance status of the genotypes tested. Furthermore, it can be used in plant breeding programs.

Table 3. Variance and broad-sense heritability of shallot genotypes.

No	Characteristic	Genetic variance (σ^2_G)	Phenotypic variance (σ^2_P)	Broad-sense heritability (h^2_{bs})*
1	Incubation period	0.89	1.62	0.56
2	Disease incidence	129.39	214.59	0.60
3	Disease severity	285.65	377.75	0.76
4	Spot diameter	3.62	5.16	0.70

Note: *low heritability $h^2_{bs} \leq 0.2$; medium $0.2 \leq h^2_{bs} \leq 0.5$; high $h^2_{bs} > 0.5$

Grouping genotypes and testing the resistance of various shallot varieties to anthracnose through artificial inoculation revealed that the low value of environmental variables contributed significantly to this study. This was attributed to the performance of shallot varieties in responding to stress induced by the pathogen *C. gloeosporioides*. The low heritability value in the disease resistance test indicates that the susceptibility that appears is the result of the influence of the environmental conditions that are inoculated with the disease (Dalarosa et al., 2022). This study has found a high heritability value in the population tested, so the genetic influence of resistance is quite significant. According to Poehlman & Allen (2003), characteristic traits such as resistance to disease are highly influenced by the environment, which causes low heritability. Orozco et al., (2013) stated that heritability results from the proportion of genetic diversity to phenotypic diversity. As found in this study, the heritability of disease resistance characters was found in the high category. This study shows that with a high disease severity but a higher genetic proportion compared to the environment, it suggests that the genotype tested has a potential source of resistance to be passed on to the next generation. The group formed indirectly results from a representation of a genetic proportion more dominant than the influence of the environment.

The findings in this study were discovering potential sources of resistance to anthracnose disease in shallots, namely the genotypes 'Rubaru', 'Maja Cipanas', 'Agrihorti I' and 'Batu Ijo'. The 'Rubaru' genotype has suitable resistance dimensions compared to other genotypes. However, this genotype has a weakness where it is reported that it is challenging to undergo the flowering stage (Fairuzia et al., 2022; Triharyanto et al., 2018). However, other resistant genotypes, such as Maja Cipanas and Batu Ijo, are also potential candidates for anthracnose resistance. So these findings elight the way for the shallot plant breeding process in developing varieties resistant to anthracnose disease.

CONCLUSIONS

K-means cluster analysis based on the characteristics of resistance to the incubation period, disease incidence, and disease severity classified Bima Brebes, Slupu Merah, Katumi, Sakato, Srikayang, Biru Lancor, Pancasona, Kuning, and Violeta varieties as susceptible shallot varieties. On the other hand, Batu Ijo, Maja Cipanas, Rubaru, and Agrihorti I were resistant to anthracnose. Maja Cipanas and Rubaru exhibited greater consistency across all traits resulted as good sources for genetic resistance. The heritability of the incubation period, the incidence of the disease, the severity of the disease, and the diameter of the spots were in the high category, indicating that the diversity of resistance responses that appear is the influence of genetic factors.

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