

Correlation between Qualitative Characteristics and Genotype Resistance of Local and Introduced Pepper Varieties Against Anthracnose Disease

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ABSTRACT

This study was aimed at obtaining information related to genetic parametric against anthracnose diseases caused by *Colletotrichum capsici*, which attacked several pepper genotypes. The research was conducted in a net house at the Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh, from July to December 2017, and it was divided into two experiments: 1) planting the peppers which carried out in a net house. This experiment employed Randomized Block Design (RBD) Non-Factorial and 2) anthracnose resistance assay. This assay applied using Completely Randomized Design (CRD) Non-Factorial with the only factor observed: 11 pepper genotypes (IPBC15, IPBC15D2, IPBC15D3, IPBC15D4, Udeng, Lamando Lapaben, Super Amando, Lanyoe, Lado F1, Kastilo F1, and PM999 F1), with three replications. The results showed that the genotypes IPBC15, IPBC15D2, IPBC15D4, Udeng, Lamando Lapaben, Lado F1 exhibited moderate resistance, while genotypes IPBC15D3, Super Amando, Lanyoe, Kastilo F1 and PM999 F1 were susceptible to the disease. The wide variety of genetic and high heritability was found in stem length, flowering age, length of fruit stalk, fruit length, fruit diameter, and skin thickness, while narrow and moderate genetic variability was found in stem diameter and harvest age. The coefficient of genetic diversity in stem length, length of fruit stalk, and fruit length was found to be moderate and expressed low diversity in stem diameter, flowering age, and skin thickness. Stem diameter and skin thickness also showed a negative correlation to disease severity.

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1. INTRODUCTION

Pepper plants (*Capsicum annum* L.) are one of the horticultural commodities which have been widely developed in Indonesia for its economic value. Statistics Indonesia (2017) confirmed that the yield of fresh pepper was reached up to 1,045,587 tons in 2016, a significant rise up to 405 tons compared to yield in 2015. This increase was contributed by the increase of agricultural land up to 2,557 ha compared to in 2015. However, Indonesia has also experienced decreased productivity of up to 18 tons/ha. One factor affecting this productivity decline is pest and disease attack. Anthracnose is an important fungal disease to pepper plants. It attacks both mature and immature plants resulting in extensive loss of pepper yield (Lee, et al., 2010).

Syukur, et al. (2012) stated that it is important to collect pepper germplasm from superior varieties, landrace, and plant introduction as an initial of pepper plant breeding. These findings will be used to improve the plant's genetics. Genetics and Plant Breeding Laboratory, Department of Agrotechnology, Faculty of Agriculture, Universitas Syiah Kuala has been successfully collected several genotypes of Aceh peppers, from both introduced and hybrid varieties. These genotypes were Udeng, Lamando Lapaben, Super Amando, Lanyoe, which collected by farmers from Bener Meriah, Aceh. Introduced genotypes observed were IPBC₁₅, IPBC₁₅D₂, IPBC₁₅D₃, IPBC₁₅D₄ obtained from IPB collection, while F1 hybrid varieties tested such as Lado, Kastilo dan TM999 obtained from several agricultural shops.

This study was aimed at obtaining information related to genetic parametric against anthracnose diseases caused by *Colletotrichum capsici*, which attacked several pepper genotypes.

2. MATERIALS AND METHODS

The research was conducted at a net house in the Faculty of Agriculture, Universitas Syiah Kuala, from July to December 2017.

The materials used in this research were 11 pepper genotypes: IPBC15, IPBC15D2, IPBC15D3, IPBC15D4, Udeng, Lamando Lapaben, Super Amando, Lanyoe, Lado F1, Kastilo F1 and TM999 F1, cow manure, green manure, NPK fertilizer Mutiara, polybags, rice husks, soil, labeled papers, isolates of *Colletotrichum capsici*, PDA media cotton, plastic wrap, disinfectant Bayclin, alcohol 70%, distilled water, some sheets of scribbling papers from newspapers and tissues. The tools used were Laminar Air Flow Cabinet, autoclave, Erlenmeyer, Bunsen burner, Petri dishes (diameter 9 cm), forceps, cock borer 0.5 mm, analytical balance, hoes, sowing trays, scissors, measuring tape, plastic cups, hand sprayer, watering cans, staplers and stationery.

2.1 Seed Sowing

The seeds were sowed 1.5 cm in-depth, one seed for one hole in the tray, which has provided with a mixture of topsoil and green manure (ratio 1:1, v/v). The mixture than needed to be sifted to soften the soil. The soils were watered in the morning and evening. After a week, the seeds were then replanted to bigger polybags (size 10 kg) with ratio topsoils and green manure 1:2 (v/v). When the seeds are grown and produced 4-5 leaves (30 days), they were replanted to another 10 kg/polybag. A day before replanting, the soils needed to be watered to have it compressed. The replanting process was done in the evening to avoid high transpiration.

Fertilization was applied one week after planting (WAP) using liquid NPK fertilizer (ratio 16:16:16) with a dose of 10 g/L. The fertilizer (250 ml) was applied to each plant once a week. The plants also needed to be maintained regularly through watering, replanting the plants, stake installation, and pruning. The best time to harvest peppers was when they turned red, but it still has green color in it, usually 75-85 days after sowing. It is better to pick the peppers in the morning and not in the afternoon or evening.

Parameters observed were leaf shape, plant performance, flower position, fruit skin surface, stem diameter, dichotomous distinction, flowering age, the length of fruit stalk, length of fruit, fruit diameter, fruit skin thickness, and first harvest period.

2.2 Isolation and Multiplication of *C. capsici*

The distilled water (250 ml) was mixed with 10 g PDA into Erlenmeyer, and the mixture should be stirred to have a homogenous solution, and then covered it with

aluminum foil and sterilized using autoclave at 121°C for 30 minutes. Petri dishes and cock borer also needed to be sterilized. Sterilized PDA media (25 ml) were then placed into each petri dish and just let them dry and hardened. The isolates of *C. capsici* were placed into the middle of each petri dish using cock borer. After that, the Petri dishes had to be closed tightly using plastic wrap. These isolates were put in incubation room for maintenance for a week until the spora appeared.

2.3 Disease Resistance Assay

The peppers were washed with disinfectant before being inoculated. The isolate of *C. capsici* (diameter 0.4 cm) was infested onto the injured tissue of independent 10 pepper fruits. The treatment was replicated three times. The inoculated fruits were then placed in plastic trays. To keep them moisturized, these plastic trays needed to be covered with tissue papers which had been applied with distilled water. These inoculated peppers then were covered with plastic wrap and were incubated for seven days at 25 °C. The parameters observed were incubation time and disease severity with following modified formula by Hewidyarti (2011):

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

Where:

- DS = Disease severity
- n = number of infected plants
- v = category of infection/class rate
- N = number of plants observed
- V = maximum severity class rate

Table 1. Determination of class rates and disease severity

Rate	Disease severity (%)
0	0 (no plants infected)
1	1-20
2	21-40
3	41-60
4	>60

Table 2. Classification of plant resistance against anthracnose disease

Category	Disease severity (%)
Very resistant	$0 \leq X \leq 10$
Resistant	$10 \leq X \leq 20$
Moderate	$20 \leq X \leq 40$
Susceptible	$40 \leq X \leq 70$
Very susceptible	$X > 70$

Correlation between characteristics was calculated using covariance analysis with the following formula:

$$r(X_1X_2) = \frac{cov.X_1X_2}{\sqrt{V(X_1)V(X_2)}}$$

where:

$r(X_1X_2)$ = Correlation between characteristics X_1 and X_2

cov. X_1X_2 = Covariance between X_1 dan X_2

$v(X_1)$ = Variance X_1

$v(X_2)$ = Variance X_2

2.4 Data Analysis

All data collected were subjected to proper statistical analysis of variance (ANOVA), and mean values of treatments were differentiated by using Tukey's HSD (Honestly Significant Difference) at probability level 5%.

3. RESULTS AND DISCUSSIONS

3.1 Qualitative Characteristics

Qualitative characteristics indicate plant morphological characteristics that often be distinguished by class or category, resulted from one or two genes (monogenic) called major genes and a slight influence of the environment (Mangoendidjojo, 2003). The results of the parameters observed were presented in Table 3, following

the guidelines provided by IPGRI (1995). There were two types of leaf shape observed in this study: 1) ovate-shaped leaf, found in genotypes IPBC15, IPBC15D2, IPBC15D3, and IPBC15D4, 2) lanceolate-shaped leaf was found in genotypes Udeng, Lamando Lapaben, Super Amando, Lanyoe, Lado F1, Kastilo F1, and TM999 F1. The prostrate plants were demonstrated by genotypes IPBC15, IPBC15D2, IPBC15D3, and IPBC15D4, and erect plants were possessed by genotypes Lamando Lapaben, Super Amando, Lanyoe, Lado F1, Kastilo F1, and TM999.

For the flower position, intermediate flowers were observed in genotypes IPBC15, IPBC15D2, IPBC15D3, and IPBC15D4, while pendant positions were revealed by genotypes Udeng, Lamando Lapaben, Super Amando, Lanyoe Lado F1, Kastilo F1, and TM999 F1. Also, the smooth surface of pepper exhibited by genotype Udeng and Kastilo F1, semi wrinkled by genotypes Lamando Lapaben, Super Amando, Lanyoe, Lado F1 and TM999 F1, while genotypes IPBC15, IPBC15D2, IPBC15D3, and IPBC15D4 demonstrated wrinkled surface.

Table 3. Qualitative characteristics of 11 pepper genotypes

Genotype	Leaf shape	Plant performance	Flower position	Fruit surface
IPBC15	Ovate	prostrate	Intermediate	Wrinkled
IPBC15D2	Ovate	prostrate	Intermediate	Wrinkled
IPBC15D3	Ovate	prostrate	Intermediate	Wrinkled
IPBC15D4	Ovate	prostrate	Intermediate	Wrinkled
Udeng	Lanceolate	compact	Pendant	Smooth
Lamando Lapaben	Lanceolate	erect	Pendant	Semiwrinkled
Super Amando	Lanceolate	erect	Pendant	Semiwrinkled
Lanyoe	Lanceolate	erect	Pendant	Semiwrinkled
Lado F1	Lanceolate	erect	Pendant	Semiwrinkled
Kastilo F1	Lanceolate	erect	Pendant	Smooth
TM999 F1	Lanceolate	erect	Pendant	Semiwrinkled

3.2 Pepper resistance against anthracnose disease

The results of ANOVA (Table 4) revealed that the pepper genotypes significantly affected the incubation time, but it did not have an effect on disease severity. The results showed that the fastest incubation time was exhibited by genotypes Lanyoe, Super Amando, and Kastilo F1, and it has no significant difference compared to genotypes IPBC15D2, IPBC15D3, IPBC15D4, Udeng, Lamando Lapaben, Lado F1 and TM999 F1. However, it had a great difference in genotype IPBC15. On average, these 11 pepper genotypes possessed an incubation period only 2-3 days after inoculation, but it took four days for genotype IPBC15 to be incubated by *C. capsici*. Incubation time indicates the time elapsed exposure to a pathogenic organism, a chemical, or radiation, and when symptoms and signs are first to appear. The longer the incubation

time is, the longer time needed by pathogens to infect the plants. Also, the shorter the incubation time is the shorter time required by the pathogens to infect the plants.

According to Hidayat, et al. (2004), differences in the appearance of anthracnose disease symptoms indicate differences in the time taken by to cause an infection. The emergence of a symptom indicates an interaction between the pathogen and the host. The initial stage of penetration and infection are important occurrence which contributed to host colonization by a pathogen, and it triggers necrotic activity.

Resistance classification helps us to determine the disease severity caused by these pathogens. The smaller the value of disease severity, it means the more resistant the genotype is to a disease. The results of this research showed that there were two categories of resistance level possessed by 11 pepper genotypes tested, moderate and

susceptible. Moderate resistance was shown by genotypes IPBC15, IPBC15D2, IPBC15D4, Udeng, Lamando Lapaben, and Lado F1, while genotypes IPBC15D3, Super Amando, Lanyoe, Kastilo F1 and TM999 F1 considered as susceptible genotypes.

Nura (2015) confirmed that genotype IPBC15 and mutant genotype IPBC15D2 were resistant to anthracnose disease, but genotypes IPBC15D3 and IPBC15D4 were identified to be moderate. However, the results of the study done by Syukur, et al. (2013) revealed different results. They reported that genotype IPBC15 was moderate to the attack of *Colletotrichum acutatum* (PYK04 isolate). Surprisingly, Hakim, et al. (2014) examined that this genotype was susceptible to the exposure of *C. acutatum* (BGR027 isolate).

This is in line with the study of Suganda (2000), who found that the cause of resistant genes did not appear is

due to these genes are often controlled by a number of minor genes which depend on its quantity. This quantity greatly depends on the environment condition. Agrios (2005) also evaluated that the expression of resistance showed by each genotype was different. It happened due to the influence of the environment and genes of the genotype. Environmental conditions influenced these differences, pathogenic isolates, plant genetics, the technique of inoculation and plant characteristics, or physiology (Oh, et al., 1999). Kim, et al. (2007) also said that it requires a long time and so much effort to create the anthracnose-resistant varieties from recessive genes. Therefore, it is better to expose them to an 'open pollination' way. The selection to create these varieties can be done in the next generations produced, aiming at forming horizontal resistance to plant disease.

Table 4. Incubation time and disease severity

Genotype	Incubation time (days)	Disease severity (%)	Resistance level
IPBC ₁₅	4.00 b	29.17	Moderate
IPBC ₁₅ D ₂	2.97 ab	31.87	Moderate
IPBC ₁₅ D ₃	2.88 ab	43.33	Susceptible
IPBC ₁₅ D ₄	2.94 ab	30.00	Moderate
Udeng	3.31 ab	32.50	Moderate
Lamando Lapaben	2.00 ab	34.17	Moderate
Super Amando	1.57 a	51.67	Susceptible
Lanyoe	1.53 a	45.83	Susceptible
Lado F1	2.87 ab	35.00	Moderate
Kastilo F1	1.57 a	46.67	Susceptible
TM999 F1	1.67 a	46.67	Susceptible
Tukey's HSD (5%)	0,70	-	

Mean values in the same columns followed by the same letters did not differ significantly, as determined by Tukey's HSD at probability level 5%.

3.3. Correlation between qualitative characteristics and pepper resistance against anthracnose disease

The results shown in Table 5 showed that harvest age, dichotomous similarity, and length of fruit stalk had a significant positive correlation to disease severity. In contrast, skin thickness and fruit diameter demonstrated a significant negative correlation to disease severity. The insignificant correlations to disease severity were exhibited by flowering age, fruit length, and stem diameter.

The closeness of the relationship between characters is shown by the correlation value (r), which is between -1.00 and +1.00 and the value of 0.00 indicated there was no relationship between the two variables. This means that the value of -1.00 and so on indicates a negative correlation, and the value of +1.00 indicates a positive correlation, while the value of 0.00 indicates no

correlation between the observed characteristics (Shaban, 2005). Hapsari, et al. (2010) confirmed that if the correlation approaches 1.00, it means that the relationship between the characteristics is closer.

The results also showed that increasing growth of stem diameter, fruit diameter, and thickness of the fruit skin had reduced the percentage of disease severity. It can be said that disease severity is mostly influenced by stem diameter, fruit diameter, and fruit skin thickness. Ramadhan (2014) stated that if there are two characteristics observed showed a positive correlation, it could be attributed to the increased number of another characteristic. On the contrary, if two characteristics showed a negative correlation, it could be attributed to the decrease of another characteristic.

Correlation is the degree of closeness of relations between two or more characters. Correlation between characters is very useful in the application of indirect selection. Falconer & Mackay (1996) also found that the

correlation coefficient is the basic benchmark for determining the closeness of relationships between characters, whether each character observed has a close relationship or not. Correlation analysis can provide

additional information about the presence of certain characters, which are important components that affect a character.

Table 5. Correlations between quantitative characteristics to anthracnose resistance

Characteristics	Dichotomous similarity	Flowering age	Length of fruit stalk	Fruit length	Fruit diameter	Skin thickness	Harvest age	Disease severity
Stem diameter	0.08 ⁿ	-0.55**	-0.05 ⁿ	-0.15 ⁿ	-0.08 ⁿ	0.09 ⁿ	-0.35 ⁿ	-0.04 ⁿ
Dichotomous similarity		0.52*	0.91**	0.67**	-0.88**	-0.76**	0.33 ⁿ	0.48*
Flowering age			0.64**	0.61**	-0.61**	-0.77**	0.78**	0.42 ⁿ
Length of fruit stalk				0.84**	-0.97**	-0.78**	0.44*	0.50*
Fruit length					-0.87**	-0.66**	0.41 ⁿ	0.18 ⁿ
Fruit diameter						0.77**	-0.44*	-0.51*
Skin thickness							-0.70**	-0.54**
Harvest age								0.67**

ⁿ Non-significant, *Significant at probability level 1%, **Significant at probability level 5%.

CONCLUSIONS

1. There were different resistance levels exposed by each genotype. Genotypes IPBC15, IPBC15D2, IPBC15D4, Udeng, Lamando Lapaben, and Lado F1 varieties exhibited moderate resistance against *C. capsici* while genotypes IPBC15D3, Super Amando, Lanyoe, Kastilo F1 and TM999 F1 were susceptible to this pathogen.
2. There was a wide genetic variety and high heritability exhibited by dichotomous similarity, flowering age, length of fruit stalk, fruit length, fruit diameter, and skin thickness, and narrow and moderate genetic variability were found in stem diameter and harvest age.
3. There was a correlation between the quantitative pepper characteristics of the resistance of anthracnose caused by *C. capsici*.
4. From the results of this study, only five genotypes demonstrated moderate resistance against anthracnose disease. Therefore, further research and improvement of plant characteristics are needed to be done.

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