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Estimation of genetic parameters of resistance to Anthracnose disease in several genotypes of ornamental Chili (*Capsicum annuum* L.)

S Hafsah[1*], A Marliah[1], P B Safhira[1], A I Pratama[1] , Nura[1] , and **S S Fazillah[1]**

[1]Agrotechnology, Faculty of Agriculture, Syiah Kuala University (USK), Jln. Teuku Nyak Arief Darussalam, Banda Aceh 23111, Indonesia

***e-mail:** sitihafsah@usk.ac.id

Abstract. Ornamental chilies have attractive and varied shapes and fruit colors, so they are liked by consumers. However, ornamental chili plants are susceptible to disease, especially anthracnose. One control that can be done is by assembling resistant cultivars. Resistant cultivars can be determined by estimating genetic parameters to obtain selection characters for resistance to anthracnose disease. The purpose of this study was to determine the genetic parameters of anthracnose disease resistance in several genotypes of ornamental chili plants. This study used a non-factorial randomized block design with four chili genotypes, namely Ayesha, Nazla, Violetta and Syakira, each of which was repeated three times. The results showed that heritability values with high criteria were found in the parameters of fruit length, fruit diameter, weight per fruit, number of fruits per plant, fruit weight per plant, productivity, incidence of ripe chili disease and disease intensity of ripe chili.

1. Introduction

Ornamental chilies are often cultivated in urban environments where land is limited but still want to look beautiful around the yard. Therefore, ornamental chilies are often used to beautify the environment by becoming a landscape design element. According to [1] this increase has had a big positive impact on ornamental plant traders, because the income they earn has increased by 40% compared to before. Likewise, according to [2] nationally, exports of ornamental plants from January to April 2019 amounted to 1,470 tons, an increase of 28.5% compared to 2018 in the same period.

Ornamental chilies are susceptible to disease attacks, especially Anthracnose. Anthracnose is one of the main types of diseases of chili plants, where the highest percentage of pest attacks that attack chili plants are yellow virus 26%, anthracnose 29%, fruit flies 17%, curly virus 16%, and thrips 12% of the existing planting area [3]. [4] stated that the initial indication of anthracnose disease is small blackish spots like water on the surface of the affected fruit, followed by soft rot. Heavy attacks cause all the fruit to shrivel and dry out, thereby reducing the number of chilies, both in quality and quantity [5]. The decrease in the number of fruit gives the impression of a plant that is not lush and the beautiful impression of ornamental chilies appears to be lost. This causes consumer interest in cultivating ornamental chilies to decrease.

Control of anthracnose generally uses synthetic fungicides or pesticides. Improper use of pesticides can pollute water, soil, and ecosystems, and result in resistance over time. Therefore, chemical control is not appropriate to carry out. [6] stated that plant breeding programs are a potential solution in overcoming the problem of anthracnose, namely by creating resistant cultivars through improvements in desired characters. Genetic diversity analysis is the first step in developing superior chili varieties. According to [7] in the process of improving plant characters, plant character selection activities are needed. Character selection is carried out by looking at heritability values through estimating genetic parameters.

Estimating genetic parameters serves to provide information that is very necessary to improve the character of a population, such as resistance to anthracnose disease and information about genetic control is needed to determine effective selection methods so that they can be used in subsequent breeding processes [8]. [9] stated that the resistance characteristics of chili plants to anthracnose are still unstable. Apart from that, ornamental chili cultivation is still limited and has not yet become a major commodity. One of them is to obtain selection characteristics for disease resistance [10]. Therefore, research is needed to estimate the genetic parameters of several ornamental chili genotypes on anthracnose resistance characters which aims to determine the genetic parameters of anthracnose resistance in several genotypes of ornamental chili plants.

2. Materials and method

This research consists of planting and resistance testing which was carried out from June 2022 to November 2022. Planting was carried out at the Aceh Agricultural Technology Assessment Center (BPTP). Resistance testing was carried out at the Genetics and Plant Breeding Laboratory, Department of Agrotechnology, Faculty of Agriculture, Syiah Kuala University. The genotypes used were Ayesha, Nazla, Violetta and Syakira which came from IPB

2.1. Research procedure

2.1.1 Making potato dextrose agar (PDA) media. The potato skins were peeled then rinsed with water and sliced and then weighed 200 g. Next, the potatoes are boiled with 500 ml of distilled water until it boils. The boiled potatoes are filtered to extract the juice. Then 500 ml of distilled water is boiled with 15 g of agar and 15 g of dextrose, stirred until dissolved. After dissolving, the agar and potato extract are combined in a 1 liter measuring cup. The solution was transferred to an Erlenmeyer, wrapped in heat-resistant plastic, aluminum foil and rubber until airtight. Next, the PDA solution was sterilized using an autoclave for 15 minutes at a temperature of 121°C. After sterilization, it is cooled until the PDA solution is ready to be poured into petri dishes. Storage of petri dishes containing PDA solution is carried out in a Laminar air flow cabinet so that they remain sterile and left to solidify.

2.1.2. Fungus Isolation. Pathogenic fungi were isolated from chilies infected with anthracnose. Chilies should be cleaned by soaking them for one minute in alcohol and then rinsing twice with distilled water. Rinse the chilies and dry them on sterile paper towels. Each external part of the plant that is identified as being attacked by the fungus Colletotrichum gloeosporioides is cut and planted in a petri dish with Potato Dextrose Agar (PDA) growing medium. Then the petri dish was closed and the edges of the petri dish were coated with plastic wrap. Until spores are formed, maintain the pure isolate culture of C. gloeosporioides in the incubation room for one week.

2.1.3. Fungus Identification. The pure isolate that has been obtained from the isolation activity will be observed for the morphology of the fungus colonies both on the top surface and under the growth medium, then described including the shape, form of the colony and color. Using a needle, pieces of mycelium are taken and placed on a glass slide to be observed under a microscope. The morphology of mycelium and conidia was identified by matching their morphology.

2.1.4. Inoculation of the fungus Colletotrichum sp. The chili fruit used was approximately 8 weeks old and free from fruit rot infection. Before inoculation, the chili fruit is selected and washed clean using distilled water, then the fruit is dried in the air before inoculation is carried out. The inoculation method used is attaching a culture of the pathogen *C. gloeosporioides* and wounding the tissue, namely by attaching a piece of fungus culture using a cock borer from a pure culture isolate of *C. gloeosporioides*. One inoculation point was made for each chili fruit for fruit length < 4 cm, namely in the middle of the fruit and two inoculation points if the fruit length was > 4 cm, namely at the base and tip of the fruit. To maintain humidity, the plastic tub is lined with tissue, then the tissue is sprayed with distilled water using a hand sprayer. Then the chilies that had been inoculated with *C. gloeosporioides* were placed on a wire in a plastic tray and covered using plastic wrap and black tarpaulin, then incubated at room temperature for 7 days.

2.2. *Experimental design*
This research used a non-factorial randomized block design with four chili genotypes, each repeated three times so that there were 12 experimental units. Each experimental unit consisted of 5 experimental plants so that the total was 60 plants. The mathematical model of experimental data analysis for the non-factorial RAK Randomized Block Design is as follows:

$$Y\_(ij =)\ \mu+\beta\_i+G\_j+\varepsilon\_ij \quad (1)$$

Note:
Yij = response or observation value from the ith treatment and jth replication
μ = general average value
βi = influence of the ith genotype (i= 1,2,3,4)
Gj = influence of the jth repetition (j= 1,2,3)
Eij = influence of experimental error from the i-th experiment and j-th repetition.

Data were analyzed using Analysis of variance (ANOVA). If the F test results show a real effect, then the analysis continues with the Least Significant Difference test at the 5% level ($BNT_{0.05}$)

2.3. Data analysis
2.3.1. The genetic variation. The observation data is then analyzed to determine the genetic variation (σ2g) calculated using the formula:

$$\sigma^2 g = \frac{(KTg-KTe)}{n} \quad (2)$$

2.3.2. The standar deviation of genetic variation. Standard deviation of genetic variation uses the formula:

$$\sigma_{\sigma^2 g} = \sqrt{\left[\frac{2}{n^2}\right]\left[\left(\frac{KTg^2}{dbg+2}\right)+\left(\frac{KTe^2}{dbe+2}\right)\right]} \quad (3)$$

Note:
KTg = Middle square of genotype
KTe = Middle square error
n = repetition
dbg = degree of freedom of genotype
dbe = degree of error freedom

2.3.3. KKG and KKF. The coefficient of genetic diversity (KKG) and the coefficient of phenotypic diversity (KKF) (Qosim et al., 2000) are estimated using the following formula:

$$KKG = \frac{\sqrt{\sigma 2g}}{x} \text{ x } 100\% \quad (4)$$

$$KKF = \frac{\sqrt{\sigma 2f}}{x} \text{ x } 100\% \quad (5)$$

2.3.4. Heritabality value. Another analysis carried out was calculating the broad sense heritability value (h2bs) as follows:

$$h^2bs = \frac{\sigma 2g}{\sigma 2f}\, x\, 100\% \quad (6)$$

Note:
h2bs = Heritability
σ2f = phenotypic variance = (σ2g + σ2e)
σ2g = genetic variation
σ2e = KTe = environmental variety
X = mean genotype
dbg = degree of freedom of genotype
dbe = degree of error freedom

3. Results and discussion

3.1. Incubation Period for Anthracnose Disease

Incubation period of IPB ornamental chili anthracnose disease on young and ripe fruit. The Violetta genotype has an average incubation period for young fruit which tends to be longer, namely 4.40 days, although statistically it is not significantly different from the other test genotypes. The Nazla genotype has an average incubation period for ripe fruit which tends to be longer, namely 3.53 days, although statistically it is not significantly different from the other test genotypes.

Table 1. Average incubation period for test genotypes against anthracnose disease on young and ripe chili fruit

Genotype	Incubation Period for Young Fruit (days)	Incubation Period for Ripe Fruit (days)
Ayesha	4,07	3,33
Nazla	3,67	3,53
Violetta	4,40	3,40
Syakira	4,00	2,80

Symptoms after inoculation are shown in all parts, in the injured areas of both young and ripe fruit and the fruit stalk. This is in accordance with the statement of [11], that anthracnose disease caused by the fungus C. gloesporioides can attack all parts of the plant. However, on ripe fruit the symptoms in the form of spots develop more quickly than on young fruit. According to [12] the development of spots will occur more quickly if inoculation is carried out on ripe chilies. Variations in the emergence of symptoms are determined by climate variables, pathogen isolates, plant genetics, inoculation techniques, and plant physiology or characteristics.

The greater the incubation period value indicates the longer the time needed for the pathogen to infect the plant, conversely, if the smaller the incubation period value, the faster the time required for the pathogen to infect the plant. [13] stated that differences in the appearance of symptoms of anthracnose have implications for differences in the time required by the pathogen for the infection process and pathogenicity. The appearance of symptoms of a disease indicates that an interaction has occurred between the pathogen and the host.

3.2 Incidence of Anthracnose Disease

The incidence of IPB ornamental chili anthracnose disease on young and ripe fruit. The Syakira and Ayesha genotypes have an average value of disease incidence in young fruit which tends to be lower, namely 53.33%, although statistically it is not significantly different from the other test genotypes. The Nazla genotype has an average value of disease incidence in ripe fruit which tends to be lower, namely 86.67%, although statistically it is not significantly different from the other test genotypes.

In the genotype of different plants, their resistance to a particular disease and the level of fruit maturity are not the same. This is evident in the occurrence of fruit diseases where young fruit genotypes Ayesha and Syakira show a vulnerability resistance level. In contrast, in the occurrence of diseases in mature fruits, genotypes Ayesha and Syakira exhibit a very vulnerable resistance level. According to [14], a plant's ability to form undesirable structures reflects its resistant traits, such as the construction of a thick cuticle layer, the organization of tissues with thick cork-walled cells, and the production of toxic chemicals capable of killing pathogenic microbes.

Young chili fruit has resistance criteria that tend to be better than mature chili fruit. This is thought to be the thickness of the cuticle layer on young chilies. It would be difficult for the virus to infect the thick cuticle layer due to its structure. Epidermal cell walls can inhibit the entry of fungi and other microbes, therefore the thicker the cuticle layer, the more resistant the plant is to disease penetration [15].

The four test genotypes on young and ripe fruit did not show the criteria for susceptible and moderate resistance. [16] stated that the absence of resistance genes is because these genes are regulated by minor genes and are quantitative in nature so they are susceptible to environmental factors. [17] also added that environmental influences or the genetic background of a genotype can be a factor in the expression of resistance characters in each different genotype.

Table 2. The average incidence of the test genotype for anthracnose in young and ripe chili fruit

Genotype	Disease Incidence in Young Fruit (%)	Criteria	Disease Incidence in Ripe Fruit (%)	Criteria
Ayesha	53,33	Susceptible	100,00	Highly Susceptible
Nazla	86,67	Highly Susceptible	100,00	Highly Susceptible
Violetta	73,33	Highly Susceptible	86,67	Highly Susceptible
Syakira	53,33	Susceptible	100,00	Highly Susceptible

3.3. Intensity of Anthracnose Attacks

Intensity of IPB ornamental chili anthracnose attacks on young and ripe fruit. The Syakira genotype has an average value of attack intensity on young fruit which tends to be lower, namely 73.33%, although statistically it is not significantly different from the other test genotypes. The Violetta genotype has a lower average attack intensity value on ripe fruit, namely 88.89%, which is significantly different from the other test genotypes.

Resistance to disease can be measured based on the intensity of the attack and the occurrence of the disease. The correlation between the intensity of disease attack and the occurrence of the disease is such that the lower the disease intensity, the lower the disease occurrence, which influences the disease resistance level of a genotype. This can be observed with the lowest intensity of disease attack shown by the Syakira genotype, correlating with the lowest occurrence of disease in young fruit in the Syakira genotype. Similarly, in mature chili plants, the lowest intensity of disease attack and disease occurrence are shown by the Violetta genotype. This fact indicates that the Syakira and Violetta genotypes tend to exhibit better resistance compared to other genotypes.

Table 3. Average attack intensity of the test genotypes against anthracnose disease on young and ripe chili fruit

Genotype	Intensity of Attack on Young Fruit (%)	Intensity of Attack on Ripe Fruit (%)
Ayesha	84,44	98,89b
Nazla	88,89	98,89b
Violetta	94,44	88,89a
Syakira	73,33	100,00b
BNT (0,05)	-	5,76

Note: Numbers followed by the same letter in the same column are not significantlydifferent in the BNT test at the 5% level

Young chili fruits are more resistant to anthracnose attacks compared to mature chili fruits. At the fruit ripening stage, the structural and functional resistance of each genotype varies. According to the research by [18], among 20 different genotypes at different stages of fruit ripeness, different resistance levels are observed, with young fruits being more resistant to anthracnose. This is because mature chili fruits contain more carbohydrates compared to young ones [19]. According to [17], fungi cannot grow without carbohydrates. In fruits with high carbohydrate content, fungi grow faster compared to young fruits. The higher disease severity in mature fruits indicates fungal growth.

3.4. Estimation of genetic parameters in several genotypes of IPB ornamental Chili

The values of all resistance traits exhibit genetic diversity with narrow criteria because all traits have genetic variance values smaller than two times the standard deviation of genetic variance. These genetic variance values indicate that the tested genotypes have consistent traits.

High heritability values are found in parameters such as fruit length (98.32%), fruit diameter (94.86%), fruit weight per fruit (91.17%), number of fruits per plant (56.31%), fruit weight per plant (77.48%), productivity (77.48%), occurrence of disease in mature chili (75.00%), and disease intensity in mature chili (89.77%). Moderate heritability values are observed in parameters like plant height (21.20%), stem diameter (36.71%), fruit flesh thickness (45.12%), incubation period of young chili fruit (26.237%), incubation period of mature chili fruit (21.42%), occurrence of disease in young chili fruit (50.00%), and disease intensity in young chili fruit (31.57%). Low heritability values are found in the parameter of plant height (9.02%). These values indicate the extent to which the traits are influenced by genetic factors in the tested genotypes.

High heritability values, in a broad sense, indicate that genetics have a stronger influence in determining plant traits than the environment. In quantitative traits, high heritability values observed in fruit length, fruit diameter, fruit weight per fruit, the number of fruits per plant, fruit weight per plant, and productivity indicate that these traits are more dominantly influenced by genetic factors than environmental factors. This suggests that the observed traits in the tested plants can be easily passed on to their offspring, allowing for selection to be carried out in early generations.

Low heritability values indicate that environmental factors have a greater influence than genetic factors, whereas high heritability values suggest the opposite [20]. Traits related to the resistance of the occurrence of disease in mature chili and the intensity of attacks in mature chili show high heritability values. This indicates that genetic factors have a stronger influence on these traits. Therefore, there is an opportunity for genetic improvement in these traits, with the hope of providing better resistance.

Table 4. The estimation of genetic diversity and genetic variability in anthracnose disease resistance traits and quantitative traits.

Character	σ^2 g	$\sigma_\sigma{}^2$ g	$2\sigma_\sigma{}^2$ g	Criteria $2\sigma_\sigma{}^2$ g	σ^2 p	$h^2_{(BS)}$ (%)	Criteria $h^2_{(BS)}$
Plant height	0,13	1,18	2,36	Narrow	1,52	9,02	Low
Dichotomous height	0,06	0,23	0,46	Narrow	0,31	21,20	Medium
Stem diameter	0,04	0,08	0,16	Narrow	0,12	36,71	Medium
Fruit length	0,69	0,44	0,89	Narrow	0,71	98,32	High
Fruit diameter	2,08	1,39	2,78	Narrow	2,20	94,86	High
Thick fruit flesh	0,003	0,004	0,009	Narrow	0,01	45,12	Medium
Weight per fruit	0,02	0,01	0,03	Narrow	0,02	91,17	High
Number of fruits per plant	32,47	38,57	77,15	Narrow	57,66	56,31	High
Fruit weight per plant	54,99	45,59	91,18	Narrow	70,97	77,48	High
Productivity	0,05	0,04	0,09	Narrow	0,07	77,48	High
Incubation period in young chili fruit	0,02	0,06	0,13	Narrow	0,09	26,27	Medium
Incubation period for ripe chili fruit	0,02	0,07	0,15	Narrow	0,10	21,42	Medium
Disease incidence in young chili fruit	133,33	181,25	362,70	Narrow	266,67	50,00	Medium
Disease incidence in ripe chili fruit	33,33	28,65	57,30	Narrow	44,44	75,00	High
Disease intensity in young chili fruit	25,30	57,62	115,25	Narrow	80,14	31,57	Medium
Disease intensity on ripe chili fruit	24,38	17,23	34,46	Narrow	27,16	89,77	High

Note: σ^2 g = genetic variety, $\sigma_\sigma{}^2$ g = standard deviation of genetic variance, σ^2 p = variety of phenotypes, $h^2_{(BS)}$ = broad sense heritability, genetic diversity is wide if it is genetically diverse > 2 x $\sigma_\sigma{}^2$ g (Pinnaria et al., 1995). Low heritability = $0< h^2_{bs} <20$, medium = $20\leq h^2_{bs}<50$, high $h^2_{bs}\geq50$ (Zen dan Bahar, 1995).

The values of the Coefficient of Genotypic Variation (GCV) with relatively broad criteria are found in parameters like fruit length (29.9%), fruit weight per plant (30.27%), and productivity (30.27%). GCV values with relatively narrow criteria are observed in parameters such as fruit diameter (18.6%), fruit weight per fruit (21.45%), the number of fruits per plant (16.55%), and the occurrence of disease in young chili (17.32%). GCV values with very narrow criteria are present in parameters like plant height (1.70%), dichotomous height (2.65%), stem diameter (4.48%), fruit flesh thickness (7.29%), incubation period of young chili (3.96%), incubation period of mature chili (4.56%), occurrence of disease in young chili (17.32%), occurrence of disease in mature chili (5.97%), intensity of attacks in young chili (5.89%), and intensity of attacks in mature chili (5.10%). These values reflect the extent of genetic variation for each parameter, with broader criteria suggesting higher genetic variation.

Characteristics that exhibit wide genetic variation criteria indicate that these traits have a wide genetic distance between genotypes. Wide genetic diversity facilitates the selection of genetic materials because a diverse genetic source is available for the purpose of improving plant traits to create new superior varieties. On the other hand, narrow genetic variation indicates that the character has a narrow genetic distance between the tested genotypes. High values of the Coefficient of Genotypic Variation (GCV) suggest that the genetic diversity of the character within the population is significantly broad and diverse. The higher the KKG value, the more different the character is among the tested genotypes, making the selection process in plants more advantageous, and vice versa [21].

Table 5. The genetic coefficient of variation (GCV) and the phenotypic coefficient of variation (PCV) for quantitative traits.

Character	GCV (%)	Criteria	PCV (%)	Criteriaa
Plant height	1,70	Narrow	5,67	Narrow
Dichotomous height	2,65	Narrow	5,76	Narrow
Stem diameter	4,48	Narrow	7,39	Narrow
Fruit length	29,9	Quite spacious	30,24	A bit narrow
Fruit diameter	18,6	A bit narrow	19,16	Narrow
Thick fruit flesh	7,29	Narrow	10,85	Narrow
Weight per fruit	21,45	A bit narrow	22,47	Narrow
Number of fruits per plant	16,55	A bit narrow	22,06	Narrow
Fruit weight per plant	30,27	Quite spacious	34,38	A bit narrow
Productivity	30,27	Quite spacious	34,38	A bit narrow
Incubation period in young chili fruit	3,96	Narrow	7,45	Narrow
Incubation period for ripe chili fruit	4,56	Narrow	9,85	Narrow
Disease incidence in young chili fruit	17,32	A bit narrow	24,49	A bit narrow
Disease incidence in ripe chili fruit	5,97	Narrow	8,44	Narrow
Disease intensity in young chili fruit	5,89	Narrow	16,15	Narrow
Disease intensity on ripe chili fruit	5,10	Narrow	5,91	Narrow

Note: KKG: Narrow (0%<X≤10.94%), A bit narrow (10.94%<X≤21.88%), quite spacious (21.88%<X≤32.83%), wide (32.83%<X43.77%), dan very wide (X>43.77%). KKF: sempit (0%<X≤24.94%), A bit narrow (24.94%<X≤49.71%), quite spacious (49.71%<X≤74.71%), wide (74.71%<X≤99.65%), and very wide (X>99.65%)(Qosim *et al.*, 2000).

Quantitative traits and disease resistance show narrow to moderately narrow values for the Coefficient of Genotypic Variation (GCV), except for the parameters of fruit length, fruit weight per plant, and productivity. This indicates that these traits have narrow genetic distances and are not very diverse. Therefore, selecting based on these traits would be less effective because the likelihood of success in improving these traits is very low. On the other hand, the parameters of fruit length, fruit weight per plant, and productivity have moderately broad GCV values, classifying them as traits with wider genetic diversity. The more diverse a trait is within a population, the greater the variation in the genetic control of that trait within the population. The higher the genetic control of a trait, the greater the likelihood of obtaining genotypes with superior traits through selection [22].

A plant trait with a high coefficient of genetic variation (GCV) is not always associated with high heritability values, and vice versa. In Tables 16 and 17, the trait of disease occurrence in mature chili has a narrow GCV (5.97) but very high heritability (75.00), indicating that the GCV and heritability values do not necessarily have a linear relationship of the same magnitude. This is due to environmental factors influencing the GCV value. Low genetic variation and high heritability values in a plant trait indicate that the trait is homogeneous within the population, and its occurrence is strongly controlled by genetic factors [22].

PCV values with rather narrow criteria were found in the characteristics of fruit length (30.24%), fruit weight per plant (34.38%), productivity (34.38%) and the incidence of young chili disease (24.29%). The PCV values with narrow criteria were found in the characters of plant height (5.67%), dichotomous height (5.76%), stem diameter (7.39%), fruit diameter (19.16%), fruit flesh thickness (10, 85%), weight per fruit (22.47%), number of fruits per plant (22.06%), incubation period of young chili fruit (9.85%), incubation period of ripe chili fruit (9.85%), incidence mature chili disease (8.44%), young chili attack intensity (16.15%), mature chili attack intensity (5.91%).

The Phenotypic Diversity Index (PCV) describes the level of diversity in a visible characteristic. Low phenotypic diversity indicates that the tested trait has a homogenous phenotype, while a high phenotypic diversity value indicates that the tested trait has a diverse phenotype. Quantitative traits and disease resistance in the tested genotype show a narrow to moderately narrow PCV value, indicating a uniform phenotype appearance for that trait. According to [23], traits with narrow diversity and uniform appearance suggest that they have low GCV and PCV values.

4. Conclusions

Parameters such as fruit length, fruit diameter, fruit weight per unit, number of fruits per plant, fruit weight per plant, productivity, occurrence of mature chili disease, and intensity of mature chili disease show high heritability values. There are currently no tested genotypes that exhibit resistance or moderate resistance to the variable of anthracnose disease, thus further research is needed using more diverse genotypes.

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