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Diallel Analysis of Cocoa (*Theobroma cacao* L.) Resistance to *Phytophthora palmivora* in Indonesia

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Abstract

For the program towards the development of resistant cacao (*Theobroma cacao* L.) to the black pod rots disease caused by *P. palmivora*, the diallel crossing analysis is expected can be used to provide genetic parameters on quantitative traits. The objective of the present study was to determine genetic parameters of cacao resistance to the disease caused by *P. palmivora*, using half diallel crosses. The crosses used five cocoa clones as parental clones (ICCRI 3, TSH 858, DR 1, ICS 13, and Sca 6) having resistance levels from susceptible to resistant. The experiment was arranged in a Randomized Block Design with three replications. The treatments were 10 hybrids (F_1) and 5 parental clones. Observation was conducted 3 days after inoculation to the spot area caused by *P. palmivora* infection. The results showed that there was no interaction between genes in determining the resistance to black pod rots disease caused by *P. palmivora*. The resistance was more affected by the additive gene action. Characters of resistant to the disease were controlled by the recessive genes. The results in the present study also showed that the dominant genes were more in the parents. It is expected that the opportunity to produce cocoa hybrid owned by ICCRI 3 and Sca 6. Furthermore, the estimated value of broad sense heritability (h^2BS) and narrow sense heritability (h^2NS) was high for the spot area, while, based on the disease intensity it was moderate to high.

Keywords: Cocoa, genetic parameters, resistance, *P. palmivora*

1. Introduction

Black-Pod Rot (BPR) disease in Indonesia, caused by *Phytophthora palmivora*, is one of the main diseases affecting the cocoa (*Theobroma cacao* L.) in the most production areas. The disease occurs in the fields not only during in the wet season, but also in the dry season (McMahon & Purwantara 2004; Rosmana *et al*. 2006). Therefore, the cocoa development program in the country is addressed mainly to find out the new superior cocoa cultivars having high yields and resistance to the disease (McMahon & Purwantara 2004; Suhendi *et al*. 2005) as many studies reported that the breeding programs have considered being the most economical, environmentally friendly, and effective control method for controlling the disease (Iwaro *et al*. 2004). In this regards, Iwaro *et al*. (1999) reported that the resistance to black pod attack is polygenically inherited and it could be improved by recurrent selection.

Studies have shown that cocoa cultivars have shown consistent differences in levels of infection (Adomako 2006; Bhavani *et al*. 2007). For instance, Bhavani *et al*. (2007) studied the reaction of 225 different cocoa types against Phytophthora pod rot by artificial inoculation of the pathogen *P. palmivora* on detached cocoa pods. They found that none of the cocoa types screened were immune to the disease. However, the cocoa types differed each other in the percentage of pod area infection. Moderately susceptible reaction was recorded with 64 cocoa types and the rest were susceptible. Meanwhile, Adomako (2006) who studied the combining ability analysis of black pod disease incidence in cocoa genotypes in two field trials reported that there were significant differences between cocoa genotypes in the levels of black pod disease. However, there is still very little of such information available resulted from studies in

Indonesia.

To support the cocoa breeding program in the country, there is a necessity to obtain the genetic information of genes that control the resistance character to *P. palmivora*. For the program towards the development of resistant cocoa to the disease, the diallel crossing analysis is expected can be used to provide genetic parameters on quantitative traits (Viana *et al*. 2001; Hayman 1960). The diallel analysis has been demonstrated for many crops (Darera *et al*. 2007; Dhliwayo *et al*. 2005; Gwata *et al*. 2005; Viana *et al*. 2001). In these regards, therefore, the objective of the present study was to determine genetic parameters of cocoa resistance to the disease caused by *P. palmivora*, using half diallel crosses.

2. Materials and Methods

2.1 Genetic Materials

The field experiments were carried out in the Kaliwining Experimental Station of Indonesian Research Center for Coffee and Cocoa, Jember, East Java (50 m asl), from January 2008 up to March 2009. Propagation and maintenance of *P. palmivora* isolates were conducted in the Laboratory of Plant Protection of the Indonesian Research Center for Coffee and Cocoa. The isolate of *P. palmivora* used was a pure culture taken from Lubuk Basung, West Sumatera, which was endemic area of *P. palmivora* in West Sumatera. The pathogen was propagated at V-8 agar media.

The five cocoa clones used as parents to develop F_1 hybrid population were grown in the Germplasm Plantation of the Indonesian Research Center for Coffee and Cocoa, Jember since 1994. Each clone consisted of five plants. The characteristics of each parental clone are presented in Table 1.

Table 1. Characters of parent cocoa clones used to develop F_1 hybrid population in the study

Clone	**Clonal productivity**		**Resistance to infection of**
	Pod yield	**Bean size**	***P. palmivora***
ICCRI 3	High	Big	Resistant/Slightly resistant
TSH 858	High	Big	Slightly susceptible
DR 1	High	Big	Susceptible
ICS 13	High	Moderate	Slightly resistant
Sca 6	Moderate	Small	Resistant/Slightly resistant

Crosses between parents were carried out in a controlled manner (by hand pollination). The combinations of crosses were arranged in combination of half with cross number of 10 F_1 crosses (Table 2).

Before planting, F_1 beans were extracted from the pods, and then germinated within germination boxes. The uniform and healthy germinated beans were selected and planted in nursery media. The selected germinated beans were planted in the poly-bags (20 x 15 cm), containing 3 kg mixed media of soil, sand, and manure in the ratio of 2:1:1. The seedlings were placed in a glasshouse and watered every morning and evening until one month old.

Table 2. Half diallel crosses with five parents to develop F_1 hybrid

♀ / ♂	ICRI 3	TSH 858	DR1	ICS 13	SCA 126
ICRI3	-	×	×	×	×
TSH858		-	×	×	×
DR1			-	×	×
ICS 13				-	×
SCA 126					-

Note: × = F_1 hybrid

2.2 Experimental Design and Observations

The experiment was arranged in a Randomized Block Design with three replications. The treatments were 10 hybrids and 5 parental clones. Each experimental unit consisted of 20 cocoa seedlings, so overall there were 900 (15 x 3 x 20) seedlings.

Pathogen cultures were incubated in a dark room with a temperature of 26^0C. The actively growing mycelia were ready for use 12 days after inoculation. To produce zoospore inoculums, the cultures were grown on V-8 agar media. The zoospores were harvested 12 days after inoculation. Zoospores were separated from the mycelia and put in the refrigerator at 4^0C for 5 minutes to allow the germination of zoospores occurred. Afterwards, 10 ml of sterile water was added into the media. And then, the cultures were diluted to the density of 10^4-10^5 zoospores/ml. The seedlings used were kept in a transparent plastic house until one-month old. Each seedling was inoculated with a piece of agar (0.5 cm^2) containing mycelia that had been previously prepared. The inoculated seedlings were put within a transparent plastic house having 90% moisture content. Observation was conducted six days after inoculation by measuring the length and width of the spots on the cocoa leaf surface using millimetre paper. The measurements were carried out every day and finished when the seedlings died due to the inoculation.

In the study of the disease intensity, the seedlings used were also one month old. The treatments were 10 F_1 hybrids and 5 parental clones. Each experimental unit was also consisted of 20 cocoa seedlings with three replications. The inoculation was done by spraying leaf surfaces with 10^4-10^5 zoospores/ml. The inoculated seedlings were put within a transparent plastic house having 90% moisture content. The measurements were conducted six days after inoculation by measuring the symptom percentage on leaf surface and determining the index of disease intensity. The measurements were carried out every day and finished when the seedlings died due to the inoculation. Spotting symptom was observed by using disease symptom scoring parameter refers to the modified Fry (1983) method as described in Table 3. The values of disease intensity were used to classify the seedlings into five categories as shown in Table 4.

Table 3. Spotting symptom score on infected cocoa leaf by *P. palmivora*

Score	**Attack**	**Symptom**
0	Healthy	0% leaf infected
1	Very mild	<5% leaf infected
2	Light	5-10% leaf infected, chlorotic/necrotic but no leaf fall, lenticels swollen
3	Moderate	10-25% leaf infected, chlorosis, necrotic, leaf fall, lenticels swollen
4	Slightly heavy	25-50% leaf infected, chlorosis, necrotic, leaf fall, lenticels swollen
5	Heavy	50-75% leaf infected, chlorosis, necrotic, leaf fall, lenticels swollen
6	Very heavy	>75% leaf infected, chlorosis, necrotic, leaf fall, lenticels swollen, seedling died

Table 4. Grouping of cocoa resistance to *P. palmivora*

Category	Disease intensity (%)
Resistant	0-30
Slightly resistant	31-50
Moderate	51-65
Slightly susceptible	66-80
Susceptible	81-100

From the obtained score, the disease intensity expressed as diseases severity determined by the following formula:

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

Where: DS = disease severity, v = disease score of sample i^{th}, n = number of plant sample with a particular score, V = the highest score, and N = total number of samples.

2.3 Data Analysis

Estimations on genetic parameters of cocoa resistance to *P. palmivora* were determined by diallel analysis using Hayman's approach following Singh & Chaudhary (1979) procedure.

3. Results and Discussion

3.1 Gene Interaction

Analysis of diallel cross to estimate genetic parameters can be carried out if there are significant differences between genotypes based on the F test on the spot width of disease symptom of *P. palmivora*. The analysis of variance (ANOVA) results in Table 5 showed that diallel cross analysis gave a highly significant difference between genotypes in the parameter of spot width due to *P. palmivora* inoculation. This suggests that the estimation of genetic parameters can be applied to the cocoa genotypes tested (Singh & Chauddhary 1979).

Table 5. Analysis of variance of cocoa resistance to *P. palmivora*

Source of variation	Degree of freedom	Sum square	Mean square	F-value
Replications	2	1518,0526	759,0263	2,4965^{ns}
Genotypes	14	19883,5342	828,4806	2,7249**
Error	28	14593,7457	304,0364	
Total	44	35995,3324		

Note: ** = highly significant, ns = not significant

The results of regression coefficient test b (Wr, Vr) was not significantly different for the observed character (Table 6), indicating that there was no interaction between genes in controlling the spot area in the cocoa resistance to *P. palmivora* disease. Sousa & Maluf (2003) who worked on diallel analysis and estimation of genetic parameters of hot pepper also found that here was no interaction among genes for the total fruit yield. This result suggests that one of the assumptions in diallel cross analysis could be met (Hayman 1954; Singh & Chaudhary 1979).

Table 6. Estimation of genetic parameters for cocoa genotype resistance to *P. palmivora*

Genetic parameters	Value	Explanation
Covariance-variance regression (b (Wr, Vr))	0.4493	ns
Additive effect (D)	0.0386	**
Fr mean (F)	0.0188	ns
Dominance effects (H_1)	0.0989	**
Proportion of dominance due to positive/negative effects of genes (H_2)	0.0700	**
F_1 deviation from average parent (h^2)	0.1497	**
Mean degree of dominance $(H_1/D)^{1/2}$	1.6003	Over dominance
Proportion of dominance genes to recessive genes ($H_2/4H_1$)	0.1768	
Environmental effects (E)	0.0017	
Proportion of dominance to recessive genes (Kd/Kr)	1.3594	Gene dominance
Number of gene groups (h^2/H_2)	2.1396	
Narrow-sense of heritability (h^2_{NS})	0.5589	high
Broad-sense of heritability (h^2_{BS})	0.9600	high

Note: ** = highly significant, ns = not significant

3.2 The effects of Additive (D) and Dominance (H_1)

The effects of additive (D) and dominance (H_1) had very significant contributions to the cocoa resistance to the black pod rot disease for the clones used (Table 6). In the present study, the effect of the dominance (H_1) was greater than that of the additive effects (D). This suggests that the resistance to the disease caused by *P. palmivora* in cocoa crossing was more influenced by the additive gene action. Lamour and Hausbeck (2000) who studied on mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan Cucurbit Fields also found that the dominance effect (H_1) was greater than the additive effect (D).

Genetic variance of additive is the main cause of similarity between relatives (between parent and offspring). While, the genetic variance of dominance is the major cause of dissimilarity between relatives. This variance is the main basis for heterosis and combining ability. Thus, only the additive and dominance gene actions that determine the variance of resistance to *P. palmivora* disease. In the present study, the additive gene action was smaller than the dominance gene action (Table 6). This suggests that genetic variance is more determined by dominance gene action (Hayman 1954).

3.3 Gene Distribution in the Parents

Distribution of parental genes showed that the H_2 value of genes that determine hereditary of resistance to black pod rot disease was not spread evenly in the parents (Table 6). This can be seen by the very significant H_2 value. The proportion of positive genes is shown by the value of H_1 against H_2. If $H_1 > H_2$, so the number of positive genes is more than negative genes, whereas, if $H_1 < H_2$, then the number of negative genes will be more than positive genes (Hayman 1954). The cocoa resistance to black pod rot disease caused by *P. palmivora* was determined by the positive genes. This indicated by the value of $H_1 > H_2$, suggesting that the parents that carry different genes will give different response to the resistance of black pod rot disease.

3.4 Dominance Level

The effect of the dominance $(H_1/D)^{1/2}$ was more than one (1,6003), indicating an over-dominance (Table 6). According to Hayman (1954), the $(H_1/D)^{1/2}$ value that more than one indicates the over-dominance, whereas, the value between zero and one shows partial dominance (partial dominance or recessive).

3.5 Proportion of Dominance to Recessive Genes

The value of Kd/Kr reflects the amount of dominant genes in the parents. If Kd/Kr > 1, it means the dominant genes

are more in the parents. Conversely, when Kd/Kr < 1, it means the recessive genes are more in the parents (Singh & Chaudhary 1979). The results in Table 6 shows that the value of Kd/Kr was 1.3594, indicating that the dominant genes were more in the parents. This result indicates that the dominant gene used as parents to produce better offspring may be difficult to achieve. Therefore, in the breeding program to produce cocoa hybrid, it is suggested to combine recessive parents.

3.6 Direction and Order of Dominance

The order of dominance of parents (based on Wr + Vr) for resistance to black pod rot disease caused by *P. palmivora* is presented in Figure 1. Clone ICS 13 was the parent having the most dominant genes (16.68), followed by clone DR 1 (68.65). While, the other three clones (TSH 858, Sca 6, and ICCRI 3) had the most recessive genes. Based on the order of dominance, when a parent is closer to zero, it has most dominat genes. Whereas, when a parent is farther from zero, it has most recessive genes (Singh & Chaudhary 1979; Sousa & Maluf 2003).

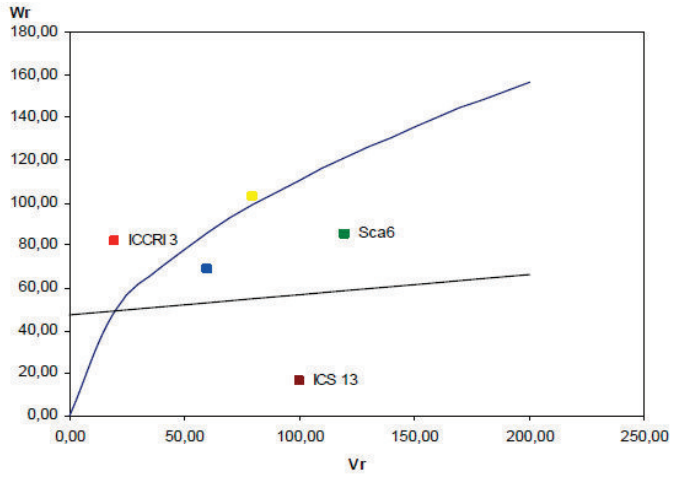

Figure 1. Relation of covariance (Wr) and variance (Vr) for cocoa resistance to black pod rot disease caused by *P. palmivora*

Moreover, based on the order of dominance of the parents, it was possible that the recessive parents to have opportunity to produce good hybrid in terms of the heterosis. If it is proven, so it is expected that the opportunity to produce cocoa hybrid owned by ICCRI 3 and Sca 6.

3.7 Number of Genes Controlling Characters

The resistance of cocoa plant to black pod rot disease caused by *P. palmivora* was controlled by the recessive genes. Number of the controlling genes is reflected in the value of (h^2/H_2). Number of the controlling genes for the cocoa resistance to black pod rot disease caused by *P. palmivora* was 2.1396 (Table 6).

3.8 Heritability

The results of the analysis using variance of phenotypes and additive genetic showed that based on the spot area as the results of *P. palmivora* inoculation, the estimated value of broad sense heritability (h^2BS) for the character of resistance to black pod rot disease caused by *P. palmivora* was 0.9600, while the estimated value of narrow sense heritability (h^2NS) was 0.5589 (Table 7).

Table 7. Heritability in the broad sense (h^2_{BS}) and in the narrow sense heritability (h^2_{NS}) for the resistance based on the spot area and disease intensity to *P. palmivora*

Character	Heritability	
	h^2_{BS} (%)	h^2_{NS} (%)
Spot area	96.00	55.89
Criteria	High	High
Disease intensity	50.46	20.95
Criteria	High	Moderate

Meanwhile, based on the disease intensity, the heritability in broad and narrow senses was high and moderate. Hanson (1963) classified the heritability as follows: $h^2 > 50\%$ is high, $20\% \leqslant h^2 \leqslant 50\%$ is moderate and $h^2 < 20\%$ is low. According to this classification, the heritability based on the components of spot area was high. Heritability in the narrow sense indicated the extent of the role of the additive gene performance. Hanson (1963) stated that the heritability in the narrow sense only describes the extent of the genetic variance in relation to the phenotypic variance.

4. Conclusions

There was no interaction between genes in determining the resistance to black pod rots disease caused by *P. palmivora*. The resistance was more affected by the additive gene action. Characters of resistant to black pod rots disease caused by *P. palmivora* were controlled by recessive genes. The results in the present study showed that the dominant genes were more in the parents. It is expected that the opportunity to produce cocoa hybrid owned by ICCRI 3 and Sca 6. The estimated value of broad sense heritability (h^2BS) and narrow sense heritability (h^2NS) was high for the spot area, while, based on the disease intensity it was moderate to high.

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