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Diallel Analysis of Chili (*Capsicum annuum* L.) Resistance to *Phytophthora capsici* Leonian

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ABSTRACT

To accomplish the study of genetic parameters of chili resistance to P. capsici, thirty cross combinations from six parents' crosses were made by full diallel method. The resistance was assessed based on the incidence of disease, following the inoculation of 28 days-old plant of chili. Inoculation was done by dropping 5 mL of inoculum (10^5 zoospore mL^{-1}) at the base part of each plant. There was non-allelic interaction and overdominance effect. Chili resistance to P. capsici controlled by one positive gene. The parents contained more dominant gene, with dominance order were IPB-C4, IPB-C10, IPB-C15, IPB-C9, IPB-C8, and IPB-C2. The prediction of the possible limits of selection if homozygote genes assemble on one individual was 0.676-0.691. Broad-sense and narrow-sense heritability values for the traits were high.

Keywords: chili, diallel analysis, genetic parameter, Phytophthora capsici

ABSTRAK

Untuk mempelajari parameter genetik ketahanan cabai terhadap P. capsici telah dibentuk 30 kombinasi persilangan cabai dari 6 tetua menggunakan metode full dialel. Ketahanan dinilai berdasarkan kejadian penyakit, inokulasi dilakukan setelah tanaman berumur 28 hari. Inokulasi dilakukan dengan memberikan 5 mL inokulum pada dasar masing-masing tanaman. Terdapat interaksi non alelik dan efek overdominan. Cabai yang tahan terhadap P. capsici dikendalikan oleh 1 gen positif. Tetua mengandung lebih banyak gen dominan, dengan urutan dominansi IPB-C4, IPB-C10, IPB-C15, IPB-C9, IPB-C8, dan IPB-C2. Prediksi batas kemungkinan seleksi ketika gen-gen homozigot berkumpul dalam satu individu adalah 0.676-0.691. Heritabilitas arti luas dan arti sempit untuk kejadian penyakit tergolong tinggi.

Kata kunci: analisis dialel, cabai, parameter genetik, Phytophthora capsici

INTRODUCTION

One of the main and important disease which reduces the production of chili is Phytophthora blight caused by *Phytophthora capsici* Leonian (Kurt and Emir, 2004; Demirci and Dolar, 2006). The fungi of *P. capsici* could attack chili at every stage and every part of plant. Disturbances in seedling phase may lead to the death. In mature stage, growth disorder may causes root rot, stem cancer, leave blight, and fruit rot (Demirci and Dolar, 2006).

One of the best ways to protect chili plants from *P. capsici* epidemic is by assembling resistant chili varieties through plant breeding programs. In order to obtain varieties which contain the desired traits, the genetic information of *P. capsici* resistances is needed. Genetic behavior of genes which control the resistance character to *P. capsici* can be studied through the estimation of genetic parameters. One of genetic parameters estimation method that has been commonly used was diallel mating analysis (Yunianti, 2007).

Diallel mating is a genetic crossing design which is commonly used to separate genetic effects from the environmental effects (Murtaza, 2005). Diallel mating technique was developed to obtain information about genetic mechanism involved in early generations (Khan and Habib, 2003). Diallel mating enables us to execute a systematic and complete genetic analysis, since at a full diallel with reciprocal and selfing treatment, population will be formed near the Hardy-Weinberg equilibrium of a random mating population (Fehr, 1987). Diallel mating analysis is also useful in estimating additive and dominant effects of a population which then can be used to infer genetic variances and heritability (Baihaki, 2000).

This method has been widely used to study the genetic analysis in pepper (de Sousa and Maluf, 2003; Geleta *et al*., 2006; Sujiprihati *et al*., 2007; do Rego *et al*., 2009; Kamble *et al.*, 2009; Daryanto *et al.*, 2010), barley (Kakani *et al.*, 2007), peanuts (Novita *et al*., 2007), wheat (Singh *et al.*, 2003; Inamullah *et al*., 2006), cassava (Owolade *et*

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al., 2006), papaya (Hafsah *et al.,* 2007), and pea (Kalia and Sood, 2009). The objective of this research is to study the genetic parameters of chili resistance to *P. capsici*.

MATERIALS AND METHODS

The experiments consisted of field and laboratory experiments. The basic population of chili was made in a private field at Sinarsari village, Darmaga, Bogor. Propagation and maintenance of fungi cultures performed at Plant Clinic and Laboratory Plant Protection Department of IPB. Screening of chili resistance to *P. capsici* was performed at Plant Breeding Laboratory, Department of Agronomy and Horticulture, IPB. The study lasted from January 2006 until April 2007.

A pure culture collection of *P. capsici* TG01 was used as fungi isolate, obtained from Dr. Widodo's collection (Department of Plant Protection, Faculty of Agriculture IPB) which was taken from endemic areas of *P. capsici* in Tegal, Central Java. The pathogen was propagated at V-8 agar media.

To develop diallel population, six chosen chili parents, namely IPB-C2, IPB-C4, IPB-C8, IPB-C9, IPB-C10, and IPB-C15, were planted (5 plants for each parent) without experimental design. Planting was done using black aluminum-painted plastic mulch with standard cultivation procedures. The number of crosses made are [n (n-1)/2] = [6(6-1)/2] = 15 F_1, [n (n-1)/2] = [6(6-1)/2] = 15 F_{1R}. Therefore, the total number of genotypes which were tested is 36 genotypes, consisted of 6 parents, 15 F_1 and 15 F_{1R}. Crossing and selfing for parent's propagation were conducted using the method of Yunianti (2007). Each genotype was made for male and female parents that would be crossed with another genotype.

The diallel experiment was arranged in randomized block design using single factor (36 genotypes of chili) with two replications. Seeds were sown in seedling trays with 72 cells. Commercial ready-to-use growing media provided as seedling media after steam pasteurized for 3 hours at 150 °C. Each line had 24 plants transplanted with two replications; hence each line consisted of 48 plants.

Inoculum was prepared according to Yunianti *et al*. (2007) procedures. Inoculation was conducted to 28 days-old (determined from seeding date) seedlings, by dropping 5 mL of inoculums (contain 10^5 zoospore mL^{-1}) at the base part of plants with pipette. Inoculated plants were incubated at standard temperature and watered twice a day or as needed to keep the media moist. Disease incidence (DI) was observed 7 days after inoculation. To ensure that the emerging symptoms were due to the infection of *P. capsici*, the infected stem were isolated at V-8 juice media for 5 days, and then observed microscopically for pathogen existence. The resistance classes were grouped as follows: resistant (0-20% infected); slightly resistant (21-50% infected); susceptible (51-80% infected); very susceptible (80-100% infected).

Data of chili resistances to *P. capsici* were taken using the formula [1-(DI/100)]. Estimations on genetic parameters of chili resistance to *P. capsici* were determined by diallel analysis using Hayman's approach following Singh and Chaudhary (1979) procedure.

RESULTS AND DISCUSSION

Estimation on genetic parameters using diallel mating analysis can be processed only if the analysis of variance showing that the mean square among genotypes is significantly different (Singh and Chaudhary, 1979). In this experiment, the result from analysis of variance showed that mean square for resistance to *P. capsici* was significantly different (Table 1). This showed that the estimation of genetic parameters for chili resistance to *P. capsici* could be continued to proceed.

The t-test to examine the value of regression coefficient of b (Wr, Vr) showed a significantly different result (Table 2). This fact indicates the role of gene interactions in determining the genetic variances (Hayman, 1954; Singh and Chaudhary, 1979). On diallel mating, a complementary type of interaction will shift the regression line (Wr, Vr), increasing the value of $(H_1/D)^{1/2}$, supressing the h^2/H_2, but had little effects on gene frequencies estimator $(H_2/4H_1)$ (Hayman, 1954). In the studies using basic population design, Yunianti (2007) reported some complementary genes interaction on the characteristic of chili resistance to *P. capsici.*

The additive (D) and dominance (H_1) influence gave contribution to the resistance to *P. capsici*. Since the influence of dominance is greater than additives effects ($D < H_1$), it can be concluded that the action of genes is over-dominance. The same result was also reported by Lamour and Hausbeck (2000). The dominance effects were also indicated from the value of H_1. H_1 value in this experiment is greater than one. According to Hayman (1954), H_1 greater than one indicates an over-dominance.

The distribution of genes in parents can be obtained from the value of H_2. The genes that determine the

Table 1. Analysis of variance of chili resistances to *P. capsici*

Source of varian	Degree of freedom	Sum of squares	Mean square	F-value
Replication	1	0.0002	0.0002	0.0490
Genotype	35	26.745	0.0764	21.3580**
Error	35	0.1252	0.0036	
Total	71	27.999		

** Significantly different

Table 2. The estimation of genetic parameter of chili resistances to *P. capsici* using diallel mating analysis

Genetic parameters	Value	t-value	t-table	Explanation
b (Wr, Vr)	0.4493 ± 0.1515	b = 0 : 2.9658	27.760	**
		b = 1 : 3.6349		**
D	0.0386 ± 0.0085	45.362	19.600	**
H_1	0.0989 ± 0.0216	45.762	19.600	**
H_2	0.0700 ± 0.0193	36.238	19.600	**
F	0.0188 ± 0.0208	0.9053	19.600	ns
h^2	0.0521 ± 0.0130	40.072	19.600	**
E	0.0017 ± 0.0032	0.5413	19.600	ns
$(H_1/D)^{1/2}$	16.003			
$H_2/4H_1$	0.1768			
Kd/Kr	13.594			
h^2/H_2	0.7443			
r	-0.115			
h^2bs	0.9600			high
h^2ns	0.5589			high
YD	0.6906			
YR	0.6762			

Note: ns = not significant; ** significantly different

inheritance of resistance to *P. capsici* did not spread evenly in the parents; although the parents used for population studies have been selected from various resistant classes. It could be seen from the value of H_2 which was significantly different.

The proportion of positive genes to negative genes was shown from the value of H_1 to H_2. If $H_1 > H_2$ the amount of the positive genes would be more than the negatives, otherwise if $H_1 < H_2$ then the amount of the negatives would be more than the positives one. Genes which are more involved in determining the character of resistance to *P. capsici* are the positive genes, and it is reflected in the value of $H_1 > H_2$. The proportion of the positive genes to the negatives can also be seen from $H_2/4H_1$ value.

The numbers of dominant genes in parents are reflected in the value of Kd/Kr. When Kd/Kr > 1, the genes are more dominant in the parents. On the other hand, if the Kd/kr < 1 then the amount of recessive genes is more in the parents. In this experiment Kd/Kr is greater than one, indicating the amount of dominant genes are more existing in the parents. The number of dominant genes is also reflected by the positive F value.

The results of the calculation of r (Wr + Vr < Yr) in this experiment is negative. This shows that the high quantification value is dominant to the low one. The order of parents domination (based Wr + Vr) for resistance to the *P. capsici* is IPB-C4 (0.001), IPB-C10 (0.010), IPB-C15 (0.014), IPB-C9 (0.016), IPB-C8 (0.018) and IPB-C2 (0.030) respectively (Figure 1). The order of dominance was also reflected by the position of parents and the position of one parent to zero. When the position of a parent is closer to zero, then it contains most dominant genes. Vice versa, more far a parent position to zero, then it mostly contains recessive genes. IPB-C2 is a parent which contains mostly recessive genes, because it is the farthest parent from zero. IPB-C4 contains the most dominant genes, since it is the closest one to zero.

Average deviation of F1 from most parents was significantly different; this is indicated by the significantly different of h^2 values. The groups number of genes which control resistance to *P. capsici* and cause dominances to at least one group of genes, indicated by the value of $h^2/H_2 = 0.7443$. Expected value of broad sense heritability (h^2_{bs}) of chili resistance against *P. capsici* in this experiment was high (0.960). It showed the variance of symptoms appeared, was mainly controlled by genetic factors. Expected value of narrow sense heritability (h^2_{ns}) resistance to *P. capsici* was also high (0.569). It shows that the proportion of additives variance in determining the resistance was high enough, according to the previous explanation that the roles of additives were significantly different.

The upper limit if all homozygous dominant genes accumulate in a single individual plant (YD) was 0.691. Meanwhile, the lowest limit if all homozygous recessive genes accumulate in a single individual plant (YR) was 0.676. It show that selection in this population provides opportunities to generate *P. capsici* resistant varieties, since these values were somewhat rely on the resistant criteria.

Finally, the results of this research enable us to obtain information regarding the interaction of genes and over dominance action in determining the chili resistant to *P. capsici*.

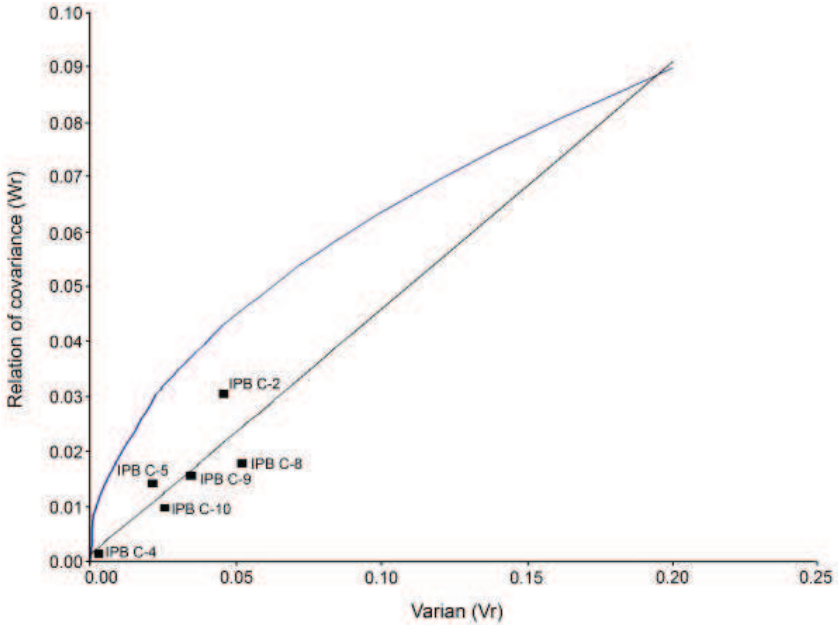

Figure 1. Relation of covariance (Wr) and variance (Vr) for chili resistances to *P. capsici*

CONCLUSIONS

Resistant characters are controlled by a group of positive genes. Parents which were used as studied population contain more dominant genes than recessive genes. The order of parent's dominance from the most dominant was IPB-C4, IPB-C10, IPBC-15, IPB-C9, IPB-C8 and IPB-C2 respectively. Resistant boundary when dominant homozygous genes gathered in one individual was 0.691, while those for the recessives ones was 0.676. Expected value of broad sense heritability (h^2_{bs}) and narrow sense heritability (h^2_{ns}) is high in this category.

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