Estimation of Genetic Parameters for Resistance to Begomovirus on Chilli Pepper Based on Joint Scaling Test

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ABSTRACT

Begomovirus infection has been reported from various important crops, including chilli pepper. The use of resistant chilli pepper cultivars is considered as the most effective way to control this disease. Study was conducted using two structured families (parental, F1, F2, back cross) derived from IPBC12 x 35C2 and IPBC10 x IPBC14, which IPBC12 (resistance), IPBC10 (moderate resistance), IPBC14 (moderate susceptible), and 35C (susceptible) to Begomovirus. Segunung isolate from Begomovirus infected plants was inoculated to the population using *Bemisia tabaci* as the vector. Results showed that begomovirus resistance on chili pepper was polygenic in nature, with extra chromosomal effect characterizing the IPBC12 x 35C2 family. For IPBC10 x IPBC14 family, the resistance was full dominant with additive, dominant, additive x additive and dominant x dominant gene actions, whereas for the IPBC12 x 35C2 family was over dominant with additive, dominant and additive x additive gene actions. Broad sense heritability was high on both family, but narrow sense heritability was low on IPBC10 x IPBC14 family. Therefore, recurrent selection should be considered as method for the development of hybrid cultivars with Begomovirus resistant.

Key words: Begomovirus, chili, pepper resistance

INTRODUCTION

Begomovirus is a virus which may damage cultivated plants. Infection of Begomovirus has been reported in beans plant (Garrido-Ramirez *et al.,* 2000), cotton (Naveed and Zahid 2007), cassava and tomato (Lapidot and Friedman 2002). In Indonesia, yellow curled leaf virus which attacked chili pepper was reported for the first time by Hidayat *et al.* (1999). In addition to infecting cultivated crops, the virus can infect weed (Salati *et al.,* 2002). The virus has also infected tobacco in Indonesia and caused crop failure (Aidawati *et al.,* 2002). This disease epidemic is influenced significantly by *Bemisia tabaci viruliferous* acting as a vector. It has been reported that one whitefly can cause virus infection (Sulandari *et al.,* 2004).

The safest control method which can be expected to succeed in controlling yellow curled leaf in chilli is the use of resistant varieties (Green and Kim 1994). Virus-resistant plants were obtained through the selection of germplasm and crossing between parents. The success of selection is determined by the amount of genetic variability in selected populations. Genetic inheritance is highly important in determining plant breeding strategies in order to have an effective character improvement program. Until now, researches on the genes controlling resistance to Begomovirus have been limited, and no research has been done on chilli. It is reported that the tomato plant resistance against Begomovirus is controlled by many genes (Pico *et al.* 1996, 1999 *In* Lappidoth and Friedmann 2002), while in bean plant (*Phaseolus vulgaris*) the genes are additive (Morales and Singh 1991 *In* Lappidoth and Friedmann 2002). Seo *et al*., (2004) suggests that bean plant resistance against Bean dwarf mosaic

geminivirus is controlled by a single dominant gene. Monci *et al*. (2005) also suggests bean plant resistance against Begomovirus is controlled by a single dominant gene.

Breeding resistance of tomatoes and beans against Begomovirus has been studied intensively abroad. Resistant varieties of beans and tomatoes have been widely produced, but no resistant variety of chilli has been reported. This research was aimed to study the genetic control of the inheritance of resistance against Begomovirus in chilli causing yellow curled leaf disease. The information obtained is expected to give an idea of breeding program which are effective and efficient to assemble superior cultivars resistant to Begomovirus in chilli.

MATERIALS AND METHODS

The research was conducted in a screen house at Agriculture Laboratory, University of Bengkulu and at a greenhouse in Cikabayan, Bogor Agricultural University. The research used genetic material derived from a collection of genetics and plant breeding Division, Bogor Agricultural University and collection from plant breeding division at University of Bengkulu. Genetic material consisted of six populations, ie P1 (parent 1), P2 (parent 2), F1 (offspring to 1), F2 (offspring to 2), BCP1 Back cross with parent 1) and BCP2 (back cross with parent P2). The six population was formed from two cross combinations: (1) parent resistant to Begomovirus (IPBC12) x parent susceptible (UNIB C GTS1); (2) parent moderately resistant (IPBC10) x parent rather susceptible (IPBC14). Twenty plants of P1 and P2, 20 F1, 200 F2, 50 BCP1 and 50 BCP2 were tested.

Individual-transmission method was used to transmit disease. Imagoes whitefly used as vectors were taken from cotton crops in the greenhouse experiment station in Cikabayan, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University. The Imagoes were maintained on cotton plants and allowed to lay eggs in captivity prevented from insects attack. The whitefly stadium used in transmission was the imago.

Plants were germinated in seedling trays until the age of 10 days and then were transferred to polythene bags measuring 30 x 40 cm. Inoculation was performed when the plants were 20 days old. The source of inoculum was tomato plants positively infected with Begomovirus, ie the 'Segunung' isolates. Some branches of the plants were covered with a cylindrical mica lid, the top of which was covered with gauze for ventilation. Then, a number whitefly inoculums were transferred to the plant to obtain feeding acquisition period for 24 hours. The transmission method was the following: the plants were closed with plastic cups with a diameter of 15 cm, the bottom of which was perforated and covered with gauze pads with a hole, 1 cm in diameter for whitefly entrance. Inoculation was done by covering the plants with plastic cups; then, 10 whiteflies which had obtained a 24-hour feeding acquisition period was taken using a glass pipette and was put on each individual plant and left for 48 hours (inoculation period). Then, the insects were killed by spraying water mixed with detergent. After that, the plants were maintained in the greenhouse until they showed symptoms. The severity of symptoms when the plants were in the period of first harvest was recorded by giving a score of 0-5 (Ganefianti *et al*., 2008).

Analyses of the data included: 1) Extrachromosomal effect. The medians of F1 and its resiprocal were tested with t-test at 5% level If the variances of F1 and F1R were homogeneous, the population of the two families can be combined in subsequent analysis. Homogeneity of variance was tested using F test; 2) The degree of predominance. It was calculated on the basis of potential ratio (hp) proposed by Petr and Frey (1966); 3) Mendelian Inheritance. It was analyzed using chi square test; 4) Estimation of Genetic Components. Analysis of the average generation was done to determine the most appropriate genetic model describing the relationship of the average generation, using the joint scaling test. (Mather and Jink, 1982); 5) Heredibility. Estimation of broad sense heritability was calculated based on the formula of Allard (1960) and the narrow sense heritability was calculated following Warner (1952).

RESULTS AND DISCUSSION

Extra Chromosomal Efffect

In populations of IPBC12 x UNIB C GTS1 crossing, there was a significant difference between F1 and F1R populations, but not between IPBC10 x IPBC14. The significant difference between F1

and F1R in populations IPBC12 x UNIB C GTS1 indicated that there was a extrachromosomal effect in the inheritance of resistance to Begomovirus in chilli. Characters of resistance are controlled by genes outside nucleus. But the population of IPBC10 x IPBC14 showed that Begomovirus-resistant character was controlled by genes contained in the nucleus. The extrachromosomal effect was shown in all family F1 phenotype observations and F1R at IPBC12x35C. Both of these families showed different phenotypes in term of symptom types, in which the disease intensity of F1 was 2.00% (resistant), while the disease intensity of F1R was 29.00% (susceptible). On the contrary, in the crossing of IPBC10 x IPBC14, there was no maternal effect. In this population, phenotype appearances of F1and F1R family were not different: the disease intensities of F1 and F1R were respectively 6.00% and 8.00% (moderately resistant). Thus, the two populations of chilli had different Begomovirus-resistant genes.

Some researchers who have conducted experiments on genetic resistance to Tomato yellow leaf curl Begomovirus (TYLCV) have reported different results. Friedmann et al. (1998) reported that resistance of TY171 strains derived from Lycopersicon peruvianum was controlled by at least three genes. Resistance in L.hirsutum was controlled by two to three recessive additive genes (Vidavsky and Czosnek, 1998), at L.pimpenellifolium by a single major gene (Vidavsky et al.,1998). Resistance in L. hirsutum used by AVRDC (Asian Vegetable Reseach and Development Center) was controlled by two genes with epistasis effects (Hanson et al. 2000). The different results of suggest that differences in the method, the place and the population used can influence the results. There has been no report on the effect of extrachromosomal on the heritance of Begomovirus-resistance in chilli pepper plants. The inheritance of resistance to Begomovirus in tomato plants was generally controlled by genes tin the nucleus. The influence of maternal was expressed by Owolade et al. (2009) in cassava genotypes resistance to Colletotricum gliesporioides manihotis f sp. In another study, Jumbo and Carena (2008) reported that out of all corn characters studied, only one character, ie ear height, showed maternal inheritance. Voichita and Ioan (2009) suggests that there is a reciprocal effect on the inheritance of grain quality of sweet corn, indicating there was an interaction between the nucleus and cytoplasm gene.

The Degree of Dominance

The potential ratio values in character scores symptom types in the population of IPBC12 x UNIB C GTS1 and IPBC10 xIPBC14 were respectively 1.24 and 1.0 (Table 1). Results of calculation of potential ratio in the population IPBC12 X UNB C GTS1 indicated that the chilli pepper resistance to Begomovirus infection was over-dominant, in which the average scores /symptom types of F1 was located close to that of the resistant parent, even some averages of F1scores /symptom types of F1 were smaller than that of parent resistant. This means that the expression of mild symptoms (resistant parent) was more dominant than the expression of severe symptoms (susceptible parent). In the cross of IPBC10 x IPBC14, the potential ratio value charater scores/ symptom types of chilli pepper plants to Begomovirus infection was fully dominant (Table 1). These results are in contrast to the tomato resistance to TYLCV (*Tomato yellow leaf curl Begomovirus*) which is controlled by a dominant gene with partial dominant gene action (Lapidot e*t al*., 2000). Mazyad *et al*. (2007) suggested that tomato plant's tolerance is controlled by a recessive gene with partial recessive gene action.

Table 1. The mean and standard error of scores/type symptoms characters in parent (P), filial 1 (F1), parent average (MP) on chili pepper infected by Begomovirus, and the potential value ratio

	IPBC12x35C	IPBC10xIPBC14
P1 (parent 1)	0.30±0.47	0.4±0.49
P2 (parent 2)	1.95±1.46	1.2±1.48
F1 (filial 1)	0.10±0.30	0.4±0.94
MP(mead parent)	1.125	0.8
Hp(high parent)	1.24	1.00
	Over dominant	Full dominant

Estimation of Genetic Component

No test on Mendellian compatibility ratio was done in population of IPBC12 x UNIB C GTS1 because there was a extrachromosomal effect. The result of normality test showed that the distribution of F2 frequency was normal with only single peak. Therefore, in this population, biometric analysis

was conducted to determine the gene action using the joint scaling test. But, in the population of IPBC10 x IPBC14, normality test showed data abnormality with multi peak values, indicating there were influences of major genes and minor genes. Subsequently, in this population, two approaches were used, i.e. Mendellian genetic analysis (to track the role of major genes) and biometric analysis (to examine the role of minor genes).

On IPBC10 x IPBC14 population, Chi Square test (χ^2) showed the observed ratio was in accordance with Mendelian ratio for two genes. The corresponding ratio was 13 resistant: 3 susceptible (χ^2 value= 0.051 and 0.016; χ^2 table = 3,841). Therefore, the frequency ratio scores / symptom types in the cross of IPBC10 x IPBC14 followed Mendelian ratios for the two genes, which means that chilli pepper resistance to Begomovirus on this cross combinations is likely controlled by two sets of genes, with epistasis dominant and recessive gene action. The results of back cross showed 3: 1 ratio.

Some experimental results showed that tomato's resistance to Tomato yellow leaf curl Begomovirus (TYLCV) was controlled by at least one to two genes (Mazyad *et al*. 2007). Another experiment conducted by Vidavsky and Czosnek (1998) showed that the TYLCV resistance in tomato was controlled by two to three genes. Nainar and Pappioha (2002) reported that the resistance-tomato to TYLCV was controlled by three genes. Lappidoth et al. (2000) also found that the resistance-tomato to TYLCV had a ratio of 7:64, meaning that there were at least three genes that control resistance.

Table 2. The results of Mendellian ratio compatibility test on score/symptoms type in F2 population

Population		O	E	Hipothesis	χ^2 value	*Back cross*
IPBC10 x IPBC14	Resistant	161	150	3:1	3.381ns	3:1
	Susceptible	39	50			
	Resistant	161	162.5	13:3	0.051ns	
	Susceptible	39	37.5			

To determine the genes action that controls the character scores/type of symptom, joint scaling test was done. Based χ^2 test, there were three genetic models compatible with cross of IPBC12 x 35C on the character of the score / type symptoms, namely m [d] [h] [i], the model m [d] [h] [i] [l], and the model m [d] [h] [j] [l] because the χ^2 value were significant at the 5% level. The result of t-test for each individual genetic component revealed that m [d] [h] [i] model had all significantly different values of genetic components: additive genes effect, dominant and additive x additive interaction; while the other two compatible models had one non significant ly different genetic component.

The results demonstrated that the most suitable model for the character scores / type of symptoms due to infection Begomovirus chili was the model m [d] [h] [i] because all the genetic components contributed significantly to this genetic model (Table 3). The additive component (d) contained in this population make it possible that the population is used to establish pure line breeding chilli pepper as the final target. Selection in this population will effectively accumulate the desired additive genes. The additive component on the character of the score / type of symptoms had a negative sign, which means the average at P1 is smaller than P2. The dominant components [h] was negative, meaning that the low expression scores / symptom type are more dominant than high expression sore/symptom type.

Because of the presence of dominant effect (h), theoretically, it can be assumed that this population can be used to create hybrid of chilli. This can also be seen from the fact that the average value for the F1 population scores / symptom types (0.100) is smaller than the average value of the two parents (1,125). This indicates the presence of an over-dominant effects which may result in heterosis effect.

There are two genetic models suitable for cross of IPBC10 x IPBC14 on scores /symptom types character, namely m [d] [h] [i], and the model m [d] [h] [i] [l], because the t-tests were not significant at 5% level. The results of t-tests for each individual genetic component showed that the model m [d] [h] [i] [l], all the genetic components, namely additive and dominant genes, additive x additive interaction and dominant x dominant interaction had significant effect. The results showed that the most suitable model for the character scores/symptom types on chilli pepper infected by Begomovirus

was the model m [d] [h] [i] [l] because all the genetic components contributed significantly to this genetic model and had the smallest χ^2 value (Table 3).

The type of interaction in this population is a duplicate interactions because a opposite sign between in the value of the genetic component aditive [d] with additive x additive interaction, also the dominant value [h] with dominance x dominance interaction [l]. According to Mather and Jink (1982) when the two components are the same sign, then the type of interaction that dominates is the type of complementary. Based on the estimation of the genetic component can be inferred type of interaction which dominates the appearance of duplicate scores / type symptoms because the opposite sign.

Table 3. Estimation of genetic component scores/symptom types characters in the most compatible genetic model on based joint scaling test model

Crossing	model	M	[d]	[h]	[i]	[l]	χ^2
IPBC12 x 35C	m [d] [h] [i]	5.14±0.22 **	-0.71 ±0.14**	-5.04 ±0.25**	-4.11±0.28 **	-	1.345ns
IPBC10x IPBC14	m [d] [h] [i] [l]	- 0.72±0.48 ns	-0.51 ±0.14**	3.07±1.39**	1.61±0.49 **	-1.94 ±0.99	1.16ns

The Estimate of Heritability

On cross of IPBC12 x UNIB C GTS1, the estimate of broad sense heritability score/ symptom types character was categorized as high (74.54%). This indicated that variability of symptoms was controlled by genetic factors, while the environmental effect was little. The narrow sense heritability estimate was high, ie 52.52%. This meaned that the proportion of additive variance in determining the phenotype appearance scores / symptom types was also high. The contribution of additive genetic variance to total genetic variance was 70.45% (proportion h2ns to h2bs) (Table 4). Additive genetic variance properties can be fixed through selection (Falconer 1991), and the selection can be done in early generations.

On cross IPBC10 x IPBC14 broad sense heritability was high, indicating that the variability scores / symptom types are more driven by environmental rather than genetic factors. The narrow sense heritability was low, meaning that the proportion of genetic additive variance than the total genetic varians was very small. This indicated that contribution to total genetic variance was derived from the dominant and interaction variances.

Table 4. Broad sense heritability (h_2^{bs}) and narrow sense heritability (h_2^{ns}) estimation on score/symptom type character at two cross.

Cross	h_2^{bs}(%)	h_2^{ns}(%)	h_2^{ns}/ h_2^{bs} x 100%
IPBC12 x 35C	74.54	52.52	70.45
IPBC10 x IPBC14	64.16	0.00	0.00

CONCLUSION

1. Chilli pepper resistance to Begomovirus was controlled by polygenic in IPBC12 x UNIB C GTS1 population and by two genes in populations of IPBC10 x IPBC14. There were extra-chromosomal effects in inheritance of resistance to Begomovirus in cross of IPBC12 x UNIB C GTS1.

2. The genes controlling resistance to Begomovirus in chilli pepper were dominant. The degree of dominance was categorized as over dominance.
3. The genes acting in chilli pepper resistance to Begomovirus in IPBC12 x UNIB C GTS1 population were additive, dominant and additive x additive interaction. In IPBC10 x IPBC14 population the action genes were additive action, dominant, additive x additive interaction and dominance x dominance interaction.
4. The broad sense heritability was high, while the narrow sense heritability was low to high.

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