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Diallel Analysis of Chili Pepper Resistance to Melon Aphid (*Aphis gossypii* Glover) Infestation in Seedling Phase

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Abstract

Aphis gossypii Glover is one of important insect pest in Indonesia. Genetic analysis of resistance to *A. gossypii* is required in plant breeding program to obtain host-plant resistance cultivar. Diallel analysis was used to estimate genetic parameters for chili pepper resistance to *A. gossypii* infestation in early generation. The objective of this research was to estimate genetic parameters of chili pepper resistance to A*. gossypii* infestation with diallel crossing design. The F1 and parent plants were arranged in randomized competed block design with three replication. Resistance lines was measured using choice test laboratory screening techniques. Two aphids were infested per plant and stopped 12 days after first infestation. Different lines respond was detected as shown by significant numbers of aphid per leaf, total aphid per plant, and total winged aphid per plant. There were no maternal effect and resistance were controlled by recessive and polygenic genes. Gene effects for resistance to aphid's infestation were additive and dominance. Dominance effect larger than additive effects. Broad-sense heritability values were high but narrow-sense heritability values were very low

Keywords: additive, dominance, half diallel, heritability, recessive

A. Introduction

The cultivation of chili pepper in lowland area has many obstacle such as high insect pest infestation. Melon aphid, *Aphis gossypii*, is one of the most important insect pest in low altitude and humid areas (Messelink, Bloemhard, Sabelis, & Janssen, 2013) and estimated can reduce ranged from 56 to 65% of chili pepper yields when no insectisides are taken (Fereres, Avilla, Collar, Duque, Fernández-Quintanilla, 1996). Cultural practices such as crop rotation, field sanitary, and delayed planting dates, are not always effective to control this pest. The development of host plant resistant remains the most effective and reliable management strategy against insect pests and may also increase the suppression of the pest development in combination with biological control (Maharijaya & Vosman, 2015).

Genetic information on resistance to melon aphid infestation in chili pepper is required to obtain high-yielding varieties with melon aphid-resistant and it can be done by estimation of genetic parameters. One of the methods used for genetic parameter estimation is the diallel cross analysis (Syukur, Sujiprihati, Koswara, & Widodo, 2013). The diallel cross method is known as a systematic and defensive genetic evaluation in view of the potential for crossing in the early generations (Johnson, 1963). Diallel mating design have been used primarily to estimate genetic variances when parents are either random individuals or inbred line from a random-mating population in linkage equilibrium (Hakizimana, Ibrahim, Langham, Haley, & Rudd, 2004).

Diallel mating design has an advantages to estimate the general combining ability (GCA), specific combining ability (SCA), additive and dominance effects, genetic variance and heritability (Roy, 2000). On the other hand, Combining ability can be analyzed by Griffing method (Griffing, 1956), while genes action, genetic component and heritability estimated by Hayman method (Hayman, 1954). GCA being a measure of additive gene action while SCA is due to non-additive (dominant or epistatic) gene action (Nsabiyera, Ssemakula, Sseruwagi, Ojiewo, & Gibson, 2013). Diallel crossing design has used in many plant species such as chili pepper (do Reˆgo, do Reˆgo, Finger, Cruz, & Casali, 2009; Daryanto, Sujiprihati, Syukur, 2010; Sitaresmi, Sujiprihati, Syukur, 2010; Syukur *et al*., 2013; Nsabiyera *et al*., 2013; Ganefianti, Hidayat, Syukur, 2015), papaya (Hafsah, Sastrosumarjo, Sujiprihati, Sobir, & Hidayat, 2007), maize (Vivek, Odongo, Njuguna, Imanywoha, Bigirwa, Diallo, & Pixley, 2010), tomato (Elsayed Ay, Henriques, Mizbuti, & Carneiro 2011; Saleem, Asghar, Iqbal, Rahman, & Akram, 2013) and wheat (Hakizimana, Ibrahim, Langham, Haley, & Rudd, 2004; Malla, Ibrahim, & Glover, 2009).

Diallel crosses have been used extensively to study the genetics of disease resistance in chili pepper such as chili resistance against Cercospora (Nsabiyera *et al*., 2013), Anthracnose (Syukur *et al*. 2013), and Begomovirus resistance (Ganefianti *et al*. 2015) but it has not been done on the evaluation of pest resistance in chili pepper. Evaluation of Alfafa resistance to *Acyrthosiphon pisum* with diallel crossing was reported by Bournoville, Carre, Julier, Landre, & Ecalle (2001) and the resistance of maize to the *Busseola fusca* reported by Beyene, Mugo, Gakunga, Karaya, Mutinda, Tefere, Njoka, Chepkesis, Shuma, & Tende, (2011).

This article analyzed gene's action, genetic components, combining ability, and heritability. of chili pepper plant resistance to aphid infestation. This study was to obtain information about the genetic parameter of chili pepper resistance to aphid infestation using half diallel analysis to found out host-plant resistence genotype. Host-plant resistance genotypes can be useful for keeping number of aphid population under economically damage level.

B. Methodology

Plant materials: The plant material used in this study was five lines genotypes which selected from 21 genotypes of chili pepper (*Capsicum annuum* L.) from previous study (Daryanto, Syukur, Maharijaya, & Hidayat, 2017). Genotype IPB 20 selected as low aphid infestation whereas IPB C3, IPB C4, IPB C5, and IPB C313 as high aphid infestation. They are collection of Bogor Agricultural University. These lines were crossed in all possible combination without reciprocals (half diallel). The plants were grown from seeds in plastic tray with 50 holes and placed in insect-tight box. The seeds were sowed on each holes of plastic tray containing a mix of growing medium (coco peat: soil: green manure; 1:1:1 v) and did not use insecticide during this experiment to avoid insecticide effects on the treatment.

Aphid colonies: Melon aphids were collected from pepper cultivation at Unifarm of Bogor Agricultural University, Indonesia followed by the identification of the species to ensure that the aphid colonies were A. gossypii Glover. The specific identification keys for A gossypii were the black cornicles, cauda lighter than cornicle, and the antennal tubercles were weakly developed.

The identification was based on the identification key guides of Blackman & Eastop (2014). Imago were cultured on susceptible pepper plants and propagated in insect-tight box, temperature of 28 ± 2oC; RH 65 ± 10% (Daryanto *et al*., 2017). Routine maintenance by moving the adult aphids to susceptible pepper plants were done when the aphid population had seen too crowded.

Choice test: Five parents and 10 F1 were conducted during the seedling phase of pepper (4-6 leaves or 5 weeks after sowing), in an insect box. Two adult wingless-aphids (apterous) were transferred with a soft brush to the leaves of the seedlings. Aphids were allowed to migrate, feed, and reproduce freely. A chili pepper resistance test to aphid infestation was conducted in Plant Breeding Laboratory, Department of Agronomy and Horticulture, Bogor Agricultural University. The experiment was designed in a randomized complete block design with pepper genotypes (five parents and 10 F1) as treatment with three replications. Observation was done at 12 days after infestation by counting the number of aphids on each genotype.

Statistical analysis: Normality test and Bartlett's test at 5% level of significance were done to meet the assumption εij ~ N (0, 2); error normal spread, the mean μ, and variance homogeneous. Furthermore, the data were tested by ANOVA (F-test), when the treatments significantly difference, genetic parameter estimation was continued using two approaches, the Hayman and Griffing methods (Singh & Chaudhary, 1979).

C. Result and Discussion

Maternal effect test (female parent) was done by comparing of morphological character and aphid's infestation on chili pepper genotype. Maternal effect used for early clarification of diallel method which need to involve reciprocal genotype (F1R) or not. The test results showed F1 population was not significantly different to F1R (Table 1 & Table 2). It's mean that the phenotype and aphid's infestation respon of F1 and F1R chili pepper genotypes was relatively similar. The absence of maternal effects indicated that the resistance of chili pepper to aphid infestation are controlled by genes in the nucleus.

The absence of maternal effects on chili pepper meet with assumption of no reciprocal effect occur, so analysis can be done by methods 2-Griffing (Singh & Chaudhary, 1979) and Hayman analysis (1954). Method 2-Griffing or half diallel is using population F1 and parents without involving F1R. The advantage of this method is result of predicting genetic parameters and combining ability as well as Method 1-Griffing, while the population used is less. Griffing's 2 method has been used in the estimation of corn resistance against stemborer (Beyene *et al*. 2011), phytopthora blight in tomatoes (Elsayed *et al*., 2011), and spotting Cercospora in chili pepper (Nsabiyera *et al*., 2013).

Table 1. Mean and homogenity of seedling leaf morphology in F_1 and F_{1R} chili pepper crossing of IPB C20 x IPB C333

Character	Genotype	Mean	Anova test	t-student [1)]
Leaf width	F_1	1.87	0.67 ns	1.34 ns
	F_{1R}	1.94		
Leaf lenght	F_1	3.88	1.35 ns	1.93 ns
	F_{1R}	4.22		

[1)] ns: not significantly different at P < 0.05

Table 2. Mean and homogenity of aphid infestation in F_1 and F_{1R} chili pepper crossing of IPB C20 x IPB C333

Aphid infestation	Genotype	Mean	Anova Test	t-student [1)]
Aphid per leaf	F_1	43.9	0.73 ns	0.38 ns
	F_{1R}	40.9		
Aphid per plant	F_1	124.0	0.39 ns	0.52 ns
	F_{1R}	142.5		

[1)] ns: not significantly different at P < 0.05

The ANOVA showed significant variation among genotypes based on analysis of variance (F-test) of melon aphid infestation on chili pepper genotypes (Table 3). Therefore, genetic parameter estimation using the diallel cross analysis can be determined for all characters.

Table 3. Analysis of variance (mean square) of melon aphid infestation on chili pepper genotypes

Aphid infestation character	Mean square [1)]
Aphid per plant	16.712**
Aphid per leaf	1.925 **
Winged aphid	0.629 **

[1)] **: significantly different at $P < 0.01$

The gene interaction can be seen from b regression coefficient (Wr, Vr) on Table 4. The result of b value for all characters were not distinct to one. Therefore, no gene interaction occurs in determining resistance to melon aphid infestation on chili pepper half diallel population. The result showed that chili pepper resistance to melon aphid infestation was caused by allelic gene that was one of diallel cross analysis assumption.

The non-additive (H1) showed dominant effect because regression coefficient (b value) known no epistasis effect. The addictive effect was showed only on winged aphid (0.30) but it was still lower than its dominance value (0.56). This suggested that the melon aphid infestation in chili pepper plants was influenced by action of dominant gene rather than additive gene. This result was consistent with the evaluation of genetic inheritance of chili pepper resistance to melon aphid infestation on six generation population (Daryanto *et al*., 2017).

The gene distribution on parents can be seen from H2 value. The genes that determine the ability of melon aphid infestation on chili pepper spread evenly within the parents on the total aphid per plant. This was reflected from not significant value of H2. This situation is in accordance with the term of the diallel analysis where the genes incorporated in the parent (Roy, 2000). Meanwhile, aphid infestation per leaf and winged aphid did not spread evenly within the parents which showed significant value of H2. Positive genes proportion will be apparent from comparison of H1 to H2 value. If value of H1 > H2, most genes were positive; on contrary, if H1 < H2 negative genes were more than the positive ones. Most genes determining susceptible to melon aphid infestation were positive gene.

Table 4. Estimation of genetic parameter of chili pepper to melon aphid infestation using the Hayman method of diallel analysis.

Genetic parameter	Aphid per plant	Aphid per leaf	Winged aphid[1)]
b (Wr, Vr)	-0.74 ns	-0.52 ns	0.92 ns
D	3.43 ns	0.23 ns	0.30 **
F	7.34 ns	0.46 ns	0.28 ns
H_1	25.93 *	2.81 **	0.56 **
H_2	22.00 ns	2.62 **	0.40 **
h^2	0.92 ns	-0.01 ns	0.02 ns
E	1.68 ns	0.19 ns	0.09 **
$(H_1/D)^{1/2}$	2.75	3.49	1.38
$H_2/4H_1$	0.21	0.23	0.18
Kd/Kr	2.27	1.80	2.06
h^2/H_2	0.04	-0.01	0.04
h^2bs (%)	76.63	77.39	67.63
h^2ns (%)	0.11	-2.18	31.78

[1)] b (Wr, Vr): Covariance-variance regression coefficient, D: Additive effect, F: Fr mean, H_1: Dominance effect, H_2: Proportion of dominance due to positive and negative effect of genes, h^2: F1 deviation from the average parent, E: Environment effect, $(H_1/D)^{1/2}$: Mean degree of dominance, $H_2/4H_1$ Proportion of dominance genes to recessive genes, Kd/Kr: The proportion of dominant to recessive genes, h^2/H_2: Number of groups of genes. h^2bs: Heritability in broad-sense, h^2ns: Heritability in narrow-sense, ns: not significantly different at $P < 0.05$, *: significantly different at $P < 0.05$ **: significantly different at $P < 0.01$.

The value of (H1/D)1/2 was indicated dominance effect level. According to Hayman (1954), if value of (H1/D)1/2 was more than one that indicates over dominance, whereas the value of (H1/D)1/2 between zero and one, indicating partial dominance (partial dominance or partial recessive). (H1/D)1/2 value of melon aphid infestation on chili pepper was more than one (2.75, 3.49 and 1.38), indicating over dominance effect (Table 4). In line with the results of the study of inheritance of chili pepper resistance against infestation of A. gossypii using six generations population (Daryanto *et al*., 2017), that susceptible over dominance to resistance

effect. So the resistance of chili pepper to melon aphd was actually recessive. The evaluation of chili pepper crossing to Anthracnose (Syukur *et al*., 2013) and inbreed tomato line against Phytophthora blight (Elsayed *et al.,* 2011) were controlled by recessive genes.

The number of dominance genes in parent genotypes reflected from the value of Kd/Kr. The value of Kd/Kr > 1, indicating dominance gene numbers were large in parent. On the other hand, Kd/Kr < 1, parent contains high recessive genes (Singh & Chaundhary, 1979). All charachter showed Kd/Kr values > 1 (2.27, 1.80, and 2.06), indicating more dominance genes in parent genotypes. This can be explainded because only the IPB C20 as a genotypes that carried the resistance character with minor recessice gene while the other four parent genotypes carried dominant genes.

Resistance to infestation of melon aphid was controlled by recessive genes. Their numbers of gene were reflected in the value of (h^2/H^2). The number of genes controlling resistance to infestation of melon aphid was one controlling groups (Table 4). The resistance estimation of broad-sense heritability ($h_2$bs) to three characters of melon aphid infestation in chili pepper genotypes were high with value 76.63, 77.39, and 63.67 but narrow sense heritability (h^2ns) was very low (Table 5). The high value of broad-sense heritability (h^2bs) have been able to explain well the genetic proportions of phenotypic-observed. However, the small value of narrow-sense heritability (h^2ns) showed that the proportion of the dominant (non-additive) genetic variation was greater than additives on the aphid infestation per plant and aphid per leaf. Daryanto *et al.* (2017) reported similar value of heritability of aphid infestation in chili pepper through a population of six generations. Non-additive gene action was reported to be characteristic on chili pepper resistance against spotting of Cercospores and bacteria (Nsabiyera *et al*., 2013).

Table 5. Variance analysis of general combining ability (GCA) and specific combining ability (SCA) of chili pepper genotypes to melon aphid infestation.

Source	df	Mean squares [1)]		
		Aphid per plant	Aphid per leaf	Winged aphid
GCA	4	1.651 ^{ns}	0.169 ^{ns}	4.620 **
SCA	10	7.148 **	0.803 **	2.187 ^{ns}
Error	28	1.644	0.178	0.083

[1)] ns: not significantly different at P < 0.05 **: significantly different at P < 0.01

Table 6. General combining ability (GCA) and spesific combining ability (SCA) values of chili pepper genotypes to melon aphid infestation.

Genotype	Aphid per plant	Aphid per leaf	Winged aphid
IPB C3	-0.150	0.108	-0.154
IPB C4	-0.501	-0.168	-0.135
IPB C5	0.045	-0.008	-0.010
IPB C20	-0.188	-0.130	-0.107
IPB C313	0.795	0.199	0.406
IPB C3 x IPB C4	-2.606	-0.958	-0.365
IPB C3 x IPB C5	-0.604	-0.413	0.172
IPB C3 x IPB C20	0.407	0.322	0.071
IPB C3 x IPB C313	1.137	0.714	-0.130
IPB C5 x IPB C4	3.819	1.361	0.108
IPB C4 x IPB C20	4.326	1.283	0.503
IPB C4 x IPB C313	-2.816	-0.854	-0.407
IPB C5 x IPB C20	0.093	-0.097	0.334
IPB C5 x IPB C313	1.770	0.539	0.654
IPB C20 x IPB C313	1.248	-0.978	-0.375

The selection of parents was based on high GCA values, because GCA represented the average appearance of a parent in all of its hybrid sets and was strongly associated with the action of the additive gene (Elsayed *et al*., 2011). The effect of GCA was not evident for aphid's infestation per

plant and aphid per leaf, while the winged aphid showed a very significant effect (Table 5). Generally, GCA value are indicated by large and positive values (Bournoville *et al*., 2001). However, on resistance characteristics such as to aphid infestations, the negative and large GCA values would contribute a high level of aphid resistance. Similarly reported, the negative GCA and SCA values contribute a high level of antracnose resistance in papaya (Hafsah *et al*., 2007) and high level of Wheat Steak Mosaic Virus resistence in winter wheat (Hakizimana *et al*., 2004)

Spesific combining ability (SCA) values were a reflection of the average appearance of hybrids, the crosses of two parents, strongly associated with non-additive i.e. dominant and epistasis gene effects (Elsayed *et al.,* 2011; Hakizimana *et al*., 2014). Highly significant difference were observed for SCA on character of aphid per plant and aphid per leaf while the infestation of winged aphid was not significantly different amongs hybrids (Table 5). The expected SCA value for aphid resistance character was negative as a contribution to the genotype resistance in suppressing the preferences and reproduction of melon aphid infestation in chili pepper plants. This value was in line with the low narrow sense of heritability, reflecting that the action of the dominant (non-additive) gene was more responsible for the character of aphid infestation in chili pepper plants.

The hybrid of IPB C4 x IPB C313 and IPB C3 x IPB C4 had high specific combining ability to the resistant of aphid per plant and per leaf i.e. -2.816, -2.606, -0.854 and -0.958 (Table 6). The genotypes of IPB C3 was susceptible genotype and IPB C4 was medium or low moderate category of aphid infestation in previous studies. IPB C4 was suspected has minor genes of resistance to aphid infestation so it was able to increase resistance level of IPBC3 and IPB C313 in hybrid form. IPB C4 had a good GCA on the character resistance to phytophthora blight (Yunianti, Sastrosumarjo, Sujiprihati, Surahman, & Hidayat, 2011). Performance of hybrids crossed by IPB C20 did not appear to have good SCA. It showed that aphid infestation resistant of IPB C20 controlled by recessive gene.

IPB C20 consistantly evaluated as a resistance genotypes to aphid infestation with negative GCA on all aphid infesation character, while IPB C313 consistantly susceptible to aphid infestation with positif GCA. Similar result, antixenosis test, that IPB C20 showed significantly reduced development and reproduction of melon aphid infestation and IPB C313 as suceptible genotype (Daryanto *et al*., 2017). Daryanto et al. (2010) stated that IPB C20 was not a good GCA for fruit character and chili production because IPB C20 is an ornamental chili pepper type with small fruit. Similar result was also found in the evaluation of the combined ability of corn resistance against corn stalk borer, stem borers, in which resistant maize genotype did not have a good GCA in production characters (Beyene *et al*., 2011). Futher, breeding program needs to create good ideotype on both consumption and resistance character to melon aphid.

These combining ability information indicated that resistance genes were still scattered outside of the IPB C20, so it needs to be assembled into a genotype. Convergent breeding methods can be applied to collect these minor resistance genes. One method of convergent breeding i.e, transgressive recombination can be used to collect genes that are dispersed into a genotype. This method is not much different from the pyramiding gene method for transferring some specific genes into a plant (Acquaah, 2011).

This research was the initial information about the chili pepper genetic resistance parameters of the melon aphid infestation. The experiments focused on the use of various types of chili pepper populations, Capsicum annuum species, in identifying the resistance of chilli peppers to aphid infestations. The expected output was breeding line that could be used as a resistant donor line and susceptible check for the development of chili pepper varieties.

D. Conclusion

There was no interaction between the non-allelic (epistatic) genes in the resistance of aphid infestations in chili pepper. The influence of dominance was significant on the character of aphid infestation while the additive effect was not significant. The genes that determine the character of aphid infestations dispersal evenly within the chili pepper. The dominant level that occurs was over dominance of susceptible genotype to the resistant genotype and the dominant genes were more susceptible than the resistance genes within the dialel crosses. General combining ability of the winged aphid infestation character was significantly different amongs parent genotype and IPB C3, IPB C4, IPB C5, and IPB C20 effectively becoming good GCA in suppressing the formation of winged aphid.

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F. Author Contribution

Ady Daryanto: Designed and conducted the experiment. Muhamad Syukur, Awang Maharijaya, Purnama Hidayat, supervisor and edited the manuscript.

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