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Antixenosis and antibiosis based resistance of chili pepper to melon aphid

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Abstract

The melon aphid or cotton aphid (*Aphis gossypii* Glover) is one of the major pests of pepper. Chemical based crop protection is the major way to control aphid until now. The use of resistant varieties may help to reduce the use of insecticides, together with Integrated Pest Management. The objective of this research was to identify the antixenosis and antibiosis based resistance of melon aphids in several pepper genotypes that may be explored as sources of resistance in aphid resistance breeding program of pepper. We used choice and no-choice test, and detached leaf based experiments. Antixenosis based resistance was detected as shown by significant number of aphid per leaf, total aphid per plant, and total winged aphid per plant. Antibiosis based resistance was also detected as shown by significant difference in longevity time, reproduction time, number of aphid progeny per day, and the fecundity of the melon aphid among genotypes.

Key words: *Capsicum annuum*, choice test, cotton aphid, host-plant resistance, no-choice test

Introduction

Aphis gossypii Glover (Hemiptera: Aphididae) or melon-cotton aphids, is one of the important insect pests in pepper, especially in low altitude and humid areas when no control measures are taken (Messelink *et al.*, 2013). Melon aphid is polyphagous insect, they can attack pepper, cucumber, melon, squash, cotton, citrus, coffee, cocoa, potatoes, tobacco, and some of ornamental plants (Blackman and Eastop, 2014). Melon aphids attack their host by piercing and sucking fluid epidermal cells, mesophyll of leaves, and phloem tissue using their stylet. Aphids excrete a sticky liquid called honeydew. The damages caused of honeydew can promote the sooty mold on host plants. Sooty mold is the result of association of the honeydew and fungus. If sooty mold formations were thick enough, they can inhibit the process of photosynthesis, resulting leaf yellowing, leaf curling, and ultimately lead to stunted plant growth (Tilmon *et al.*, 2011).

The life cycle of melon aphid is short, in which viviparous reproduction and parthenogenesis or asexual propagation take place (Sullivan, 2008). These cause an abundance of aphid colonies that can damage the host plants. Without the use of insecticides, infestation of melon aphid on chili pepper plants is estimated to reduce 56-65% yields (Fereres *et al.*, 1996). Aphids can also transmit 22 viruses to Solanaceae crops (Hooks and Fereres, 2006), including non-persistent viruses such as CMV (*Cucumber mosaic virus*), Potyvirus (ChiVMV), and Polerovirus (Escriu *et al.,* 2000; Pinto *et al.*, 2008).

The aphid management and control practices include chemical treatments, biological controls and cultural practices. However, up till now, the use of insecticides is the major way to control aphids. However, insecticides might also kill beneficial insects, predators, parasitoids and pollinators. Besides that, large scale application of chemical pesticides can lead to serious health and environmental problems. Melon aphids have also been resistant to many insecticides such as organophosphate and pyrethroid (Carletto *et al.*, 2010). The use of host-plant resistance is one of the best management strategy against insect pests. Incorporation of resistant varieties may be a valuable addition to the IPM system. Resistant varieties can be used together with cultural practices (*e.g.* field sanitary and crop-rotation measures) to prevent infestation.

Resistant varieties may also increase the suppression of the pest development in combination with biological control (Maharijaya and Vosman, 2015). Towards breeding for resistance against aphids, it is important to identify the resistance of several pepper genotypes to aphids. Although some important studies regarding aphid resistance in pepper have been reported before (Bosland and Ellington, 1996; Frantz *et al.*, 2004; Babu *et al.*, 2011), our current study is the first report focusing on the source of resistance in *C. annuum,* the most cultivated pepper species in the world. The main objective of this research was to select several pepper genotypes that may be explored as sources of resistance in aphid resistance breeding program of pepper.

There are three mechanisms of plant defense against pests *i.e.* antixenosis, antibiosis, and tolerance (Niks *et al.*, 2011; Maharijaya, 2013). Antixenosis or non-preference is a defense mechanism in form of morphology, phenology, and odor from the plant to reject the presence of pests. Antixenosis can be evaluated through the reduction in number of colonies of pests (Hesler and Dashiell, 2011). Antibiosis is the ability of plants to limit and reduce the proliferation of pathogens after contacting with the

plant. Antibiosis on insect are reflected in high mortality, low breeding rate of the neonate, and decreased reproductive ability of pests (Li *et al.,* 2004). Tolerance is the difference in the ability of plants to respond to pests and limiting damage per unit in presence of pests (Niks *et al.*, 2011). However these different mechanisms are not always easy to separate (Maharijaya and Vosman, 2015). Thus in our current study, we tried to identify the antixenosis and antibiosis effect of pepper plant to aphids.

Materials and methods

Plant materials: Twenty one genotypes of peppers (*Capsicum annuum* L.) from Bogor Agricultural University and The World Vegetable Center or Asian Vegetable Research and Development Center (AVRDC) collections were used for this study. The plants were grown from seeds in plastic tray with 50 holes and placed in insect-tight box. Firstly, two seeds were sown in each hole of plastic tray containing a mix of growing medium (soil: coco peat: manure; 1:1:1 v) and after two weeks, one seedling was maintained in each hole. No insecticide was used during this experiment to avoid insecticide effects on the treatment.

Aphid population: 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 identification was based on the identification key guides of Blackman and Eastop (2014). The specific identification keys for *A. gossypii* were the black color of cornicles, the pale color of cauda (cauda lighter than cornicle), and the antennal tubercles that were weakly developed (not exceeding height of medial part of frons). Adult aphids (imago) were cultured on susceptible pepper plants and propagated in insect-tight box (temperature of 28 ± 2°C; RH 65 ± 10%). Routine maintenance by moving the adult aphids to fresh susceptible pepper plants were done.

Choice test: Screening of the twenty one genotypes was 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 (choice-test). The experiment was designed in a randomized complete block design with pepper genotypes as treatment with three replications. Observation was done at 12 day after infestation by counting the number of aphids per leaf on each genotype. Further, the genotypes were categorized as follows: 8-21 aphids per leaf = very low infestation, 22-35 aphids per leaf = low infestation, 36-49 aphids per leaf = lower-medium infestation, 64-77 aphids per leaf = high-medium infestation, 78-91 aphids per leaf = high infestation, 92-105 aphids per leaf = very high infestation. Six genotypes were selected using above criteria. Selected genotypes were used further for the antixenosis and antibiosis based resistance tests.

Antixenosis based resistance test were done in a choice test setup as in previous screening test. Aphids were allowed to migrate, feed, and reproduce. Six genotypes selected based on the result of the first screening test *i.e.* IPB C5, IPB C12, IPB C20, IPB C145, and IPB C313 were used. Observation was done at 12 day after infestation by counting the number of aphids per leaf and per plant on each genotypes.

No-choice test: Antibiosis based resistance test was done in a no-choice setup using detached leaf system. Leaves of pepper, the third or fourth fully opened leaves from the top, of each genotypes were used in this experiment. Each leaf was placed in a container (6.3 x 5 cm) with addition of wet cotton to keep the leaves fresh. Each container was covered by muslin (50 meshes) for ventilation. Environmental conditions were kept at 28 ± 2°C and 65 ± 10% RH based on Satar *et al.* (2008). Observations were carried out every day until all aphids died. As initial infestation one apterous adult was placed and after 24 hours that got first newborn nymph. Nymphs, 3-5 nymphs, were maintained until be imago for testing the nymph survival and development time. Furthermore we selected one imago from nymphs that had become imago to be tested fecundity, longevity, and reproduction time. All newborn nymphs were counted and removed daily.

Nymph survival was the number of living nymphs of first birth to be imago, while the life cycle was the time interval from first instar to first instar back. Longevity time was calculated from the first newborn nymph to death of selected imago. Fecundity was the total number of nymphs (progenies) produced by an aphid during its lifetime. The experiment was designed in a randomized complete block design with pepper genotypes as treatment with three replications.

Statistical analysis: Normality test and Bartlett's test at 5% level of significance were done to meet the assumption $\varepsilon ij \sim N(0, \sigma 2)$; error normal spread, the mean μ, and variance homogeneous. Furthermore, the data were tested by ANOVA (F-test), when the treatments significantly differed, followed by Honestly Significant Difference (HSD) test. Correlation (Pearson) was performed on leaves character against aphid infestation. The statistical analysis were done using Microsoft Excel 2013, IRRI's STAR, and Minitab 15.

Results and discussion

Screening of pepper genotypes against melon aphids: There were significant differences ($P < 0.05$) in response to the number of aphids infestation per leaf among genotypes of pepper used in this study (Table 1). The range of aphid infestation was 22.9 - 95.8 nymphs per leaf. IPB C5 had the lowest number aphid per leaf with an average of 22.9 nymphs. However, it was not significantly different with IPB C145, IPB C325, IPB C324, IPB C120, IPB C313, IPB C140, IPB C4 and IPB C20, while genotype IPB C3 had the highest aphid per leaf by 95.8 nymphs and was not significantly different with IPB C19, IPB C10, IPB C142, IPB C51, IPB C15, and IPB C12. This kind of differences was also found by Frantz *et al.* (2004) in peppers against *Myzus persicae* infestation with a range of 15.5 - 115.4 nymphs per leaf. This indicates that there are clear differences among pepper genotypes for their suitability or resistance as host for aphids which might be explored as natural resistance sources in pepper. Since *C. annuum* is the major cultivated pepper species (Bosland *et al.*, 2012), the finding of resistance sources among *C. annuum* is very important considering their compatibility to transfer the resistance into commercial varieties of pepper through conventional crossing and selection.

Antixenosis based resistance: Difference was found in the number of aphid per leaf among the same genotypes in the first screening (Table 1) compared to the second choice test (Table 2). This might indicate the detection of antixenosis based resistance

Table 1. Number of aphids per leaf, 12 days after aphid infestation, in twenty one genotypes of pepper

No	Genotypes	Aphid per leaf*	Classification**
1	IPB C5	22.9 h	Low infest
2	IPB C145	23.3 h	Low infest
3	IPB C325	25.4 gh	Low infest
4	IPB C324	28.4 fgh	Low infest
5	IPB C120	28.4 fgh	Low infest
6	IPB C313	29.4 fgh	Low infest
7	IPB C140	36.8 efgh	Medium-low infest
8	IPB C4	36.9 efgh	Medium-low infest
9	IPB C20	45.7 efgh	Medium-low infest
10	IPB C9	51.5 defg	Medium infest
11	IPB C159	54.4 def	Medium infest
12	IPB C323	58.5 cde	Medium infest
13	IPB C111	59.9 bcde	Medium infest
14	IPB C322	59.9 bcde	Medium infest
15	IPB C19	72.3 abcd	Medium-high infest
16	IPB C10	76.5 abcd	Medium-high infest
17	IPB C142	81.5 abc	High infest
18	IPB C51	82.3 abc	High infest
19	IPB C15	86.1 ab	High infest
20	IPB C12	93.4 a	Very high infest
21	IPB C3	95.8 a	Very high infest

Numbers followed with same letter in column are not statistically different; Tukey test with α=0.05

** 8-21 aphids per leaf = very low infestation, 22-35 aphids per leaf = low infestation, 36-49 aphids per leaf = lower-medium infestation, 64-77 aphids per leaf = high-medium infestation, 78-91 aphids per leaf = high infestation, 92-105 aphids per leaf = very high infestation

in pepper. Antixenosis or non-preference is a defense mechanism in form of morphology, phenology, and odor from the plant to reject the presence of pests. Genotypes containing relatively lower resistance level were visited by more aphids compared to those containing higher level of resistance to aphids since the aphids will choose the genotype with lower resistance level. Genotype IPB C20, consistently, had the lowest aphids per plant compared to other genotypes (Table 2). Difference of the number of winged aphid as shown in Table 2 stressed the presence of antixenosis effect in pepper to aphids. In certain condition, such as non preference condition, adult aphid can be equipped with a pair of wings as a mechanism of dispersal colonies (Kunert *et al.,* 2005). Antixenosis was suggested to be the defense mechanism active in *C. pubescence* against *Myzuz persicae* (Bosland and Ellington, 1996). The dense hairiness of *C. pubescence* leaves may be impregnable to aphid feeding, or at least not preferred by aphids. This is not the case in our study since we did not detect hairiness in *C. annuum* leaves. Therefore the antixenosis based resistance in our study must be caused by other factors.

Table 2. The average number of aphid infestation on six genotypes choice test method

Genotypes	Total aphid/ plant	Aphid per leaf	Winged aphid
IPB C5	213.9 ab	40.5 ab	6.6 ab
IPB C12	207.5 ab	51.1 a	6.6 ab
IPB C15	191.1 ab	46.6 ab	4.6 b
IPB C20	101.1 b	21.2 b	1.7 b
IPB C145	195.9 ab	40.4 ab	6.5 ab
IPB C313	271.7 a	51.2 a	13.1 a

Numbers followed with same letter in column are not statistically different; Tukey test with α=0.05

Antixenosis based resistance on pepper cultivation may strongly protect the plant from the infestation of aphid, especialy in a mix cultivation system of pepper varieties. A strong antixenosis could reduce direct damage, virus acquistion and transmission (Mutschler and Wintermantel, 2006). However, incomplete antixenosis can enhance the spread of the viruses within a pepper crop or to other crops since it can increase insect probing and movement (Joost and Riley, 2005).

Antibiosis based resistance: Antibiosis based resistance in pepper against aphids was identified. In the no-choice test, all of the biological characters of aphid were affected by genotype except the life cycle. Life cycle was 4-5 days and did not differ significantly among the genotypes. This result is similar with previous finding on cucumbers (van Steenis and El-Khawass, 1995) and *Colocasia esculenta* var. esculenta (Agarwala and Choudhury, 2013).

Reproductive time and longevity of melon aphid on 6 genotypes were in the range of 7-12 days and 13-18 days, respectively (Table 3). On genotype IPB C20, shortest longevity and reproduction time of melon aphid (13 days and 7 days) was recorded compared to other genotypes tested whereas genotype IPB C313 caused

Table 3. Effect of six selected genotypes to biological aspect of aphid infestation by non-choice test method

Genotypes	Life cycle (day)	Longevity time (day)	Reproduction time (day)
IPB C12	4.5	15.9 b	8.4 bc
IPB C145	4.9	13.8 cd	7.9 bc
IPB C15	4.4	16.1 b	9.6 b
IPB C20	4.6	13.0 d	7.2 c
IPB C5	4.6	14.4 c	8.3 bc
IPB C313	4.6	17.9 a	11.8 a

Numbers followed with same letter in column are not statistically different; Tukey test with α=0.05

longer longevity and reproductive time (18 and 12 days) for melon aphid among the 6 genotypes tested. Short longevity and reproduction time in natural conditions suppress the development of aphid colonies (Thomson *et al.,* 2010).

There were differences in the number of progeny per day and total nymph (fecundity) during the period of reproduction among the 6 genotypes. Number of newborn aphids (progeny) per day ranged 3-5 nymphs, while fecundity ranged 23.4-53.5 nymphs (Table 4). IPB C20 genotype demonstrated the ability to suppress the aphid progeny per day and fecundity compared with IPB C313. These data supported previous experimental data on antixenosis

Table 4. Nymph survival, number of progeny per day and fecundity on six selected genotypes

Genotypes	Nymph Survival (%)	Progeny per day (nymph day^{-1})	Fecundity (nymph aphid^{-1})
IPB C12	91	4.3 ab	36.0 b
IPB C145	62	3.7 abc	29.7 bc
IPB C15	81	3.6 bc	33.5 bc
IPB C20	70	3.4 bc	23.4 c
IPB C5	73	3.3 c	26.8 bc
IPB C313	91	4.6 a	53.5 a

Numbers followed with same letter in column are not statistically different; Tukey test with α=0.05

resistance test where IPB C20 was a genotype with low aphid preference. Antibiosis influence also found in soybean against *A. glycines* by reducing fecundity on genotype resistant or tolerant (Diaz-Montano *et al.*, 2006; Hesler *et al.,* 2007).

Host plant quality is one of important factors that influence the antibiotic resistance of plants (Mottaghinia *et al.*, 2011). The ability of melon aphid to reproduce and to survive are influenced by amino acids and secondary metabolites of host plant. For example, the fecundity and survival of *A. gossypii* on *Chrysanthemum indicum* plants were positively correlated with the levels of amino acids or nitrogen in its leaves (Rostami *et al.*, 2012).

Wild relatives are already well known as good and reliable sources of resistance traits for plant genetic improvement including resistance to insect pests (Hajjar and Hodgkin, 2007; Broekgaarden *et al.,* 2011). However, the use of wild relatives as source of resistance is constrained by biological constraints such as hybrid sterility and low cross-ability, retention of undesirable traits (Hajjar and Hodgkin, 2007). Fortunately, IPB C20 is *C. annuum*, the most cultivated amongst chilli pepper varieties. Therefore, the introgression of resistance is possible through conventional crossings.

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Author contribution

AD, AM and MS conceived the project. AD performed most of the practical work and was the main author of the manuscript. AM and MS supervised the work on daily basis and contributed extensively to the manuscript. PH contributed to the writing of manuscript and assisted with the insect rearing.

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