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, make leaves yellowing, leaves curling, and ultimately lead to stunted plant growth (Tilmont et al., 2011).

The life cycle of melon aphid is short and fast, 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 estimated can 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 (Escric et al., 2000; Pinto et al., 2008).

The aphid management and control practices include chemical treatments, biological controls and cultural practices. However, up to now, the use of insecticides is the major way to control aphids. However, insecticides might also killed 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). Toward 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), however 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 number of colonies of pests (Hesler and Dashiel, 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 decrease reproductive ability of pests (Li et al., 2004). Tolerance is the difference in the ability of plants to respond to pests and limiting damage to the broader per unit where these pests (Niks et al., 2011). However these different mechanisms are not always easy to separate (Maharjaya 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 sowed on each holes of plastic tray containing a mix of growing medium (soil: cocoa peat: green manure; 1:1:1 v) and separated to be one each hole after two weeks. 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 antenral 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 when the aphid population had already seen too crowded.

**Choice tests**: 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 follow: 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 = very high infestation. Six genotypes were selected using above criteria. Those selected genotypes were used for further the antixenosis and antibiosis based resistance tests.

Further 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 single container (6.3 cm 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. Initial infestation placed one apteroous adult for 24 hours and after 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 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 εij ~ N (0, σ2), error normal spread, the mean μ, and variance homogeneous. Furthermore, the data were tested by ANOVA (F-test), when the treatments significantly difference, followed by Honestly Significant Difference (HSD) test. Correlation (Pearson) was

<table>
<thead>
<tr>
<th>No</th>
<th>Genotypes</th>
<th>Aphid per leaf</th>
<th>Classification</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IPB C5</td>
<td>22.9**</td>
<td>Low infest</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>IPB C145</td>
<td>23.3</td>
<td>Low infest</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IPB C325</td>
<td>25.4**</td>
<td>Low infest</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>IPB C324</td>
<td>28.4</td>
<td>Low infest</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>IPB C120</td>
<td>28.4</td>
<td>Low infest</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>IPB C313</td>
<td>29.4</td>
<td>Low infest</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>IPB C140</td>
<td>36.8</td>
<td>Medium-low infest</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>IPB C4</td>
<td>36.9</td>
<td>Medium-low infest</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>IPB C20</td>
<td>45.7</td>
<td>Medium-low infest</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>IPB C9</td>
<td>51.5</td>
<td>Medium infest</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>IPB C159</td>
<td>54.4</td>
<td>Medium infest</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>IPB C323</td>
<td>58.5</td>
<td>Medium infest</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>IPB C111</td>
<td>59.9</td>
<td>Medium infest</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>IPB C322</td>
<td>59.9</td>
<td>Medium infest</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>IPB C19</td>
<td>72.3</td>
<td>Medium-high infest</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>IPB C10</td>
<td>76.5</td>
<td>Medium-high infest</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>IPB C142</td>
<td>81.5</td>
<td>High infest</td>
<td></td>
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<td>18</td>
<td>IPB C51</td>
<td>82.3</td>
<td>High infest</td>
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</tr>
<tr>
<td>19</td>
<td>IPB C15</td>
<td>86.1</td>
<td>High infest</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>IPB C12</td>
<td>93.4</td>
<td>Very high infest</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>IPB C3</td>
<td>95.8</td>
<td>Very high infest</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers followed with same letter are not statistically different; Duncan 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.
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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.

Antixenosis based resistance on pepper cultivation may strongly protects the plant from the infestation of aphid, especially in a mix cultivation system of pepper varieties. A strong antixenosis could reduce direct damage, virus acquisition 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 difference 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 in the range of 7-12 days and 13-18 days (Table 3). Genotype IPB C20 made shortest longevity and reproduction time of melon aphid infestation by non-choice test method. This might indicates the detection of antixenosis based resistance 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 *Myzus persicae* (Bosland and Ellington, 1996). The dense of hairiness of *C. pubescence* was 3-5 nymphs, while fecundity was 23.4 – 54.5 nymphs (Table 7). Genotypes containing relatively lower resistance level were visited more aphids compare to those containing higher level of resistance to aphids since the aphids will chose the genotype with lower resistance level. Genotype IPB C20, consistently, had the lowest aphids per plant compared to other genotypes (Table 2).

Table 4 Nymph survival, Number progeny per day, and fecundity on six genotypes of pepper

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Nymph Survival</th>
<th>Number progeny per day</th>
<th>Fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPB C12</td>
<td>91</td>
<td>4.3</td>
<td>36.0</td>
</tr>
<tr>
<td>IPB C145</td>
<td>62</td>
<td>3.7</td>
<td>29.7</td>
</tr>
<tr>
<td>IPB C15</td>
<td>81</td>
<td>3.6</td>
<td>33.5</td>
</tr>
<tr>
<td>IPB C20</td>
<td>70</td>
<td>3.4</td>
<td>23.4</td>
</tr>
<tr>
<td>IPB C5</td>
<td>73</td>
<td>3.3</td>
<td>26.8</td>
</tr>
<tr>
<td>IPB C313</td>
<td>91</td>
<td>4.6</td>
<td>53.5</td>
</tr>
</tbody>
</table>

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

There were differences in the number of progeny per day and total nymph (fecundity) during the period of reproduction among the 6 genotypes. Range number of newborn aphids (progeny) per day was 3-5 nymphs, while fecundity was 23.4 – 54.5 nymphs (Table 4). IPB C20 genotype demonstrated the ability to suppress the aphid colonies (Thomson et al., 2010).

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<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Nymph Survival (%)</th>
<th>Progeny per day (nymph day⁻¹)</th>
<th>Fecundity (nymph aphid⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPB C12</td>
<td>91</td>
<td>4.3</td>
<td>36.0</td>
</tr>
<tr>
<td>IPB C145</td>
<td>62</td>
<td>3.7</td>
<td>29.7</td>
</tr>
<tr>
<td>IPB C15</td>
<td>81</td>
<td>3.6</td>
<td>33.5</td>
</tr>
<tr>
<td>IPB C20</td>
<td>70</td>
<td>3.4</td>
<td>23.4</td>
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<tr>
<td>IPB C5</td>
<td>73</td>
<td>3.3</td>
<td>26.8</td>
</tr>
<tr>
<td>IPB C313</td>
<td>91</td>
<td>4.6</td>
<td>53.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Total aphid/plant</th>
<th>Aphid per leaf</th>
<th>Winged aphid</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPB C5</td>
<td>213.9</td>
<td>40.5</td>
<td>6.6</td>
</tr>
<tr>
<td>IPB C12</td>
<td>207.5</td>
<td>51.1</td>
<td>6.6</td>
</tr>
<tr>
<td>IPB C15</td>
<td>191.1</td>
<td>46.6</td>
<td>4.6</td>
</tr>
<tr>
<td>IPB C20</td>
<td>101.1</td>
<td>21.2</td>
<td>1.7</td>
</tr>
<tr>
<td>IPB C145</td>
<td>195.9</td>
<td>40.4</td>
<td>6.5</td>
</tr>
<tr>
<td>IPB C313</td>
<td>271.7</td>
<td>51.2</td>
<td>13.1</td>
</tr>
</tbody>
</table>

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

| 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 \(^b\) |
| IPB C313| 4.6 | 17.9 \(^c\) | 11.8 \(^c\) |

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

Table 2 The average number of aphid infestation on six genotypes of pepper

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Life cycle (day)</th>
<th>Longevity time (day)</th>
<th>Reproduction time (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPB C12</td>
<td>4.5</td>
<td>15.9 (^b)</td>
<td>8.4 (^b)</td>
</tr>
<tr>
<td>IPB C145</td>
<td>4.9</td>
<td>13.8 (^c)</td>
<td>7.9 (^c)</td>
</tr>
<tr>
<td>IPB C15</td>
<td>4.4</td>
<td>16.1 (^b)</td>
<td>9.6 (^b)</td>
</tr>
<tr>
<td>IPB C20</td>
<td>4.6</td>
<td>13.0 (^d)</td>
<td>7.2 (^c)</td>
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<tr>
<td>IPB C5</td>
<td>4.6</td>
<td>14.4 (^c)</td>
<td>8.3 (^b)</td>
</tr>
<tr>
<td>IPB C313</td>
<td>4.6</td>
<td>17.9 (^c)</td>
<td>11.8 (^c)</td>
</tr>
</tbody>
</table>
progyni aphids per day and fecundity compared with IPB C313. These data supported previous experimental data on antixenosis resistance test where IPB C20 was a genotype with low aphid preference. Antibiosis influence also found in soybean against A. glycinus by reducing fecundity on genotype resistant or tolerant (Diaz-Montano et al., 2006; Hesler et al., 2007).

Host plant quality is one of important factor 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 positively correlated with the levels of amino acids or nitrogen in it 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; Broekgaard 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 largest cultivated of chili pepper which farmer already has planted. Therefore the introgression of resistance factors can be done through conventional crossings.

Acknowledgement

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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.

References


