Inheritance of resistance to cowpea aphid (Aphis craccivora Koch.) in IT82E-16

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Abstract

The cowpea aphid (Aphis craccivora Koch.) is one of the major insect in yardlong bean production in Thailand. Breeding for resistant to this insect is need to be done. The cross between susceptible variety of yardlong bean (Selected-PSU) and resistant variety of cowpea (IT82E-16) was made to produce F₁ and F₂ progenies. The parental line and their progenies were then screened for aphid resistance in the field and microsatellite marker segregation. Under the greenhouse condition, most F₁ plants were resistant to cowpea aphid, while F₂ populations segregated 177 resistant to 63 susceptible, fit a 3:1 ratio. Result indicated that single gene controlling resistance in IT82E-16. One microsatellite marker from primer VM 37 was found to be linked to aphid resistant gene.

Keyword: cowpea, yardlong bean, microsatellite marker, Aphis craccivora Koch

Introduction

Yardlong bean, (Vigna unguiculata ssp. sesquipedalis) is a common vegetable in Asian market and sources of protein to the people in Asia. In Thailand production area of yardlong bean was approximately 18,560-20,160 ha annually. One of a major problem for yardlong bean production in Thailand is severe infestation and damages caused by insect pests in the field. Cowpea aphid (Aphid craccivora Koch.) is an important pest of yardlong bean and cowpea (Quan, 1996; Singh and Jackai, 1985). The cowpea aphid feeding by sucking terminal shoots, flowers and pods. Several aphid infestations can cause leaf distortion, stunning and reduced pod set in the plant. In extreme cases, the infested plant dies. Not only damaging the plants, aphid also through transmit mosaic virus to plants (Atiri, 1985).

The use of resistant cultivar is the cheapest and most affective way to control insect pest in the production area. Many cowpeas accession from cowpea germplasm at the International Institute of Tropical Agriculture have been identified as resistance to A. craccivora Koch. Aphid resistance in cowpea is inherited as a monogenic dominant trait (Beta et al., 1987; Pathak, 1988). In Thailand, Benchasri et al., (2007) evaluated 24 yardlong bean and cowpea genotype for cowpea aphid resistance, and they reported that cowpea IT82E-16 displayed a high level of resistance. This finding offer promises for the development of cowpea aphid resistance yardlong bean cultivars.

The molecular markers are useful tools in plant breeding and can be utilized to select genotypes with resistant gene from segregating population. Microsatellite markers, or simple sequence repeat (SSR) have been successfully used in cowpea (Li et al., 2001). The objectives of this study are to determine the inheritance of cowpea aphid resistance and identify microsatellite markers closely linked to the cowpea aphid resistance in yardlong bean and cowpea.
**Materials and Methods**

Crossing between selected-PSU and IT82E-16 was made to produce F₁ and F₂ seeds. Seeds of selected-PSU, IT82E-16 F₁ and F₂ were planted under the screenhouse. The experimental design was a Completely Randomized Design (CRD) with unequal replications. Parental lines and its F₁ were planted in 3 replication (10 plants/replication) while F₂ was planted in 24 replications. Five cowpea aphids were released in each plant at the 30 days after planting.

**Data collection and analysis**

Number of aphids and damage on leaves and flower buds were recorded every 7 days after exposed to aphids. Visual damages were evaluated using damage scores (Smith et al., 1994) as described in Table 1.

### Table 1 Scores and the symptom description on each plant

<table>
<thead>
<tr>
<th>Score</th>
<th>Symptom descriptions</th>
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<tbody>
<tr>
<td>0</td>
<td>visual damage on leaves and flower buds &lt;10%</td>
</tr>
<tr>
<td>1</td>
<td>visual damage on leaves and flower buds 10-25%</td>
</tr>
<tr>
<td>2</td>
<td>visual damage on leaves and flower buds 26-50%</td>
</tr>
<tr>
<td>3</td>
<td>visual damage on leaves and flower buds 51-75%</td>
</tr>
<tr>
<td>4</td>
<td>visual damage on leaves and flower buds 76-100%</td>
</tr>
</tbody>
</table>

Based on these observations, the following rating scale was used to estimate aphid resistance: 0-2 = resistant (R), 3-4 susceptible (S). Chi-squared test was performed to test the goodness of fit to a 3:1 ratio in F₂.

**Microsatellite analysis**

Young leaves from each plant were collected and DNA extraction was performed by the cetyl trimethyl ammonium bromide (CTAB) protocol of Doyle and Doyle (1990). Five primer pair were used for PCR amplification. The PCR reaction was carried out in 10 µl final volumes containing 20 ng of genomic DNA, 10X Taq buffer, 0.2 µM each of the forward and reverse primers, 200 µM dNTPs and 0.7 unit of Taq polymerase. The reaction was performed by predenature at 94 °C for 2 min. The touch-down PCR for seven cycles were follow: 94 °C for 1 min, 64 °C for 30 sec, 72 °C for 1 min for 18 cycles, 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min for 30 cycles and final extension at 72 °C for 10 min. Amplification products were run on a 6% denaturing polyacrylamide gel containing 7 M urea using 0.5 TBE buffer at 1000 V. DNA bands were visualized with silver nitrate.

**Results and Discussion**

**Result**

**Inheritance of resistant gene**

Three weeks after the artificial infestation, the number of cowpea aphid was rapidly increased in all populations and decline at the 5th week. The highest average number of aphids was found in F₂ populations followed by Selected-PSU. The number of cowpea aphid observed on the F₁’s almost the
The visual score in the 3rd week was present in Figure 2. In the F1’s, damages were mild at first and progressing slowly with time, but never reaching severity scores as high as their susceptible parent. The average damage scores of selected-PSU was 3-4, while F1’s appeared to be resistance with average score 1-2 close to IT82E-16. Segregation in F2 population indicated that aphid resistance was governed by a single dominant gene. Of the 240 individuals, 177 were resistance and 63 were susceptible with an exact fit to a 3:1 ratio (Table 2).

Table 2 Phenotype segregation for resistance and susceptibility to cowpea aphid resistance in IT82E-16, Selected-PSU, F1 and F2 population as evaluated by chi-squared values to fit a 3:1 single gene model.
Microsatellite analysis among resistance and susceptible

From 4 microsatellite primer pairs (VM 31, VM 34, VM 37 and VM78) tested, VM 37 which displayed the polymorphism between the resistant and susceptible parents was then chosen to identified polymorphic between the resistant and the susceptible F₂ population. SSR primer VM 37, generated three alleles (a, b and c) in the two parents. Allele (band) a was present in the resistant IT82E-16 but absent in the susceptible Selected-PSU. This band was also present in most the members of the resistant (78%), but absent in the members of the susceptible plants (Figure 3). Using chi-square test, the segregation of microsatellite markers fit to a 3:1 segregation ratio in F₂ population.

The results of phenotype screening and molecular analysis clearly indicate that the genetic control of resistance to cowpea aphid was single dominance.

Discussion

The aphid resistance in crop plant is often qualitative rather than quantitative (Klingler et al., 2005). In cowpea, the genetics of A. craccivora resistance have been investigated. Bata et. al. (1987) and Pathak (1988) reported that two independent loci Rac1 and Rac 2 are involved in the expression of resistance to cowpea aphid. However, plant reactions to insect attack may depend on plant genotype, insect biotypes and environmental factors. Benchasri et al. (2007) studied in 24 yardlong bean and cowpea accessions and they reported that IT82E-16 displayed the highest level of resistance to cowpea aphid. From this result, we made cross between IT82E-16 and susceptible variety to cowpea aphid, Selected-PSU, to investigate the genetic control of resistance in IT82E-16. The large amount of aphid was found in Selected-PSU and some F₂ population. Results from visual damages and aphid multiplication in F₂’s, the segregation ratio for aphid resistance phenotypes strongly support the model of single dominant gene controlling to A. craccivora in IT82E-16, this finding was in agreement with Benchasri et. al. (2009). It was noted that F₂ plants with narrow leaves displayed higher level of resistance than the wider leaves. Laamari et. al. (2008) studied in broad bean and also reported that large leaves are more attractive.
to aphid than narrow leaves. Wuttiwong et al. (2010) also reported that IT82E-16 have strong antixenosis resistance against the cowpea aphid. This result may be explained by a combination of physical and chemical features in IT82E-16 involved in aphid resistance.

To develop molecular markers linked to cowpea resistance, SSR markers were first tested with the parents and applied to the F$_2$ population. From 4 primer pairs used in the initial study, VM 37-a marker tend to be linked to resistant gene in IT82E-16. However, closer linked markers may increase the selection efficiency, therefore, it is need to develop more markers and integrate them for the traits of interest.

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