
Charassri Nualsri1*, Somsak Potarot1, Jitra Jansod1, Dušan Milošević1, Kanok-on Wuttiwong1, Sorapong Benchasri2 and Veera Maneelert1

1 Department of Plant Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand 90112
2 Faculty of Technology and Community development, Thaksin University, Phatthalung Campus, Phatthalung, Thailand 93110

* Corresponding author: ncharass@yahoo.com

Abstract

Germplasm provides the raw genetic diversity for plant breeding program. Without some basic information of the accessions, breeder will not be able to use proper materials for their works. A field experiment was conducted to assess genetic diversity of 50 *Vigna unguiculata* (yardlong bean and cowpea) by morphological characteristics and RAPD analysis. Twenty-four accessions were further evaluated for aphid (*Aphis craccivora* Koch) resistance. One of those accessions, cowpea IT82E-16, tended to be tolerance to cowpea aphid. Due to genetic compatibilities, the introgression of this trait to yardlong bean will be achieved. Crossing between IT82E-16 and Selected-PSU, an susceptible variety, was made to investigate genetic of cowpea aphid resistance. Four generations including P1, P2, F1 and F2 were evaluated for cowpea aphid resistance in the field under the screenhouse. Plants are arranged in the field using Randomized Complete Block Design (RCBD) with unequal replications. The results from damage score indicated that a single dominant gene controlling cowpea resistance in IT82E-16.

Key words: *Vigna unguiculata*, *Aphis craccivora*, SSR marker, RAPD, Marker-assisted selection, Antixenosis

Introduction

Yardlong bean and cowpea belong to *Vigna unguiculata* (L.) Walp, they are most widely used legume in the tropics. Yardlong bean is also known as asparagus bean, string bean, Chinese longbean, etc. It is botanically more closely related to cowpea, but it is much more a trailing and climbing plant than the cowpea, often reaching 9-12 feet in height (USDA-ARS, 2012) and it is characterized by very long pods (30–90 cm in length). Yardlong bean is cultivated mainly for crisp and tender pods that are consumed both fresh and cooked. It is considered to be one of the most important vegetable crops in parts of Indonesia, Thailand, Philippines, Taiwan, and China (Rachie, 1985). It thought to be grown on about 300,000 ha (Timko et al., 2007). In Thailand, yardlong bean is one of economic crops grown across the country and many varieties of yardlong bean are popular home garden items.

Cowpea is one of the important food grain legumes grown in the semi-arid tropics covering Africa, Asia, Europe, United States and Central and South America. Because of high protein content in dry grain (23-29%), it is served as a cheap source of protein for the poor (Diouf and Hilu, 2005). Cowpea can adapt well to different types of soil, resistant to drought and can be used to improve soil fertility makes it an important economic crop in many developing countries particularly in Africa.
is the most important among *Vigna* in terms of planting area. Production area of cowpea about 14 million hectares worldwide and annual global production of cowpea is approximates 3.3 million tons (CGIAR, 2011). West and Central Africa is the leading cowpea producing region in the world, this region produces 64%. Nigeria is the largest producer and consumer, accounts for 61% of production in Africa and 58% worldwide (IITA, 2009). The second rank is Brazil, producing 600,000 t annually (Guazzelli, 1988). Both cowpea and yardlong bean suffer from cowpea aphids, infestations by this insect cause deleterious effects on the physiological processes resulting in reductions in growth and losses in yields. Cowpea and yardlong bean need to be improved for resistance to cowpea aphid. There is an important to identify sources of resistance and to understand the nature of resistance.

**Evolutionary and germplasm resources.**

*Vigna unguiculata* (2n = 2x = 22) is believed to have originated in Africa where a large genetic diversity of wild types occur throughout the continent, particularly southern Africa, however the greatest genetic diversity of cultivated cowpea is found in west Africa (PROTA, 2006). Pasquet, (1999) reported cowpea domesticated in Northeast Africa and a secondary centre of domestication was in West Africa and the Indian sub-continent. In present, cowpea is an essential crop in developing countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, Central and South America (Singh *et al.* 1997).

*V. unguiculata* has 11 subspecies including 10 wild perennial subspecies and one annual subspecies (ssp. *unguiculata*) (Maxted *et al.*, 2004; Pasquet, 1996b). Subspecies *unguiculata* comprising of a cultivated form (var. *unguiculata*) and a wild form (var. *spontanea*). The cultivated forms (var. *unguiculata*) of ssp. *unguiculata* are further distinguished to five following cultivar groups (cv-gr) based mainly on pod and seed characteristics (Fang *et al.*, 2007; Pasquet, 1996a).

- cultivar-group (cv-gr.) *Unguiculata*: cowpea, black-eye bean. The most widespread and economically important group of the species. They are pulse and vegetable types.
- cultivar-group. *Melanophthalmus*: The most recently recognized cultivar-group, it is based on the taxon with a thin testa and often wrinkled, and is cultivated mainly in West Africa.
- cultivar-group *Biflora*: (catjang cowpea). Mainly cultivated in South Asia (India, Sri Lanka). It is grown as a pulse or as forage crop, especially for hay and silage, and as a green manure crop. Much less variable than the true cowpea.
- cultivar-group *Sesquipedalis*: Yardlong bean, asparagus bean. It is climbing grown as vegetable, immature pods and seeds are used as a green vegetable.
- cultivar-group *Textilis*: plants cultivated for the fibres extracted from their long peduncles (Pasquet, 1998).

The selection of cowpea as a pulse as well as for fodder might have resulted in the establishment of the culti-group *Unguiculata* (Ng and Sign, 1997). There are two centers of diversity for this variable crop species: cultivated –group *Unguiculata* and wild forms in Tropical Africa and the other cultivar-groups in India/Southeast Asia (IPGRI, 2004). Cowpea was first introduced to India 1,000-1,500 years ago. India appears to be a secondary center of diversity of cowpea, since significant genetic variability occurs on the subcontinent. After its introduction to this part south of Asia, a strong selection for succulent and fleshy pod types was exerted on the crop that resulted in its modification (Kongjaimun *et al.*, 2012), making it the first subspecies to be isolated from the other *Vigna* members. Consequently, the present-day *sesquipedalis*, or yardlong bean is characterized by its very long pods, which are consumed as a green-snap vegetable bean (Ehlers and Hall, 1997; Fatokun, 1993). Yardlong bean is found widely spread throughout the tropics as a minor vegetable crop. But it is mostly cultivated in India, Bangladesh, as well as Southeast Asia, and Oceania (Pandey and Westphal, 1989). However, the center of diversity of yardlong bean could very probably be in east or southeast Asia (Borget, 1992; Grubben *et al.*, 1994).
Yardlong bean is susceptible to a wide range of diseases and insect pests. A major problem for yardlong bean production in Thailand is severe infestation and damage by various insect pests in the field such as thrips (Megalurothrips sjostedti), cowpea aphids (Aphis craccivora Koch) and pod borers (Maruca vitrata Fabricius) etc. (Bottenberg et al., 1997; Tamò et al., 1997). Among these, cowpea aphid is considered to be an important pest of yardlong bean and cowpea (Singh and Jackai, 1985). Cowpea aphids are well distributed through the tropics and have numerous hosts but primarily on legumes. They are found primarily on the growing point of the host plants including tips, flowers and developing pods and regularly attended by ants. Adults always shiny black, immatures lightly dusted with wax and light brownish (Figure 1). The damage to yardlong bean by A. craccivora is caused by both adults and nymphs (Ofuya, 1997). The aphid feeds by sucking fluid from the stem terminal shoots, petioles, flowers and pods (Figure 2). Heavy feeding kills young plants while it causes stunting, distortion of leaves, delay in initiation of flowers and reduced pod set in plants which survive attack. The cowpea aphids colony expanse very quickly in hot and dry weather. Biotypes of cowpea aphid are assumed to exist (Johnson and LeGault, 2006).

The most damaging effect of cowpea aphid may be through transmission of cowpea aphid – borne mosaic virus causes mosaic-like symptoms and results in yield loss (Atiri, 1984). Transmission of CABMV by A. crassivora has been reported as 57% (Bashir and Hampton, 1994). Foliar application of several insecticides has been reported to be effective against A. craccivora. However, insecticide application is not safe for human and increases the costs of production. To reduce chemical application, plant resistance to insects is needed to be created. It is also an environmentally safe and cost-effective pest management technique.
Germplasm collection and evaluation of V. unguiculata spp. unguiculata

We collected total 97 accessions of cowpea and yardlong bean, 60 from southern part and 37 accessions came from other parts of Thailand (Sarutayophat, 2008). The first approach in the description and classification of germplasm is morphological characterization. Morphological descriptors for cowpea were used as a reference (IBPGR, 1983). Variation in leaf, flower, pod and seed of some samples was shown in Figure 3.

Figure 3. Variation in leaves, flower color, pods, seed shape and color observed in cowpea and yardlong bean collected from southern Thailand
However, there are several disadvantages of using morphology as genetic markers: 1) morphological markers are, in some cases, associated with deleterious effects, 2) they are difficult to analyze in breeding populations, and 3) they are affected by environmental conditions (DiJkhuizen et al., 1996; Nualsri and Konlasuk, 2000). DNA markers had been found to be useful for plant characterization and are often used to estimate genetic diversity in crop species. Among DNA markers, RAPD (Random Amplified Polymorphic DNA) and Microsatellite are most popular. RAPDs provide a rapid means of identifying genetic markers to distinguish closely related species. These markers are inexpensive, rapid, and easy to use and need small amounts of DNA. Fifty accessions of cowpea and yardlong bean from the collection were further identified and evaluated for genetic relationships using RAPD markers with selected 7 primers (Figure 4). Cluster analysis by the unweighted pair group method of arithmetic means (UPGMA) showed that 50 accessions could be divided into four groups with genetic distance vary from 0.5-0.980.

Figure 4. RAPD patterns of yardlong bean and cowpea accessions using primer T12. M is 100 bp ladder, lane 1-23 are yardlong bean and cowpea accessions collected from southern Thailand

**Evaluation for cowpea aphid resistance**

To-date, breeding for resistant to cowpea aphid resistance is needed to reduce further losses from cowpea aphid outbreaks. The use of resistant cultivar is the cheapest and most effective way to control insect pest in the production area. To achieve resistant lines for insect pest, we need information on mechanisms and inheritance of resistance. These information can be utilized in selecting parents with diverse mechanisms or with different genes for resistance. Selection of appropriate breeding methodologies (pedigree, backcross, or population improvement) depending on the number of genes involved and nature of gene action (Sharma, 2009).

Breeders are forced to seek for the needed genes they are interested in and the sources of genetic variation can be landrace varieties, weedy or wild relative populations. The largest collections of *Vigna unguiculata* are held by the International Institute of Tropical Agriculture (IITA) with more than 14,000 accessions (Timko et al., 2007). Many cowpeas accessions from cowpea germplasm at the IITA or other places have been identified as resistance to *A. craccivora* Koch (Table 1). In Thailand, a preliminary evaluation was carried out in the field in 2007 (unpublished results), leading to the selection of twenty-four lines for further testing. A second study was then carried out in the screenhouse, to confirm the performance of each accessions (Benchasri et al., 2007). Resistance was evaluated by measuring differences in aphid populations and visual damages on the accessions. Five aphids were
released on each plant at 21 days after germination and number of aphid was examined every weeks. After the aphids were released on the plants, their numbers increased rapidly over time particularly from the 2nd week to the 3rd week. Selected-PSU had the highest number of aphid. The lowest number of aphid was found on IT82E-16. Results from aphid number and damage scores obtained from this experiment (Table 2) indicated aphid tolerance in those four accessions.

Table 2. Number of cowpea aphids and damage scores evaluated on 5 lines/accessions of cowpea and yardlong bean.

<table>
<thead>
<tr>
<th>Lines/accessions</th>
<th>Damage score at the 3rd week</th>
<th>Number of cowpea aphids after artificial infestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wk 1</td>
</tr>
<tr>
<td>Selected- PSU</td>
<td>3.30</td>
<td>62.33</td>
</tr>
<tr>
<td>IT82E – 16</td>
<td>1.75</td>
<td>21.67</td>
</tr>
<tr>
<td>SR 863</td>
<td>2.10</td>
<td>28.67</td>
</tr>
<tr>
<td>Khao-hinson</td>
<td>2.14</td>
<td>29.00</td>
</tr>
<tr>
<td>Suranaree 1</td>
<td>1.93</td>
<td>29.33</td>
</tr>
<tr>
<td>F – test</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>12.00</td>
<td>223.64</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>24.88</td>
<td>48.96</td>
</tr>
</tbody>
</table>

Sources: Benchasri et al. (2007)

Mechanisms of host plant resistance

Host plant resistance is a plant mechanism used to defend against attack and reduce damage from pest species. In general, there are three categories of host plant resistance that plant breeder use to develop resistance cultivars. First, antixenosis- this type of resistance is often known as non preference, resistance affects the behavior of an insect pest and usually is expressed as non-preference of the insect for a resistant plant compared with a susceptible plant. Second, antibiosis- plants have adverse effect on the biology of feeding insect. Antibiosis resistance often results in increased mortality or reduced longevity and reproduction of the insect (Teetes, 2007). Third, tolerance- plants have inherent ability to withstand the attack of the insect (Smith, 1989). Plants with an ability to tolerate insect damage at
times may produce more yield than the plants of a nontolerant susceptible cultivar at the same level of insect infestation. Tolerance often occurs in combination with antixenosis and antibiosis components of resistance (Sharma, 2009).

Wuttivong (2008) categorized of A. craccivora resistance among selected yardlong bean and cowpea, IT82E-16, Kaohinson, Suranaree 1 and SR00-863 to enhance an understanding of the underlying resistant mechanisms. The experiment on probing and feeding behavior of cowpea aphid at 45 days was investigated under choice test. The longest probing duration and the shortest feeding period were observed on IT82E-16 (Figure 5). Probably plant surface factors are involved in IT82E-16 resistance because IT82E-16 had much more length and density of trichomes presenting at the lower surface of leaves than others (Table 3). They concluded that resistance found in IT82E-16 is mainly due to antixenosis. The same finding was reported in cowpea resistant line ICV 12 by Annan et al. (2000).

**Table 3.** Length and density of trichomes in 5 accessions of yardlong bean and cowpea after 45 days

<table>
<thead>
<tr>
<th>Accessions</th>
<th>Trichome length and density (Mean±SE)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length (uM)</td>
<td>Density/cm²</td>
</tr>
<tr>
<td>IT82E16</td>
<td>298.7±9.3a</td>
<td>122±6.2a</td>
</tr>
<tr>
<td>SR00-863</td>
<td>92.1±1.4c</td>
<td>49±2.5c</td>
</tr>
<tr>
<td>Suranaree 1</td>
<td>136.6±3.5b</td>
<td>106.2±7.8a</td>
</tr>
<tr>
<td>Kao-hinson</td>
<td>94.3±1.9c</td>
<td>73.8±3.8b</td>
</tr>
<tr>
<td>Selected-PSU</td>
<td>81.9±2.5c</td>
<td>59.4±2.6bc</td>
</tr>
<tr>
<td>F-test</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.5</td>
<td>13.7</td>
</tr>
</tbody>
</table>

Sources: Wuttiwong et al. (2010)
Inheritance of cowpea aphid resistance in *V. unguiculata* spp. *unguiculata*

The findings mentioned above offer promises for the development of cowpea aphid resistance in yardlong bean cultivars. Crossing between Selected-PSU, a susceptible variety and IT82E-16 (resistant variety) was made to produce F1 and F2 populations and genetic control for cowpea aphid resistance was investigated. The experiment was arranged in a Completely Randomized Design (CRD) with unequal replications under the screenhouse. Each parental line and F1 were planted in 3 replications while F2 plants were planted in 24 replications, 10 plants/plot/replcation. At the 4th weeks after seed germination, 5 adult cowpea aphids were deposited on each plant. Results from 1-3 weeks after artificial infestation revealed that the number of cowpea aphids in all crop populations increased rapidly. The total amounts of cowpea aphids observed on F1 almost the same as on their resistant parent, IT82E-16 (3,206 and 3,115 respectively) and the highest number was recorded on F2 (5,306) followed by that on Selected-PSU (4,496). The data was shown in Figure 6.

![Figure 6. Average aphid number recorded weekly after infestation on F1, F2 and their parents of Selected-PSU x IT82E-16 Source: Potarot and Nualsri (2011)](image)

To assess the resistance to cowpea aphid, visual damage scores were assessed for each generation at five levels based on the scales suggested by Smith et al. (1994). Data regarding damage scores in parents, F1 and F2 were given in Figure 7. It was found that all 30 plants of Selected-PSU were susceptible (rating score=4) and rating score for IT82E-16 was 1. Almost all F1 plants appeared to be resistant to cowpea aphid (29 from 30 plants showed resistance with the rating score of 1-2). The individual F2 progenies segregated for cowpea aphid resistance. The scores were ranging from a 1 to 4. The phenotypic data for F2 were tested by using chi-square for the hypothesis of 3:1 resistant to susceptible ratio. The total 240 F2 individuals were classified into two categories: 177 resistances (R) and 63 susceptible (S). The probability for the expected 3 resistant: 1 susceptible segregation in F2 was not significant. The result indicated that the F2 segregation in the ratio of 3:1 ratio which fit the inheritance model of a single dominant gene. Thus the cowpea aphid resistance to IT82E-16 is controlled by a single dominant gene.
Conclusions

Genetic resources of Vigna provide sources of desirable genes. Maintenance of diverse set of landraces and wild relatives proved valuable to current or future plant breeding. Breeding for resistance to cowpea aphids is important to overcome the constraint of yardlong bean or cowpea production. From 97 accessions of cowpea and yardlong bean evaluated, only IT82E-16 displayed the highest level of resistance to cowpea aphids. Mechanisms and inheritance of resistant gene was investigated in order to obtain basic information of this trait and use for breeding program. Results indicated resistance to cowpea aphid is controlled by single dominant gene and resistance found in IT82E-16 is mainly due to antixenosis. Implementation of molecular markers for resistance to aphid (Aphis craccivora Koch) in cowpea may be useful in some breeding programs. In conclusion, future breeding program should rely on genetic diversity from landraces and wild relatives, we need to be thinking and conserving a wider range of genetic diversity before it is lost forever.

References


Evaluation and Utilization of Cowpea \((Vigna unguiculata)\) Germplasm for Varietal Improvement of Resistance to Cowpea Aphid \((Aphis craccivora)\) in Thailand


