Characterization of *Vigna unguiculata* (L.) Collected from Southern Thailand

and Its Tolerance to *Blackeye Cowpea Mosaic Virus*

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Thesis Title Characterization of *Vigna unguiculata* (L.) Collected from Southern Thailand and Its Tolerance to *Blackeye Cowpea Mosaic Virus*

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ABSTRACT

The importance of plant genetic resources and the need for screening adaptive characteristics can not be overlooked. Their vital significance for their maintenance of genetic improvement and biodiversity has been recognized worldwide. Yardlong bean and cowpea suffer from a wide range of harmful organisms, especially aphids such as cowpea aphid (*Aphis craccivora* Koch), which transmit many viral diseases, particularly *Blackeye Cowpea Mosaic Virus* (BICMV) and *Cowpea Aphid-Borne Mosaic Virus* (CAMBV). The objectives of this study were to analyse of qualitative and quantitative characteristics and screening for BICMV resistance of yardlong bean and cowpea accessions mostly collected from southern part of Thailand. In this study, we made analysis of qualitative and quantitative characterictics of 50 collected accessions. Qualitative characteristics were: growth habit, plant pigmentation, seed shape, eye color, flower color, terminal leaflet shape, pod curvature, immature pod pigmentation and mature pod pigmentation. The highest variability was found in immature and mature pod pigmentation. The eight following quantitative characteristics were recorded: days to flowering, terminal leaflet length, terminal leaflet width, pod length, pod weight, seed length and seed width. Results showed high significant differences in all quantitative characteristics. High variability was observed on important agronomic traits such as days to flowering, pod length and pod weight. Days to flowering varied from 39 to 69 days with average 51.4 days. Fourteen accessions produced flower earlier than the average value. The length of pod ranged from 12.2 cm (cowpea accession no. 5b, IT82E-16 indeterminate)) to 63.2 cm (Samchook). Fourteen accessions showed significantly positive deviation from the average value. Weight of pods, in most accessions, was in positive correlated with the pod length. All cowpea accessions except accession no.28 (Line suar) showed significantly lower value than the average for most quantitative characters. Evaluation for BICMV resistance was carried out by visual symptoms and confirmed by indirect ELISA method. Results indicated that out of 50 accessions studied, only two accessions; Trang 1 and Taitor were resistant to BICMV and these accessions will be used in yardlong bean breeding program.

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Chapter 1

Introduction

Background

Yardlong bean, *Vigna unguiculata* subsp. *sesquipedalis,* is a common vegetable in asian markets. It originated from Central West Africa and is now cultivated extensively in many countries in Southeast Asia such as Taiwan, Phillipines, Indonesia and Thailand. This crop is also widely grown in Southern China and Southern Asia (India, Pakistan and Bangladesh) (Ehlers, 1997).

In Thailand, production area of yardlong bean was estimated at 18, 560 – 20, 160 ha annually (Sarutayophat, 2007). Yardlong bean suffers from a wide range of production constraints including insect pest such as cowpea aphid (*Aphis craccivora* Koch.) (Benchasri, 2007). However, the most damaging effect of *A. craccivora* may be through transmission of viral diseases. The majority of viral diseases of yardlong bean and cowpea lead to overall stunting, reduction in leaf size, mottling, mosaic, leaf chlorosis, leaf distortion, leaf curling, vein clearing, necrotic, local lesion and death (Akinjogunla, 2008). *Blackeye Cowpea Mosaic Virus* (BICMV) is one of the most important viral pathogen of yardlong bean and cowpea in major growing regions of the world (Bashir *et al*., 2002a). The early reported of BICMV infection on cowpea was by Anderson in the U.S.A. in 1955. Yield loss up to 98% was reported when cowpea was infected with BICMV (Puttaraju and Santhosan, 2000).

Breeding for resistance to BICMV is an ongoing activity in various laboratories, however, the availability of resistant germplasm is still limited. The investigation of yardlong bean and cowpea accessions as resistant sources against BICMV is an important step to yardlong bean breeding program.

Literature Review

1. Genetic diversity

Genetic diversity refers to any variation in the nucleotides, genes, chromosomes, or whole genomes of organisms. The genome is the entire complement of DNA within the cells or organelles of the organism. The DNA is contained in the chromosomes present within the cell; some chromosomes are contained within specific organelles in the cell, for example, the chromosomes of mitochondria and chloroplast. Nucleotide variation is measured for discrete sections of the chromosomes, called genes. Thus, each gene compromises a hereditary section of DNA that occupies a specific place of the chromosome, and controls a particular characteristic of an organism (Harrison *et al*., 2006).

The presence of unique genetic characteristics distinguishes members of a given population from those of any other population. Large populations will usually have a greater diversity of alleles compared to small populations. This diversity of alleles indicates a greater potential for the evolution of new combinations of genes, subsequently, a greater capacity for evolutionary adaptation to different environmental conditions. In small populations, the individuals are likely to be genetically, anatomically, and physiologically more homogeneous than in larger populations and less able to adapt to different environmental conditions

Genetic diversity is, therefore, a key component for conservation efforts associated with population management. The genetic constitution of an organism (the arrangement of the DNA into genes on the chromosomes) is also referred to its genotype. Hence, variation that exists within the genetic constitution of an organism is often referred to as genotypic variation.

2. Diversity of *Vigna* species

The genus *Vigna* (Family *Fabaceae*) is composed of more than 200 species that are native to the warm regions of both the old world and new world and this genus is of considerable economic importance in many developing countries. All of the cultivated *Vigna* species can be grown over a wide range of environmental conditions and all provide inexpensive protein available in several edible forms. Previous researchers used to analyze relationships among Asian *Vigna* have recognized two groups within this subgenus, the azuki bean group and mungbean group. The economic *Vigna* species exhibit a number of attributes that make them particularly valuable for inclusion in many types of cropping systems. They can be grown successfully in extreme environments (e.g., high temperatures, low rain fall, and poor soils) with few economic inputs (Fery, 2002). Many of these species produce multiple edible products, and these products provide subsistence farmers with a food supply throughout the growing season as well as dry seeds that are easy to store and transport. For example, tender shoot tips and leaves of cowpeas can be consumed as soon as the plants reach the seeding stage and immature pods, and immature seeds can be consumed during the fruiting stage. Harvested dry seed of all of the *Vigna* crops can be consumed directly, and seeds of several of the crops are commonly used to make flour or produce sprouts. Plant residues can be used as fodder for farm animals. *Vigna* food products exhibit many excellent nutritional attributes and these products provide a needed complement in diets comprised mainly of roots, tubers, or cereals.

One of the important *Vigna* is *Vigna unguiculata* (L.) Walp. *V. 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) (Figure 1). 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 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).

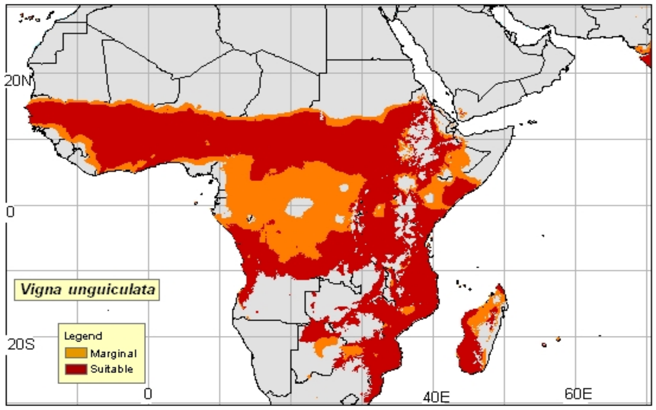

Figure 1. Distribution of *V. unguiculata* in Africa

Source: http://www.tropicalforages.info/key/Forages/Media/Html/Vigna_unguiculata.htm

- cv-gr. *Unguiculata:* cowpea, black-eye bean. The most widespread and economically important group of the sub species. They are pulse and vegetable types.
- cv-gr. *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.
- cv-gr *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.
- cv-gr *Sesquipedalis*: yardlong bean, asparagus bean. It is climbing grown as vegetable, immature pods and seeds are used as a green vegetable.
- cv-gr *Textilis:* plants cultivated for the fibres extracted from their long peduncles.

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. After its introduction to this part of South 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).

3. The important of yardlong bean and cowpea

Most taxonomists agree that yardlong bean and cowpea belong to the botanical species *Vigna unguiculata* (L.) Walp. It is widely grown in Asia and Oceania in India, China, Nepal, Bangladesh, Pakistan, Sri Lanka, Indonesia, Philippines, Thailand, Vietnam, Cambodia, Laos and Australia (Mishra *et al*.,1985) (Figure 2). Yardlong bean is an intensely cultured, vegetable crop that is grown widely in Southeast Asia (Singh and Tarawali, 1997) and is considered to be one of the most important vegetable crops in parts of Indonesia, Thailand, Philippines, Taiwan, and China (Rachie, 1985). Rubatzky and Yamaguchi (1997) estimated that yardlong bean production in China alone exceeds 250,000 ha annually.

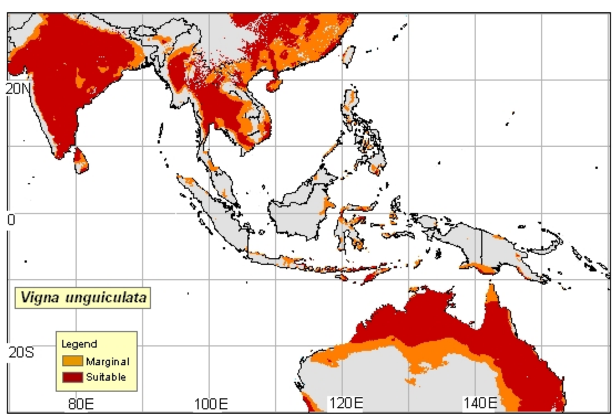

Figure 2. Distribution of *V. unguiculata* in Asia and Australia

Source: http://www.tropicalforages.info/key/Forages/Media/Html/Vigna_unguiculata.htm

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Cowpea is an important food crop in Africa and other developing countries. All the parts used as food are nutritious and provide proteins, vitamins (notably vitamin B), and minerals. The cowpea halum is also a good source of livestock feed. In the last three decades, some efforts have been put into research aimed at improving the yield of cowpea (Samson *et al,* 2008). Quin (1997) estimated the annual world cowpea crop at 12.5 million ha, and the total grain production at 3 million t. West and Central Africa is the leading cowpea producing region in the world. This region produces 64% of the estimated 3 million t of cowpea seed produced annually. Nigeria is the world's leading cowpea producing country. Other countries in Africa, e.g., Ghana, Niger, Senegal, and Cameroon, are significant producers. Outside Africa, the major production areas are Asia and Central and South America. Brazil is the world's second leading producer of cowpea seed, producing 600,000 t annually (Guazzelli, 1988).

Mahalakshmi *et al*. (2007) noted that cowpea is a drought-tolerant food legume grown in the savannah regions of the tropics and subtropics. The International Institute for Tropical Agriculture (IITA) holds the world collection of 15,003 cultivated cowpea from 89 countries in its gene bank. In excess of 12,000 samples were characterized for 28 agrobotanical descriptors. The entire collection was first stratified by country of origin and biological status. Land race samples (10,227) with information on origin and characterization data were grouped using clustering procedures. The core collection of cowpea provides an opportunity for further exploration of the cowpea germplasm for improvement of this crop.

4. Viral diseases in *Vigna unguiculata*

Viral diseases are a major limiting factor to *V. unguiculata* production in many countries. *Blackeye Cowpea Mosaic Virus* (BICMV) and *Cowpea aphid-borne mosaic virus* (CABMV) are potential threats to yardlong bean production in many regions. It can cause a yield loss of 1-98% under field conditions depending upon crop susceptibility, virus strain and the environmental conditions (Bashir *et al.*, 2002a).

BICMV belongs to the family *Potyviridae*, genus *Potyvirus.* It is a distinctive virus with flexuous filamentous particles 750 nm long. BICMV is seed-borne in cowpea, has a wide experimental host range, and is transmitted by several common species of aphid. BICMV occurs in many countries where cowpea is grown such as India (Puttaraju and Santhosan, 2000), Iran (Colnaraghi and Shahree, 2000), Pakistan (Bashir and Ahmed, 2002) and Zimbabwe (Hampton and Gubba, 1997) etc. BICMV was first described by Anderson (1955) from U.S.A. Some isolates were previously designated as CABMV (Taiwo *et al*., 1982).

BICMV causes a severe mosaic of yardlong bean (Figure 3), the severity depending on host cultivar and virus strain. Diseased yardlong bean plants show variable amounts of dark green vein banding or interveinal chlorosis, leaflet distortion, blistering and stunting. Huguenot *et al*. (1993) reported that BICMV and CABMV induce a very similar mosaic disease in cowpea. In addition, mix infections of BICMV, CABMV and *Cucumber mosaic virus* (CMV) result in severe stunting of cowpea and Rugose mosaic symptom of yardlong bean (Chang,1983), and nearly complete yield losses of cowpea. At least 36 species in 7 dicotyledonous families are susceptible to this virus, with cowpea being a major natural host.

Figure 3. Symptoms of BICMV on yardlong bean and cowpea in natural environment

The BICMV infected seed provides the initial inoculum and aphids are responsible for the secondary spread of the disease under field conditions. The virus symptoms vary with the plant genotype and virus strain. The rates of BICMV transmission by seed on cowpea and yardlong bean vary from 0-30% according to varieties (Frison *et al.,* 1990). Yardlong bean and cowpea are very often visited by aphids in vegetation period. On this way it is possible that many viruses can be transmitted, including BICMV (Dijkstra *et.al*.,1987) . The virus was identified on the basis of its host range and symptoms, particle morphology, transmission by sap, aphids, and in seed, and its serological reaction with a known antiserum.

5. BICMV identification

All viruses are difficult to identify using morphological criteria which can be time consuming, challenging, and require extensive knowledge in taxonomy. In order to improve the quality and quantity of the germplasms and to significantly reduce the infection and transmission of virus to different cultivars of cowpea, proper diagnosis and control is essential. As detection/indexing methods, the following can be used:

- *Growing out test:* in screen houses/ containment facility to determine presence/ absence of virus symptoms in the seedlings growing from the virus-infected seeds.
- *Infectivity test:* presence of virus assayed by inoculating extracts of seed or seedlings on to indicator hosts under containment facility
- *Serological tests:* most reliable and effective methods for the detection of seed borne viruses and virus from plant tissues
- *Indirect ELISA:* Enzyme-linked immunosorbent assay (Ayodele and Kumar, 2010).

Enzyme-linked immunosorbent assay (ELISA) is the most appropriate method for the detection of the virus in the seed or plant tissue for seed certification programs (Mali *et al*., 1988, Akinjogunla *et al.,* 2008). There are many different types of ELISAs such as direct and indirect ELISA (Figure 4). Indirect ELISA is a five-step procedure: 1) coat the microtiter plate wells with antigen; 2) block all unbound sites to prevent false positive results; 3) add primary antibody specific to antigen to the wells; 4) add secondary antibody conjugated with enzyme; 5) add substrate with the enzyme to develop color, thus indicating a positive reaction.

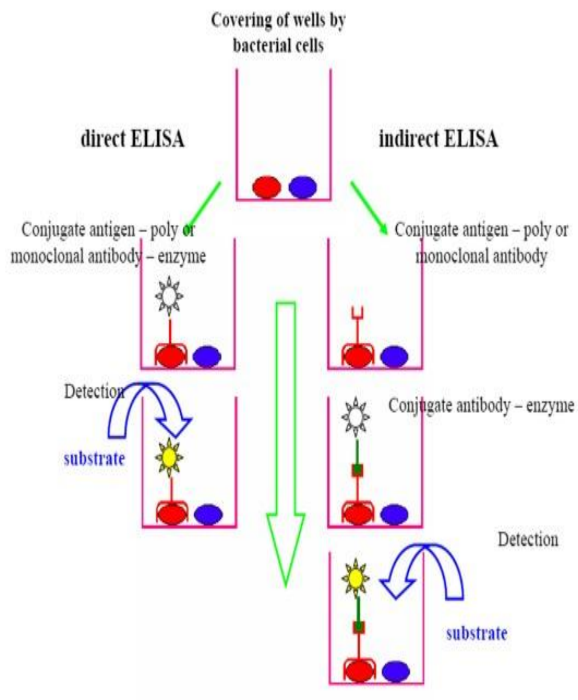


Figure 4. Working principle of ELISA test

Source:http://www.bing.com/images/search?q=direst+and+indirect+ELISA+test+images&view=detail&id=0F7055252FAAE1D15502A76B5A24516E3E35717C

Seed-borne isolates of BICMV were detected and identified from cowpea germplasm accessions by direct antigen-coating (DAC) ELISA (Hampton *et al*., 1992, Bashir and Hampton, 1996a). A panel of monoclonal antibodies in mixture has been used to detect virus in plant tissue (Huguenot *et al*., 1993). The other tests that have been employed to detect and identify BICMV isolates are the Immunodiffusion test, Immunosorbent electron microscopy (IEM), Agglutination test, Dot-immunobinding assay (DIBA), Tissue blot immunoassay (TBIA), Western blottiry, HPLC peptide profiles analysis and nucleic acid sequence analysis (Bashir and Hampton, 1996). Among all these tests, ELISA is more commonly used in many laboratories to analyze seed or plant samples collected during surveys. Huguenot *et. al*. (1993) described a diagnostic ELISA suitable for detecting the viruses of seven serotypesin infected cowpea plants. Mink and Silbernagel (1992) compared eight isolates of BCMV, five of BICMV and four of CABMV using a panel of 13 monoclonal antibodies (MAbs) raised against BCMV, BlCMV CABMV and PStV using indirect ELISA. Four MAbs detected all isolates, suggesting that their coat proteins have at least one epitope in common.

6. Controlling of viral diseases

They are various ways of controlling viral diseases for example; use of disease free planting materials. Disease free planting materials can be obtained by virus elimination through shoot tip culture or using cross protection in which mild strains of the virus is used as protective isolates. In addition, viral disease can be controlled by searching resistant genes in the crops, and resistant varieties of plants can be obtained through classical breeding or genetic engineering. In classical breeding, the cultivar will be identified for disease resistance and then crossed with the cultivar to be improved. Excellent sources of resistance are available for the breeding of resistant cultivars. Resistance to virus in cowpea is conferred by either a dominant or a recessive gene (Bashir *et al.,* 2002). Resistance to BICMV in cowpea cv. TVU 2480 was found to be governed by a single recessive gene (Taiwo *et al*., 1981) while a single dominant gene was revealed in cowpea resistant variety white Acre BVR (Quattara and Chambliss , 1991). Barsir and Hampton (1996a) reported that 5 genotypes of cowpea; IT 80S 2049, Big Boy, Corona, Serido, and Tennessee Cream #8 were immuned to seven isolates of BICMV. Barsir and Hampton (1996b) studied in 182 cowpea (*Vigna unguiculata*) pre-introductions/germplasm accessions from 12 countries. Accessions were tested under greenhouse conditions for six seed-borne viruses. Twenty-one accessions (13.3%) from eight countries were found to be seed-infected with one of the three following viruses: *Blackeye Cowpea Mosaic Virus* (BlCMV) and *Cowpea aphid-borne mosaic virus* (CABMV) potyviruses, and *Cucumber mosaic virus* (CMV). They reported that viruses belong to potyviruses group were predominant in seed infection.

Objectives

The objectives of this study were:

1. To collect accessions of yardlong bean and cowpea from southern part of Thailand, and make analysis of qualitative and quantitative characteristics and

2. To evaluate for *Blackeye Cowpea Mosaic Virus* resistance or tolerance of collected yardlong bean and cowpea on the basis of symptoms and ELISA test.

Chapter 2

Materials and Methods

1. Materials

1.1. Plant materials

Fifty accessions of yardlong bean and cowpea were used in this investigation (Table 1). Thirty-nine are local varieties from Southern Thailand, 4 are improved lines currently used, 3 accessions received from Field Crops, Research center, Ubon Ratchatani, 1 each from Kasetsart University Kamphangsan campus and Suranaree University of Technology, 2 from Malaysia and 1 from Serbia.

1.2. Field materials

1.2.1. Materials for accession collection

- Plastic bags
- Pen
- Scissors
- Refrigerator

1.2.2 Field trial material for seed collection

- Plastic pods
- Seed from collected 50 accessions
- Bamboo sticks
- Plastic ropes
- Fertilizer

- Insecticide
- Paper bags
- 70% alcohol
- Clips
- Refrigerator

1.3. Materials for morphological characterization

- Plant leaves
- Plant flowers
- Plant pods
- Seeds
- Ruler
- Scissors
- Libra

1.4. Chemicals

1.4.1. Chemicals for BICMV Inoculation

- Celite
- PBS (Phosphate buffer saline)
- H_2O
- Mortar
- Plastic spray bottle
- Ice

1.5. Planting yardlong bean and cowpea

- Plastic pods
- Plastic pipes
- Net
- Fertilizer
- Insecticide

1.6. Laboratory Material

1.6.1. Chemicals for ELISA test

1. PBS
   - 136.8 mM NaCl
   - 8 mM $Na_2HPO_4.12H_2O$
   - 1.8 mM KH_2PO_4
   - 2.7 mM KCl
2. Washing buffer (PBST)
   - PBS
   - 0.05%Tween–20
3. Blocking solution (5% skimmilk (w/v) in PBS)

4. Substrate buffer
   - Diethanolamine
   - Sodium azide
   - $MgCl_2.6H_2O$
   - Anti – BICMV polyclnal antibody (anti-BICMV PAB)
   - Goat + anti-rabbit conjugated with alkaline phosphatase (GAR)
   - p-Nitrophenyl – (PNPP)

2. Methods

2.1 Collecting of yardlong bean and cowpea accessions

The first part of the experiment was related to collect the seed accessions of yardlong bean and cowpea. During this period, seed of 40 yardlong bean and cowpea were collected from seven provinces of Southern Thailand: Patthalung, Pattani, Songkhla, Chumporn, Nakhon Si Thammarat, Trang, Phang- Nga and Ranong. Another 7 accessions were received from private company and research institute in Thailand. In addition, we received one accession from Serbia and 2 from Malaysia (Table 1).

Figure5. Map of seven provinces in which the seeds accessions were collected

Source: http://www.saltwater-dreaming.com/travel/maps/thailand-provinces.htm

2.2 Morphological characteristic evaluation

The experiment was conducted at the field of the Faculty of Natural Resources, Prince of Sonkla University during 2010 and 2011. Seed accessions of yardlong bean and cowpea were germinated in plastic pots containing soil and compost mixture in 2:1 proportion. Experimental design was arranged in Completely Randomized Design (CRD) with 3 replications, one pot/replication, 2 plants in each pot. Plants were daily watering and fertilized every 7 days. We used insecticide 2 times to protect plants from insect infestation so they can be grown in good condition to bring enough seed material for further research. Two weeks after germination, plants were fastened with bamboo sticks to prevent lodging. During vegetation period, plants were observed and qualitative and quantitative morphological characteristics were measured as followings:

Table1. Accession number and sources of yardlong bean and cowpea accessions in the present study

Accession No.	Common name	Variety	Source
1	Yardlong bean	Samchook	Suphanburi
2	Yardlong bean	KU-20	Kasetsart University
3	Yardlong bean	Selected-PSU	Prince of Songkla University
4	Yardlong bean	Chia Tai	Chia Tai Co.Ltd.
5 a	Cowpea	IT82E-9	Field Crop Research, Ubon Rachathani
5 b	Cowpea	IT82E-9	Field Crop Research, Ubon Rachathani
6	Cowpea	IT82E-16	Field Crop Research, Ubon Rachathani
7	Yardlong bean	Tahanpran	Pattani
8	Yardlong bean	Malasia 308	Malaysia

Table 1 (cont.) Accession number and sources of yardlong bean and cowpea accessions in the present study

Accession No.	Common name	Variety	Source
9	Yardlong bean	Khao-hin-Son	Royal Project, Chachoengsao
10	Yardlong bean	Yumi	Phathalung
11	Yardlong bean	Malaysia 308	Malaysia
12	Yardlong bean	Suranaree	Suranaree University of Technology
13	Yardlong bean	Cameron	Malaysia
14	Cowpea	VIG 009	Serbia
15	Yardlong bean	Unknown	-
16	Yardlong bean	Trang 1	Trang
17	Yardlong bean	SR 863	Field Crop Research, Ubon Rachathani
18	Yardlong bean	Unknown	Lansaka-Nakorn-sithammarat
19	Yardlong bean	Unknown	Nopphitam, Nakorn-sithammarat
20	Yardlong bean	Foundsai	Na Rang, Nakorn-sithammarat
21	Yardlong bean	Teenman	Na Rang, Nakorn-sithammarat
22	Yardlong bean	Or.So	Na Rang, NakhonSiTammarat
23	Yardlong bean	Unknown	Tha Sa La, Nakorn-sithammarat
24	Yardlong bean	Lebmee	Cha uat, Nakorn-sithammarat
25	Yardlong bean	Unknown	Na Yong, Trang
26	Yardlong bean	Dang	Bang Rak, Trang
27	Yardlong bean	Unknown	KhounPring, Trang

Table 1 (cont.) Accession number and sources of yardlong bean and cowpea accessions in the present study

Accession No.	Common name	Variety	Source
28	Cowpea	Line suar	Wang Wiset, Trang
29	Yardlong bean	Unknown	Naphala, Trang
30	Yardlong bean	Unknown	Naphala, Trang
31	Yardlong bean	Kampong	Si Chon, Nakorn-sithammarat
32	Yardlong bean	Foundsai	Si Chon, Nakorn-sithammarat
33	Yardlong bean	Unknown	Si Chon, Nakhon-sithammarat
34	Yardlong bean	Unknown	Si Chon, Nakorn-sithammarat
35	Yardlong bean	Unknown	Si Chon, Nakorn-sithammarat
36	Yardlong bean	Unknown	Tha Sa La, Nakorn-sithammarat
37	Yardlong bean	Taidang	ThungLan, Khlong Hoi Khong
38	Yardlong bean	Unknown	ThungLan, Khlong Hoi Khong
39	Yardlong bean	Line	-
40	Yardlong bean	Pran	Wang Phai, Chumpon
41	Yardlong bean	Trang 2	PromKhiri, Nakorn- sithammarat
42	Yardlong bean	Taitor	PromKhiri, Nakorn-sithammarat
43	Yardlong bean	Trang 3	PromKhiri, Nakorn-sithammarat
44	Yardlong bean	Dang	Ko Yao Yai, Phang-gna
45	Yardlong bean	Unknown	ThungLan, Khlong Hoi Khong
46	Yardlong bean	Unknown	Mai Kaen, Pattani

Table 1 (cont.) Accession number and sources of yardlong bean and cowpea accessions in the present study

Accession No.	Common name	Variety	Source
47	Yardlong bean	Ranong	Ranong
48	Yardlong bean	Pattani 1	Pattani
49	Yardlong bean	Pattani 2	Pattani
50	Yardlong bean	Pattani 3	Pattani

2.2.1. Qualitative characteristics :

- growth habit; determinate or indeterminate
- plant pigmentation; use as the score from 0 to 2
    - 0- no pigmentation (all plants were green)
    - 1- part of stem, pods were with purple colored splashes
    - 2- stem and pod all were purple
- flower color
- terminal leaflet shape
- pod curvature
- immature pod pigmentation
- mature pod color
- seed shape
- eye color

2.2.2 Quantitative characteristics

- days to flowering
- terminal leaflet length (mm); mean length of 10 terminal leaflets from each plant

- terminal leaflet width (mm); mean width of 10 terminal leaflets from each plant
- pod length (cm); mean pod length of the 10 pods from each plant
- pod weight (g); mean pod weight of the 10 pods from each plant
- seed length (mm); mean seed length of the 10 seeds from each plant
- seed width (mm); mean seed width of the 10 seeds from each plant

2.3 Screening of BICMV resistance sources in yardlong bean and cowpea

Seed of fifty accessions was sown in baskets with soil and compost mixture and kept in a screen house to protect accessions from insect infestation. Twenty plants were germinated for each of 50 accessions. When the seedlings were at the stage of cotyledon, plants were tested with Indirect ELISA method for virus free using anti-BICMV PAb., infected plants were removed and only healthy plant materials were left for further test. After checking the presence of BICMV in young plants, young leaves were artificially inoculated by mixture of sap infected with BICMV and celite. Celite damages the young leaves and the virus can easily enter into the plant. All seedlings were kept in the screen house for 6 weeks. After inoculation, plants were observed on the presence or absence of symptoms characteristic for BICMV, or the other viruses. Observation was done for each plant. Symptoms like systemic mosaic, stunting, distortion, interveinal chlorosis, vein-banding, distortion, blistering were recorded. After 40 days, plant materials from each of 50 accessions were collected for running ELISA test applied the protocol from Clark and Adams (1977) as followings ;

- Grinding the cowpea leaf in Carbonate coating buffer (CB) in ratio 1:5 (w/v);
- Add 50 ml of sap to each well of ELISA plate;
- Incubate ELISA plate for 1.30 hour at room temperature and remove sap;
- Wash ELISA plate 3 times with PBST;
- Add 50 ml of anti-BICMV PAb (diluted in blocking solution at ratio 1: 200 (v/v)) in each well of ELISA plate;
- Incubate ELISA plate at room temperature for 1.30 hour and remove anti-CABMV PAb;
- Wash ELISA plate 3 times with PBST;

- Add 50 ml of GAR (diluted in PBS buffer in ratio 1: 10,000 (v/v)) in each well of ELISA plate;
- Incubate ELISA plate at room temperature for 1.30 hour and remove GAR;
- Wash ELISA plate 3 times with PBST buffer;
- Add 100 ml of PNPP (1mg/ml) substrate to each well and incubate at RT for 1 hour;
- Add 100 ml of NNaOH to each well and read plate with ELISA reader at absorbance values 405 (A 405 nm)

Multiple-transfers in plant virus transmission were used for result calculation (Gibbs and Gower, 1960). Symptom expression and ELISA results were used to distinguish between resistance and susceptible plants. Plants showing the symptom of BICMV during 4 weeks after inoculation were assigned as S, plants with no symptom were assigned as N. Positive results from ELISA were considered when the average absorbency value of duplicate test wells was at least twice of known negative control. Positive results were assigned as P. Individual plants were rated as the followings (modified from Ouattara and Chambliss,1991)

PS if they developed BICMV symptoms and gave positive for ELISA.

NS if they developed symptoms but gave negative for ELISA

P if they show no symptom but gave positive for ELISA

N if they show no symptom and gave negative for ELISA

Chapter 3

Results

1. Morphological characteristic evaluation

1.1 Qualitative characteristics

Characterization and evaluation of each character were used to determine the range of variability in qualitative characteristics of yardlong bean and cowpea accessions collected from Southern part of Thailand, Malaysia and Serbia. It was found that high variability was observed in particular qualitative characteristics between collected accessions (Table 2). Variability was expected on different origin of collected accessions. Some of those accessions, which have desirable properties, can be used for further examination in breeding program because they show high level of genetic diversity. The following are result details,

1.1.1. Growth habit

For growth habit, 46 from 50 accessions (92%) had indeterminate growth habit and they belong mostly to yardlong bean accessions. Only 4 accessions or 8 % had determinate growth habit and all these 4 accessions were cowpea including accession no. 5a (IT82E-9 determinate), 6 (IT82TE-16), 14 (VIG 009) and 28 (Linesua) (Table 2 and Figure 6).

Table 2. Qualitative characters of 50 yardlong bean and cowpea accessions

Access. no.	Growth habit	Plant pigmentation	Seed shape	Eye color	Flower color	Terminal Leaflet shape	Pod curvature	Immature pod pigmentation	Mature pod color
1	Indeterminate	0	Kidney	Black	Violet	Ovate	Straight	None	Pale tan
2	Indeterminate	1	Kidney	Black	Violet	Ovate	Straight	Uniformly pigmented	Dark purple
3	Indeterminate	0	Kidney	Black	White	Ovate	Straight	None	Pale tan
4	Indeterminate	0	Kidney	Black	Violet	Ovate	Curved	None	Pale tan
5 a*	Determinate	0	Rhomboid	Black	Violet	Sub-hastate	Straight	None	Pale tan
5 b*	Indeterminate	0	Rhomboid	Black	Violet	Hastate	Slightly curved	Uniformly pigmented	Pale tan/purple
6	Determinate	0	Rhomboid	Brown	Violet	Hastate	Slightly curved	None	Pale tan
7	Indeterminate	0	Ovoid	Black	White/Violet splashes	Ovate	Straight	Pigmented valves	Pale tan with purple valves
8	Indeterminate	0	Kidney	Brown	Violet	Sub-hastate	Straight	None	Dark tan
9	Indeterminate	0	Kidney	Black	Violet	Ovate	Slightly curved	None	Pale tan
10	Indeterminate	0	Globose	Black	Violet	Ovate	Straight	None	Pale tan

Table 2 (cont.) Qualitative characters of 50 yardlong bean and cowpea accessions

Access. no.	Growth habit	Plant pigmentation	Seed shape	Eye color	Flower color	Terminal Leaflet shape	Pod curvature	Immature pod pigmentation	Mature pod color
11	-	-	-	-	-	-	-	-	-
12	Indeterminate	0	Kidney	Black	Violet	Ovate	Straight	None	Pale tan
13	Indeterminate	0	Kidney	Black	Violet	Sub-hastate	Slightly curved	None	Pale tan
14	Determinate	0	Rhomboid	Tan brown	Violet	Ovate	Slightly curved	None	Pale tan
15	Indeterminate	0	Kidney	Brown	White	Ovate	Straight	None	Pale tan
16	Indeterminate	0	Kidney	Black	White/Violet splashes	Ovate	Straight	None	Dark tan
17	Indeterminate	0	Kidney	Brown	Violet	Sub-hastate	Straight	None	Pale tan
18	Indeterminate	0	Ovoid	Brown	Violet	Ovate	Straight	Splashes of pigments	Dark tan
19	Indeterminate	1	Kidney	Brown	Violet	Sub-ovate	Straight	Uniformly pigmented	Black or dark purple
20	Indeterminate	0	Ovoid	Tan brown	Violet	Sub-ovate	Straight	None	Pale tan

Table 2 (cont.) Qualitative characters of 50 yardlong bean and cowpea accessions

Access. no.	Growth habit	Plant pigmentation	Seed shape	Eye color	Flower color	Terminal Leaflet shape	Pod curvature	Immature pod pigmentation	Mature pod color
21	Indeterminate	0	Ovoid	Brown	Violet	Sub-ovate	Straight	Splashes of pigments	Dark tan
22	Indeterminate	0	Ovoid	Black	Violet	Ovate	Straight	Pigmented valves	Pale tan/ purple straps
23	Indeterminate	1	Kidney	Brown	Violet	Ovate	Straight	Uniformly pigmented	Black or dark purple
24	Indeterminate	0	Kidney	Black	Violet	Sub-ovate	Slightly curved	None	Dark tan
25**	Indeterminate	0	---	---	---	Sub-ovate	---	---	---
26	Indeterminate	1	Kidney	Brown	Violet	Sub-ovate	Slightly curved	Uniformly pigmented	Dark brown/purple
27	Indeterminate	0	Kidney	Black	Violet	Sub-ovate	Slightly curved	None	Dark tan
28	Determinate	0	Ovoid	Black	Violet	Ovate	Slightly curved	Pigmented valves	Pale tan
29	Indeterminate	0	Ovoid	Black	White/Violet splashes	Ovate	Straight	Pigmented valves	Pale tan/ purple straps
30	Indeterminate	0	Ovoid	Black	Violet	Ovate	Straight	None	Pale tan

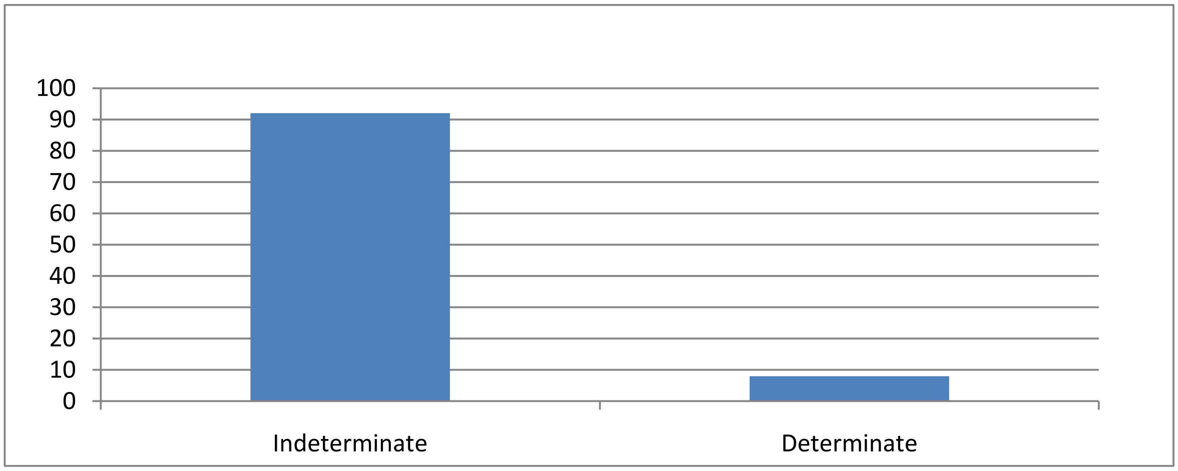
Table 2 (cont.) Qualitative characters of 50 yardlong bean/cowpea accessions

Access. no.	Growth habit	Plant pigmentation	Seed shape	Eye color	Flower color	Terminal Leaflet shape	Pod curvature	Immature pod pigmentation	Mature pod color
31	Indeterminate	0	Ovoid	Tan brown	Violet	Sub-ovate	Straight	None	Pale tan
32	Indeterminate	1	Kidney	Brown	White/Violet splashes	Sub-ovate	Straight	Uniformly pigmented	Dark brown
33	Indeterminate	0	Ovoid	Brown	Violet	Ovate	Slightly curved	None	Pale tan
34	Indeterminate	1	Kidney	Brown	Violet	Sub-ovate	Curved	Uniformly pigmented	Black or dark purple
35	Indeterminate	0	Ovoid	Tan brown	Violet	Sub-ovate	Slightly curved	None	Dark tan
36	Indeterminate	0	Ovoid	Black	Violet	Sub-ovate	Straight	Pigmented tips	Pale tan/ pigmented tips
37	Indeterminate	0	Kidney	Black	Violet	Sub-ovate	Straight	None	Pale tan
38	Indeterminate	1	Kidney	Brown	Violet	Sub-ovate	Slightly curved	Pigmented tips	Dark tan
39	Indeterminate	0	Kidney	Black	Violet	Sub-hatate	Slightly curved	Pigmented sutures	Pale tan/purple
40	Indeterminate	0	Kidney	Black	Violet	Ovate	Straight	Pigmented valves	Pale tan/purple lines

Table 2. Qualitative characters of 50 yardlong bean/cowpea accessions

Access. no.	Growth habit	Plant pigmentation	Seed shape	Eye color	Flower color	Terminal Leaflet shape	Pod curvature	Immature pod pigmentation	Mature pod color
41**	Indeterminate	0	---	---	Violet	Ovate	---	---	---
42	Indeterminate	0	Kidney	Black	Violet	Sub-ovate	Slightly curved	None	Pale tan
43	Indeterminate	1	Kidney	Brown	Violet	Sub-ovate	Straight	Uniformly pigmented	Black or dark purple
44	Indeterminate	0	Ovoid	Brown	Violet	Sub-ovate	Straight	Splashes of pigment	Dark brown
45	Indeterminate	2	Kidney	Brown	Violet	Sub-ovate	Slightly curved	Uniformly pigmented	Black or dark purple
46	Indeterminate	0	Kidney	Black	Violet	Ovate	Straight	None	Dark tan
47	Indeterminate	0	Kidney	Brown	White/Violet splashes	Sub-ovate	Straight	Splashes of pigment	Dark brown
48	Indeterminate	0	Kidney	Black	Violet	Sub-ovate	Slightly curved	None	Dark brown
49	Indeterminate	0	Kidney	Brown	Violet	Sub-ovate	Straight	None	Pale tan
50	Indeterminate	1	Kidney	Brown	White/Violet	Ovate	Straight	Splashes of pigment	Dark brown

*Plants in accession 5 has been mixed as determinate and indeterminate growth habit and from this reason accession was divided in 5a and 5b . **Accession no.25 have no flower and pod,** accession no.41 produced no pod.



Growth habit

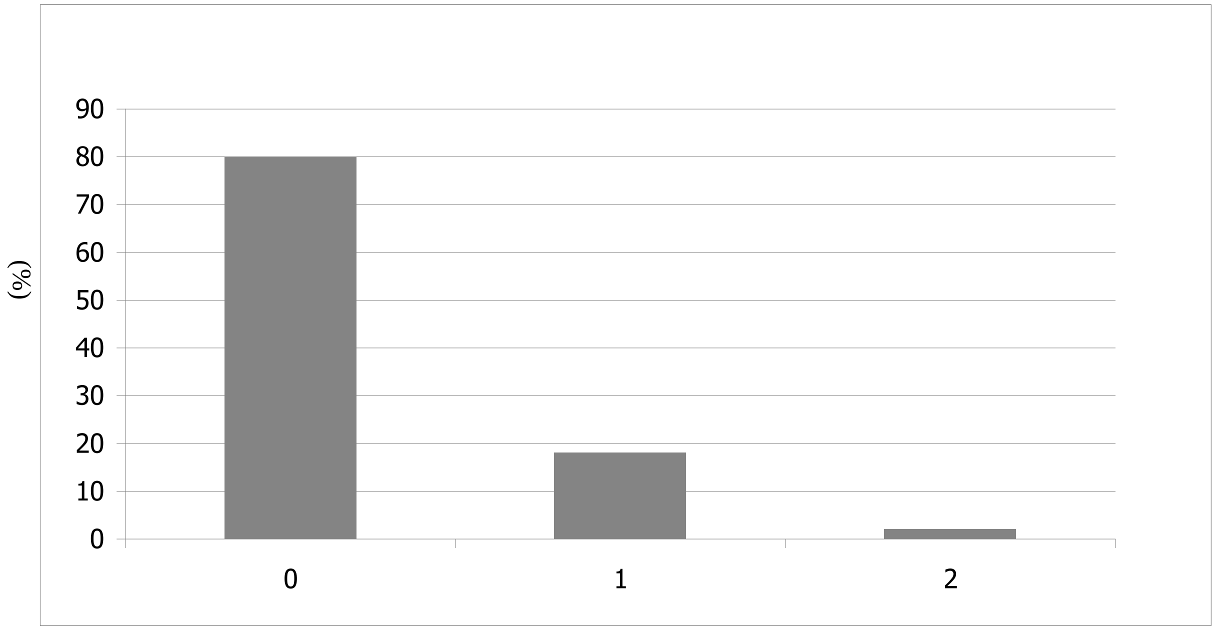
Figure 6. Frequency distribution of growth habit in collected yardlong bean and cowpea accessions

1.1.2. Plant pigmentation

Forty accessions or 80% of plants have no pigmentation. All plants were intensive green. Nineteen accessions (18%) including yardlong bean accessions no. 2 (KU 20), 19, 23, 34, 38 (unknown), 26 (Dang), 32 (Foundsai) have pigmented pods (score 1). Accession number 45, yardlong bean, unknown variety from Klong-hoikhong, has pigmented in vegetative and generative parts (score 2) (Figure 7). Pigmentation on yardlong bean and cowpea can be presented on the cotyledons, joints, flower petals, seeds pods, peduncles, stems and leaves.

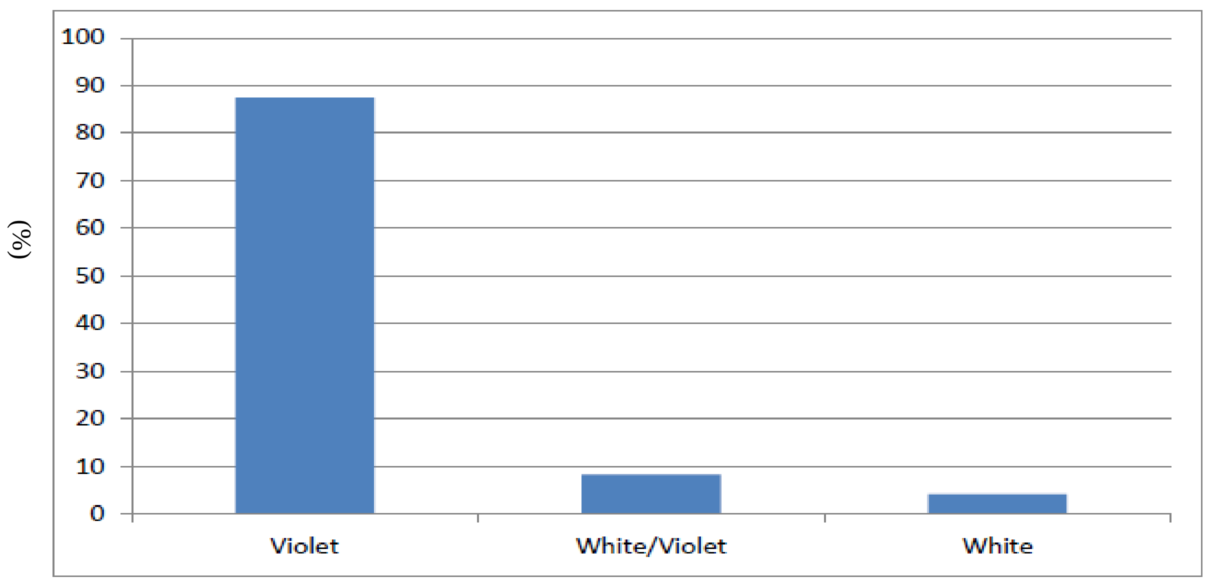
1.1.3. Flower color

Inheritance of flower color and pod color in cowpea (*Vigna unguiculata* L. Walp.) have followed a qualitative pattern. High variability founded for this trait. Flower color range from violet, white/violet to white. Most accessions in our study have a violet flower color (83.3%) (Figure 8). Yardlong bean accessions no. 7 (Tahanpran), 16 (Trang 1), 29, (unknown), 32 (Foundsai),



Plant pigmentation

Figure 7. Frequency distribution of plant pigmentation in collected yardlong bean and cowpea accessions



Flower color

Figure 8. Frequency distribution of flower color in collected yardlong bean and cowpea accessions

47 (Ranong) and 50 (Pattani 3) have a violet/white flower color (in total 12.5%). Only two accessions (4.2%) exhibited white flower color. Those are yardlong bean accessions no. 3 (Selected- PSU) and 15 (unknown).

1.1.4. Terminal leaflet shape

The observed terminal leaflet shapes of the collected accessions are presented in Table 2 and Figure 9. It showed that collected accessions have 4 forms of terminal leaflet shape: hastate,sub-hastate, ovate and sub-ovate. Almost 90 percent of accessions have ovate and sub-ovate terminal leaflet shape and the rest of accessions have hastate and sub-hastate terminal leaflet shape (10.0%). All results were shown in Table 2.

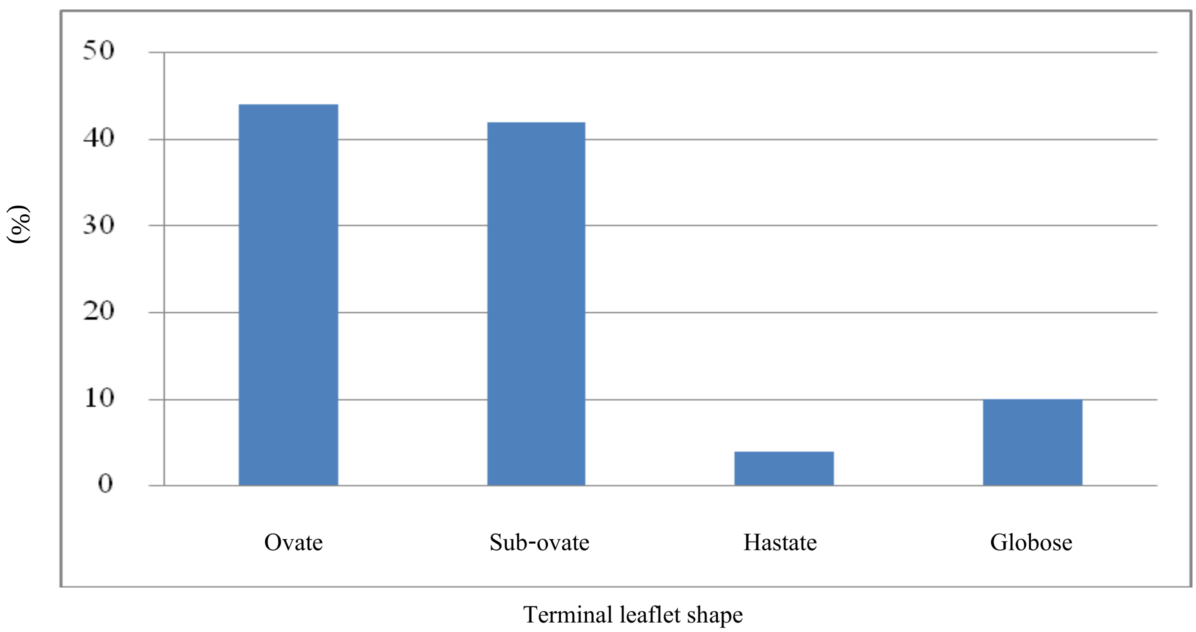


Figure 9. Frequency distribution of terminal leaflet shape in collected yardlong bean and cowpea accessions

1.1.5. Pod curvature

Pod curvature trait is important to identify pod and seed characters and usually displayed precise areas of cultivation (Pasqueth, 1998). This trait varied from straight, slightly curve to the curve. Straight pod character was the dominant pod shape in the collected accessions (62.5%). Sixteen accessions or 33.3 percent of accessions have slightly curved pod including cowpea accessions no. 5b (IT82E-9 indeterminate), 6 (IT82E-16), 14 (VIG 009), yardlong bean accessions no.9 (Kao-hinson), 13 (Cameron), 24 (Lebmee), 26 (Dang), 27, 33, 35,38,45 (unknown), accession no.28 (Line suar), 39 (line), 43 (Trang 3), and 48 (Pattani 1). Curved shape of pod was recorded on accessions no.4 (Chai Tai) and 34 (unknown) (Table 2, Figure 10).

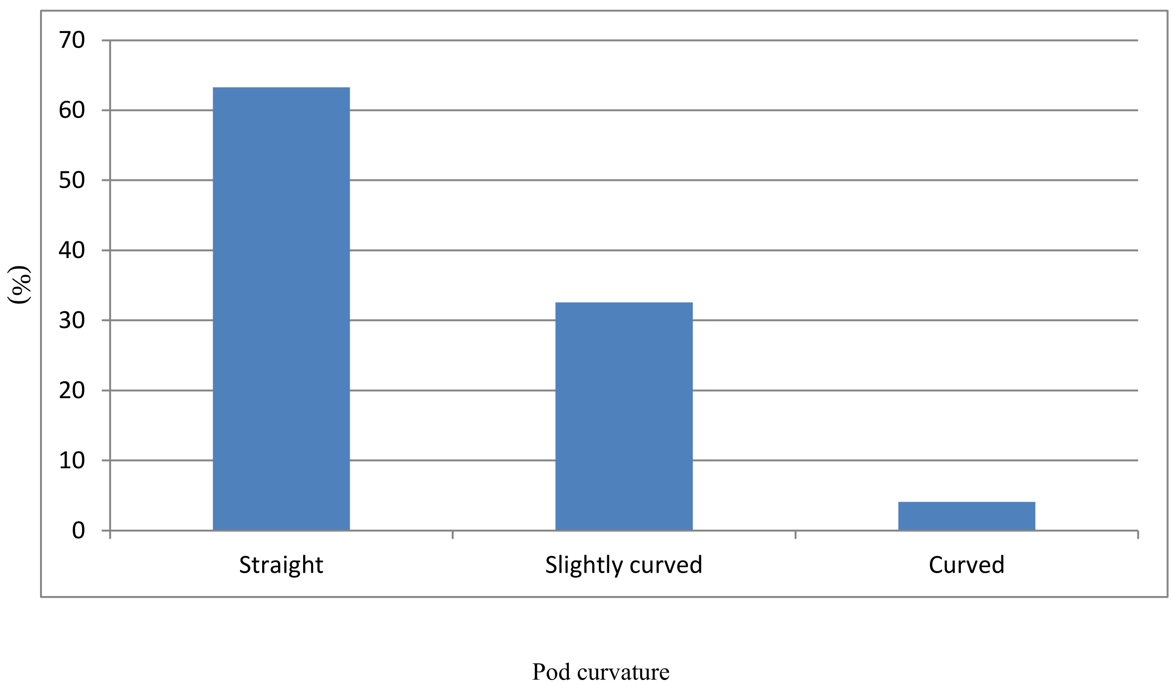


Figure 10. Frequency distribution of pod curvature in collected yardlong bean and cowpea accessions

1.1.6. Immature pod pigmentation

Pigmentation on cowpea and yardlong bean is present on the cotyledons, joints, petals, seeds pods, peduncles, stems and leaves. The pigmentation pattern of the immature pods of the accessions shown in Table 2 and Figure 11 was characterized by the presence or absence of pigment with various transitional forms. Of total 48 accessions which produced pods, 28 accessions (58.3%) have immature pod without pigmentation, all pods were green. Uniformly pigmented immature pods have 21% of accessions including yardlong bean accession no. 2 (KU-20) from Kasetsart University, cowpea accession no. 5b (IT82E-9), yardlong bean accessions no. 19, 23 and 34 (unknown), 32 (Foundsai), 43 (Trang 3), and 50 (Pattani 3). Five accessions produced pigmented valves immature pod (yardlong bean accessions no. 7 (Tahanpran), 22 (Or.So), 28 (Line suar), 29 (unknown) and 40 (Pran). Splashes of pigment immature pods was observed on four accessions including accession no. 18 (unknown), 21 (Teenman), 44 (Dang) and 47 (Ranong). Immature pod from two accessions were pigmented tips (accessions no. 36 and 38: unknown). Only one accession or 2% had pigmented sutures (no. 39: line).

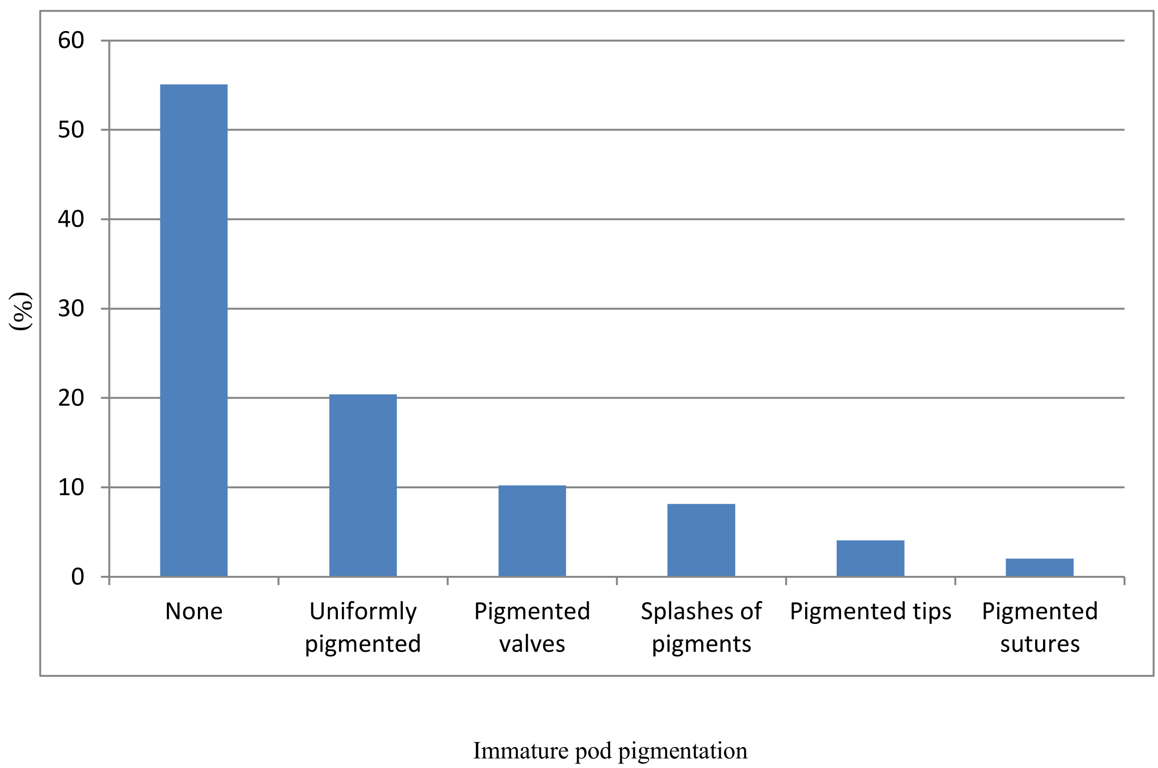


Figure 11. Frequency distribution of immature pod pigmentation in collected yardlong bean and cowpea accessions

1.1.7. Mature pod pigmentation

The color of mature pods ranged from pale tan to dark purple. Pale tan pod was observed in 43.7% of the accessions while dark tan was recorded on the 7 following accessions: yardlong bean accessions no. 16, 18, 27, 35 and 46 (unknown), 21 (Teenman), 24 (Lebmee). Black or dark purple pod was recorded on 5 accessions, they are accession no. 19, 34 and 45 (unknown), 23 (unknown), 43 (Trang 3). Dark brown pod pigmentation have 6% of accessions; accessions no. 26 (Dang) , 32 (Foundsai), 47 (Ranong) and 48 (Pattani 1). Pale tan/purple straps immature pod was observed in accessions no. 22 (Or.So) and 29 (unknown). Two accessions of yardlong bean (accessions no. 39: line) and 40 (Tahan pran) have pale tan/purple lines mature pods, while cowpea accession 5b (IT82E-9 indeterminate) has pale tan/purple mature pod color. Pale tan/pigmented tips, Pale tan/purple valves mature pod color, dark purple mature pod pigmentaion and dark purple were recorded on the following accession no. 36 (unknown), 7 (Tahan pran), 2 (KU-20) and 26 (Dang), respectively (Table 2 and Figure 12). This morphological marker showed the highest variability compared with all studied characteristics.

1.1.8. Seed shape

Kidney seed shape was a most frequent observed in collected accessions, which can be seen in Table 2 and Figure 13. In Figure 13, 30 from 48 or 62.5% of accessions have kidney seed shape. Twenty seven percent of accessions have ovoid seed [yardlong bean accessions no. 7 (Tahanpran), 18 (unknown), 20 (Foundsai), 21(Teenman), 22 (Or So), 28 (cowpea Line sur), 29, 30 (unknown), 31 (Kampong), 33, 35, 36 (unknown) and accession number 44 (Dang)]. Rhomboid seed shape has 8 % of accessions, mostly accessions which belong to cowpea including accession no. 5a (IT82E-9 determinate), 5b (IT82E-9 indeterminate), 6 and 14 (VIG 009). Just one accession has globose seed shape, it was accession number 7 (yardlong bean Tahanpran from Pattalung).

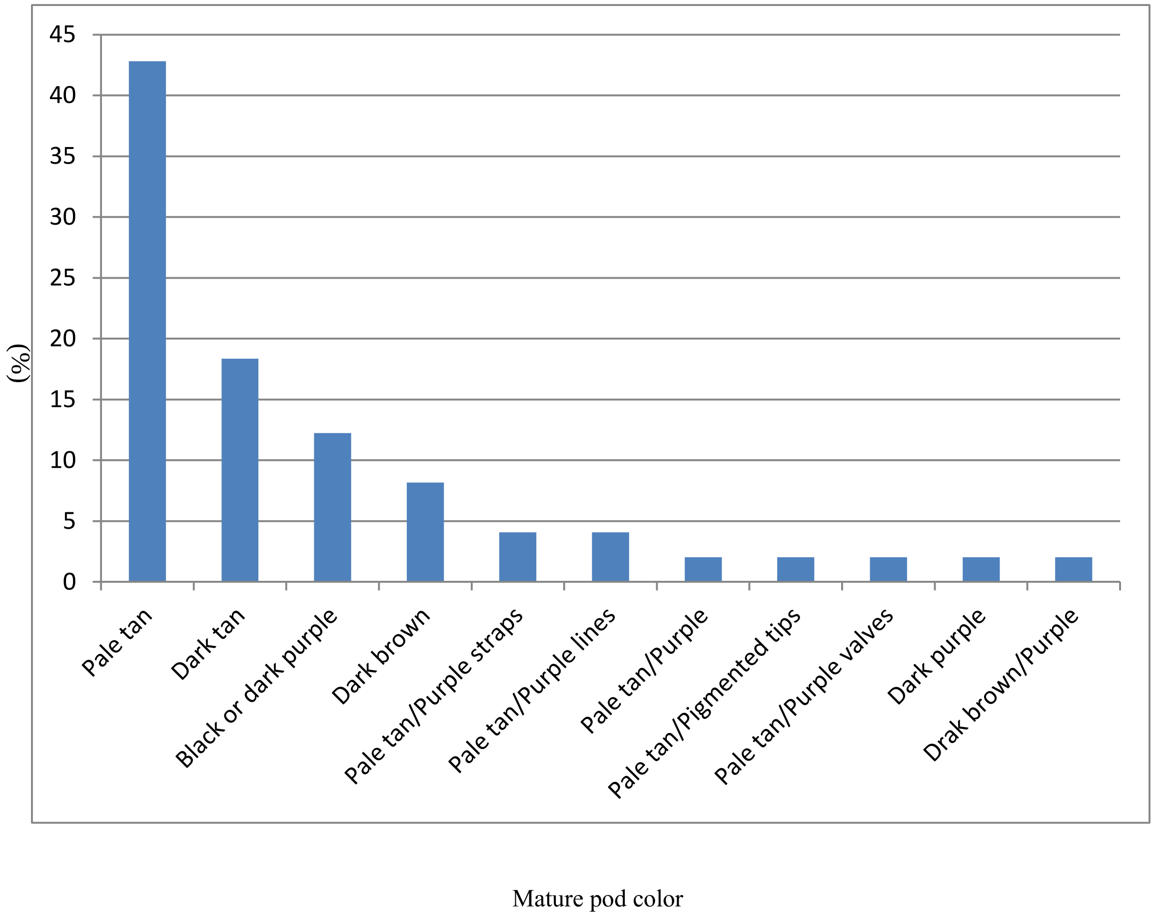


Figure 12. Frequency distribution of mature pod color in collected yardlong bean and cowpea accessions

1.1.9. Eye color

Results of seed eye color of the accessions used in this study are presented in Table 2 and Figure 14. Seed eye color ranged from black to tan brown. Black color of seed eye was dominant (57.1%), followed by brown seed eye color (36.7%) of cowpea accession no. 6 (IT82T-16), yardlong bean accessions no. 8 (Malaysia 308), 15 (unknown), 17(SR- 863), 18, 19, 23, 33, 34, 38, 45 (unknown), 21(Teenman), 26 (Dang), 32 (Foundsai), 43 (Trang 3), 44 (Dang), 47 (Ranong), 49

(Pattani2) and 50 (Pattani 3). Two accessions, no. 14 (VIG 009) and 31 (kampong) have tan brown seed eye color.

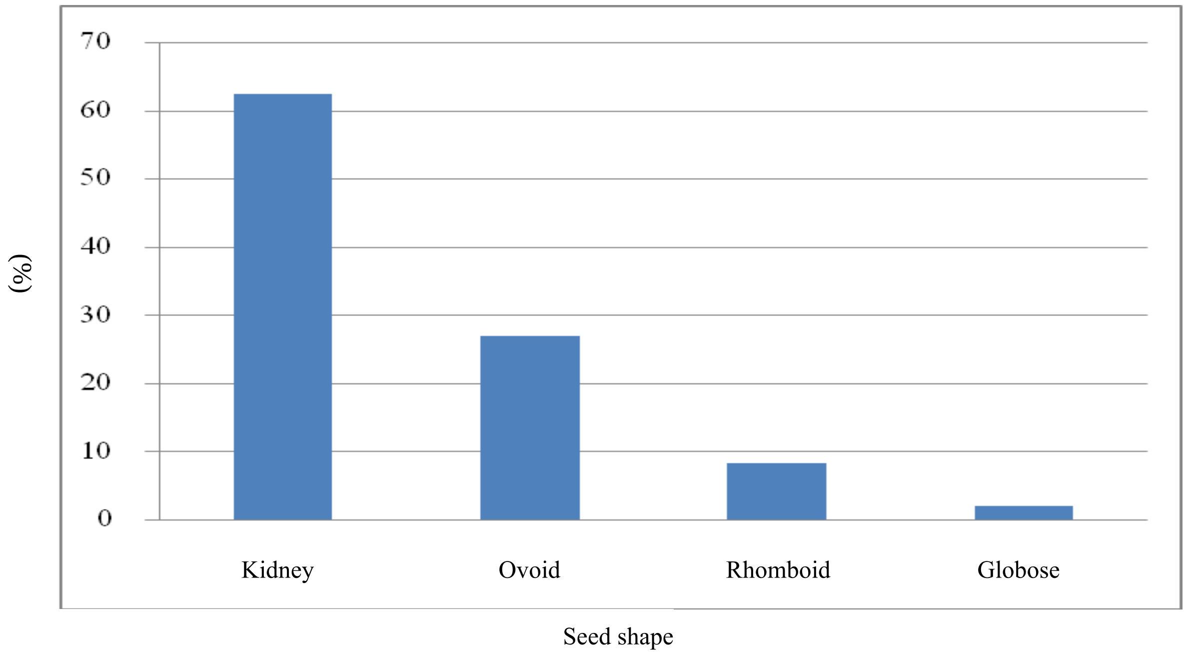


Figure 13. Frequency distribution of seed shape in collected yardlong bean and cowpea accessions

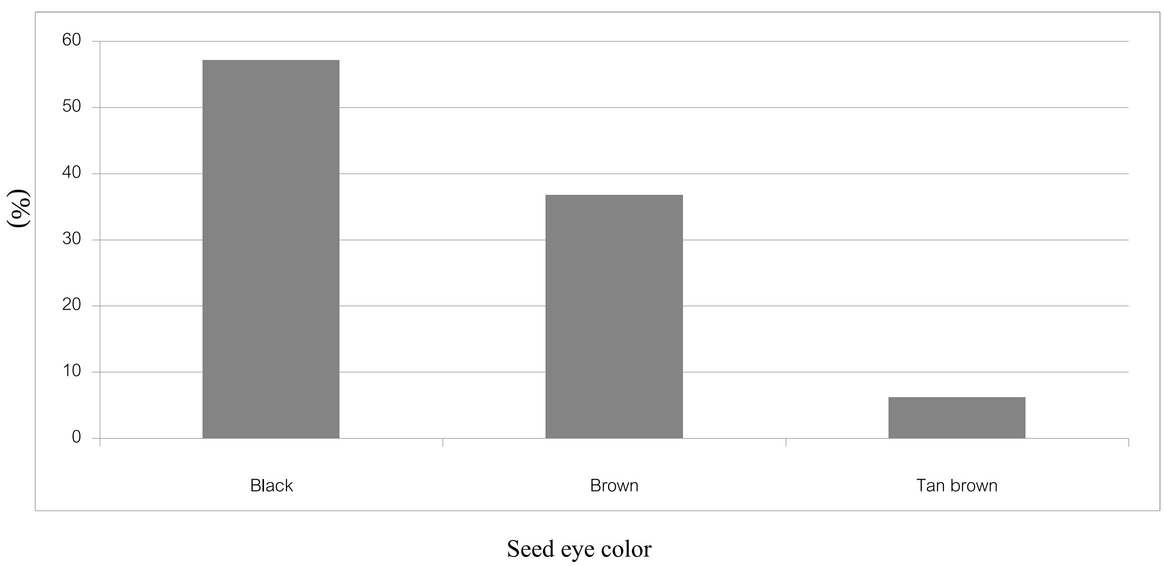


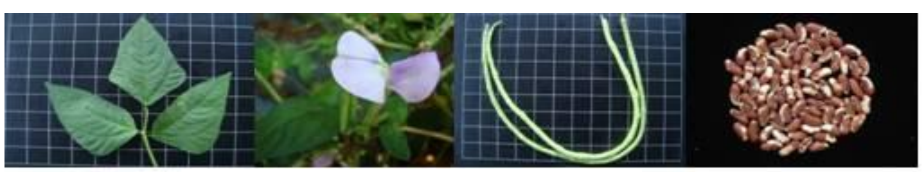
Figure 14. Frequency distribution of seed eye color in collected yardlong bean and cowpea accessions

1.2 Quantitative characteristics

Quantitative characteristics are important morphological indicator of collected accessions, mostly as a yield predisposition. In this study, quantitative characters were days to flowering, terminal leaflet length, terminal leaflet width, pod length, pod weight, seed length and seed width (Table 3).

1.2.1. Days to flowering

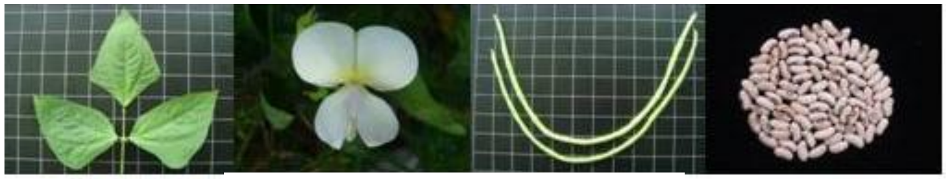
Number of days to flowering of the studied accessions was shown on Table 3. The observed traits varied depending on the sample and ranged from 39 days on accession no. 9 (Khao hinson) and no. 13 (Cameron) to 69 days on accession no. 41 (Trang 2). Mean value for number of days to flowering was 51.4 days. Highly significant positive differences were found in yardlong bean accessions no.18 (unknown), 20 (Founsai), 21 (Teenman), 31 (Kampong) 32 (Foundsai), 33, 35, 36 (unknown) , 41 (Trang 2), 43 (Trang 3), 44 (Dang), 49, (Pattani 2) and 50 (Pattani 3) and significant negative differences were in accession no. 2 (KU 20), 3 (Selected- PSU), 4 (cowpea Chia Tai), 5a (IT82E-9 determinate), 5b (IT82E-9 indeterminate), 6 (IT82E-16), 13 (Cameron), 15, 19 (unknown), 26 (Dang) , 42 (Taitor), 45 (unknown) and 47 (Ranong). The variation of flowering date was shown in Figure 16.



Accession 1

Accession 2

Figure 15. Morphological characters of 50 accessions yardlong bean and cowpea



Accession 3

Accession 4

Accession 5

Accession 6

Accession 7

Accession 8

Figure 15 (cont.) Morphological characters of 50 accessions yardlong bean and cowpea

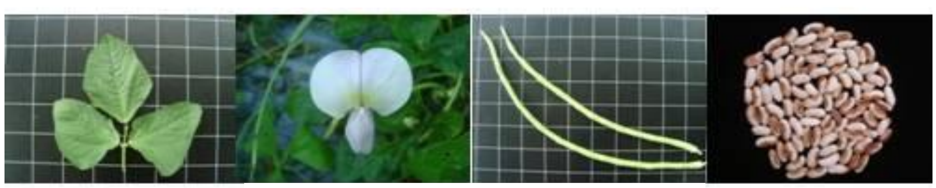
Accession 9

Accession 10

Accession 12

Accession 13

Accession 14



Accession 15

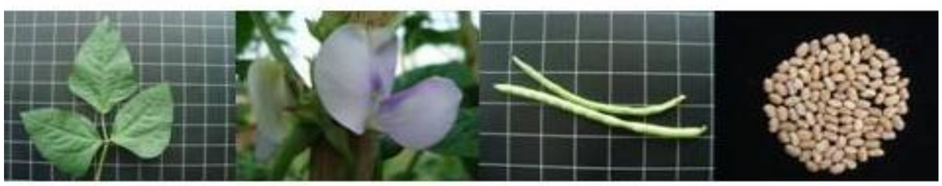
Figure 15 (cont.) Morphological characters of 50 yardlong bean and cowpea accessions

Accession 16

Accession 17

Accession 18

Accession 19

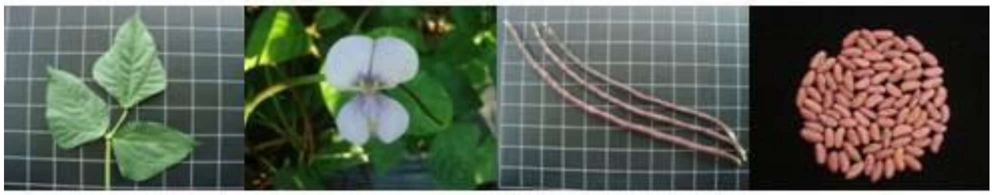


Accession 20

Accession 21

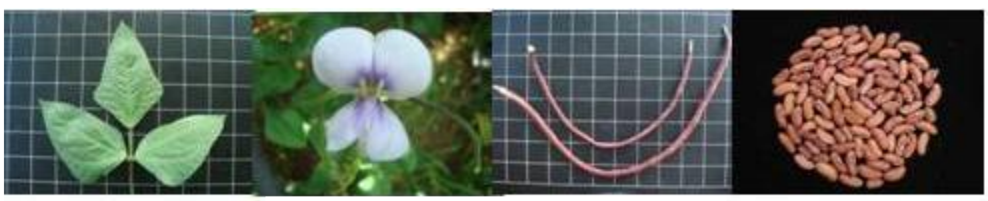
Figure 15 (cont.) Morphological characters of 50 yardlong bean and cowpea accessions

Accession 22



Accession 23

Accession 24



Accession 26

Accession 27

Accession 28

Figure 15 (cont.) Morphological characters of 50 yardlong bean and cowpea accessions

Accession 29

Accession 30

Accession 31

Accession 32

Accession 33

Accession 34

Figure 15 (cont.) Morphological characters of 50 yardlong bean and cowpea accessions

Accession 35

Accession 36

Accession 37

Accession 38

Accession 39

Accession 40

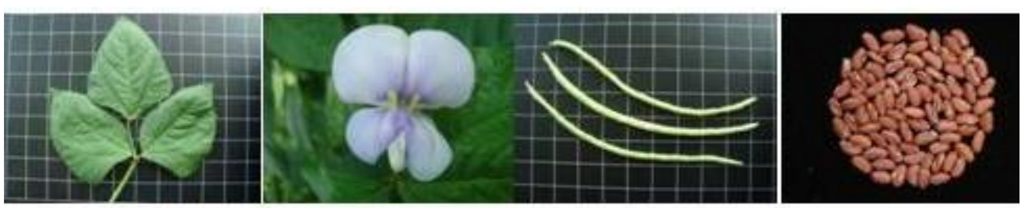
Figure 15 (cont.) Morphological characters of 50 accessions yardlong bean and cowpea

Accession 42

Accession 43

Accession 44

Accession 45

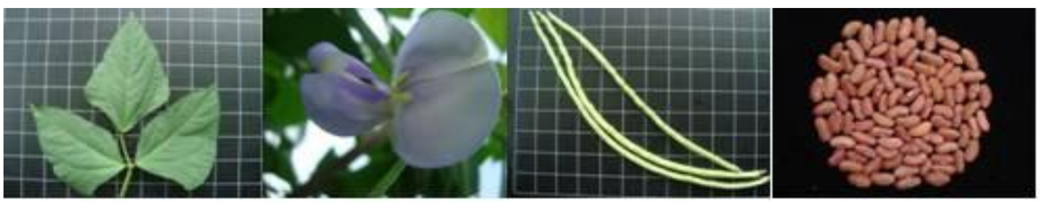


Accession 46

Figure 15 (cont.) Morphological characters of 50 accessions yardlong bean and cowpea

Accession 47

Accession 48



Accession 49

Accession 50

Accession 25

Accession 41

Figure 15 (cont.) Morphological characters of 50 accessions yardlong bean and cowpea

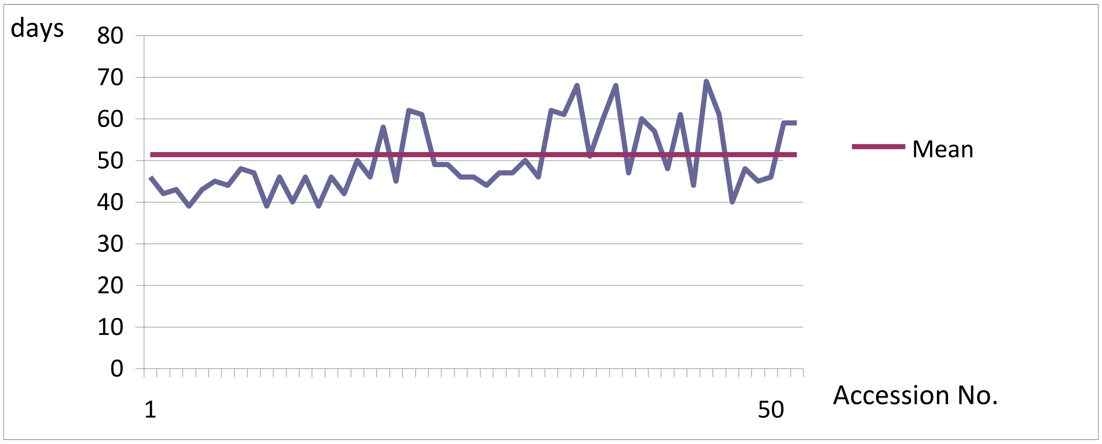


Figure 16. Frequency distribution of days to flowering in collected yardlong bean and cowpea accessions.

1.2.2 Terminal leaflet length

The lowest value for the terminal leaflet length was recorded in accession 14, cowpea from Serbia (94.6 cm) and the highest value was in accession number 49 (Patani 2) (152.6 mm). Most accessions have no significant differences in terminal leaflet length compared to the average value (123.27 mm). F- test confirmed that there exist positive significant differences in leaflet length just in one accession (accessions no. 49). Negative significant differences were revealed in accessions number 13 (Cameron) and accession no. 14 (VIG 009) (Figure 17 and Table 3).

Table 3. Quantitative characters of 50 yardlong bean/cowpea accessions in this study

Accession No.	Days to flowering (days)	Terminal leaflet length (mm)	Terminal leaflet width (mm)	Pod lenght (cm)	Pod weight (g)	Seed length (mm)	Seed width (mm)
1	46	137.3	67.0	63.2**+	29.2**+	7.02**-	5.87
2	42**-	152.0	74.0	37.8**+	12.2	11.45**+	5.87
3	43**-	127.6	68.3	50.6**+	18.8**+	11.21	5.56
4	39**-	99.0	69.0	25.0	8.0**-	9.37**-	4.70
5a	43**-	134.0	44.6**-	20.6**-	8.8**-	8.37**-	6.99**

Table 3 (cont.) Quantitative characters of 50 yardlong bean/cowpea accessions in this study

Accession No.	Days to flowering (days)	Terminal leaflet lenght (mm)	Terminal leaflet width (mm)	Pod lenght (cm)	Pod weight (g)	Seed lenght (mm)	Seed width (mm)
5b	45**-	100.3	61.3	12.2**-	3.4**-	7.65**-	5.93
6	44**-	115.0	23.0**-	19.0**-	7.2**-	7.02**-	6.29**+
7	48	132.6	99.6**+	26.2	12.4	8.35**-	5.21
8	47	133.0	59.6	51.2**+	20.6**+	12.25**+	6.01
9	39**-	117.3	69.2	28.0	11.4	12.42**+	5.23
10	46	101.6	68.0	18.0**-	6.4**-	6.78**-	5.61
11	--	--	--	--	--	--	---
12	46	125.0	87.6**+	23.6**-	8.0**-	9.92	5.26
13	39**-	96.0**-	45.6**-	47.2**+	17.0**+	11.23	5.92
14	46	94.6**-	50.0**-	18.8**-	7.2**-	7.19**-	6.01
15	42**-	101.3	73.0	37.8**+	13.4	11.74**+	5.69
16	50	137.0	84.0	38.6**+	15.6	11.35**+	5.85
17	46	107.0	42.6**-	28.4	10.2	10.36	5.71
18	58**+	117.0	69.3	25.8	10.6	8.25**-	5.60
19	45**-	116.3	55.3	44.6**+	18.2**+	12.62**+	5.66
20	62**+	122.6	71.3	18.0**-	5.8**-	8.14**-	5.76
21	61**+	107.0	51.0	19.2**-	7.2**-	8.54**-	5.71
22	49	122.3	72.3	24.4**-	12.8	9.96	5.77
23	49	108.6	66.0	37.6**+	15.2	11.5	5.20**-
24	46	126.3	58.6	35.8	12.4	10.49	5.87
25	---	112.3	78.3	---	---	---	---
26	44**-	125.6	52.3	51.4**+	28.4**+	12.26**+	5.76
27	47	121.0	78.3	32.6	13.8	11.25**+	5.71
28	47	132.6	86.0	25.6	11.0	10.57	6.21
29	50	133.0	80.6	28.8	13.2	8.69**-	5.45
30	56	116.3	71.6	27.8	9.6	9.03**-	5.27**-
31	62**+	123.0	67.6	22.2**-	11.6	10.94	6.30**+
32	61**+	118.3	63.0	30.6	11.8	9.97	4.88**-
33	68**+	137.3	79.3	34.4	21.4**+	10.45	5.36**-
34	51	121.0	68.6	32.8	11.6	10.89	5.29**-
35	60**+	117.3	63.6	25.4	12.0	10.22	6.51**+
36	68**+	129.6	86.3**+	22.4**-	8.2**-	8.56**-	4.64**-

Table 3 (cont.) Quantitative characters of 50 yardlong bean/cowpea accessions in this study

Accession No.	Days to flowering (days)	Terminal leaflet length (mm)	Terminal leaflet width (mm)	Pod lenght (cm)	Pod weight (g)	Seed lenght (mm)	Seed width (mm)
37	47	114.0	69.0	31.0	15.2	12.03**+	5.63
38	60**+	123.0	74.3	26.2	10.6	9.87**-	6.41**+
39	57	126.3	44.3	25.2	8.4**-	10.63	5.58
40	48	129.0	74.6	24.8	9.0**-	10.24	5.59
41	61**+	106.0	71.3	---	---	---	---
42	44**-	116.1	60.3	28.8	9.8	10.50	5.45
43	69**+	126.3	65.6	51.2**+	25.6**+	12.62**+	5.67
44	61**+	118.3	59.3	22.2**-	7.6**-	9.62**-	5.43**-
45	40**-	124.6	63.6	43.0**+	15.0	12.14**+	5.73
46	48	144.0	89.0**+	29.2	11.8	11.47**+	6.17
47	45**-	127.0	66.3	47.2**+	13.66	12.41**+	6.23
48	46	122.6	79.6	31.0	11.8	11.88**+	5.83
49	59**+	152.6**+	81.0	42.6**+	16.8**+	11.94**+	6.31**+
50	59**+	132.6	76.6	33.0	17.0**+	12.20**+	5.87
F-test	**	**	**	**	**	**	**
LSD 0.01	5.95	26.51	18.09	5.60	3.52	0.67	0.41
C.V. (%)	5.25	10.19	12.48	9.77	16.84	5.58	6.19

Note : **+ - positively significant difference from mean value

**- - negatively significant difference from mean value

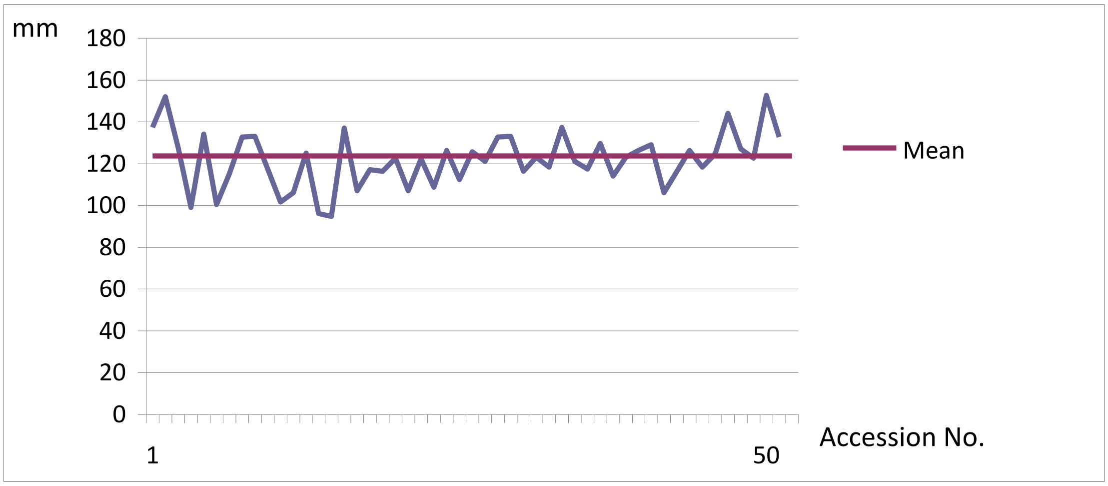


Figure 17. Frequency distribution of terminal leaflet length in collected yardlong bean and cowpea accessions.

1.2.3 Terminal leaflet width

Terminal leaflet width did not showed large variability as a trait between studied accessions. Figure 18 shows that there were significant differences between accessions have just few accessions. The mean value for leaflet width of accessions studied was 68.9 mm. Terminal leaflet width measurements showed that the accession no. 5a (IT82E-9 determinate) has the narrowest terminal leaflet width (42.6 mm) and the broadest terminal leaflet width was observed in accession no. 47, the unknown yardlong bean (99.6 mm). Yardlong bean accessions no. 7 (Tahanpran), 12 (Suranaree) and 46 (unknown) have higher value than the average. Significantly smaller leaflet width of terminal leaflet was recorded in accession no. 5a (IT82E-9 determinate), 6 (IT82E-16), 13 (Cameron), 14 (VIG 009), 17 (SR 863) and 39 (Line) (Figure 18, Table 3).

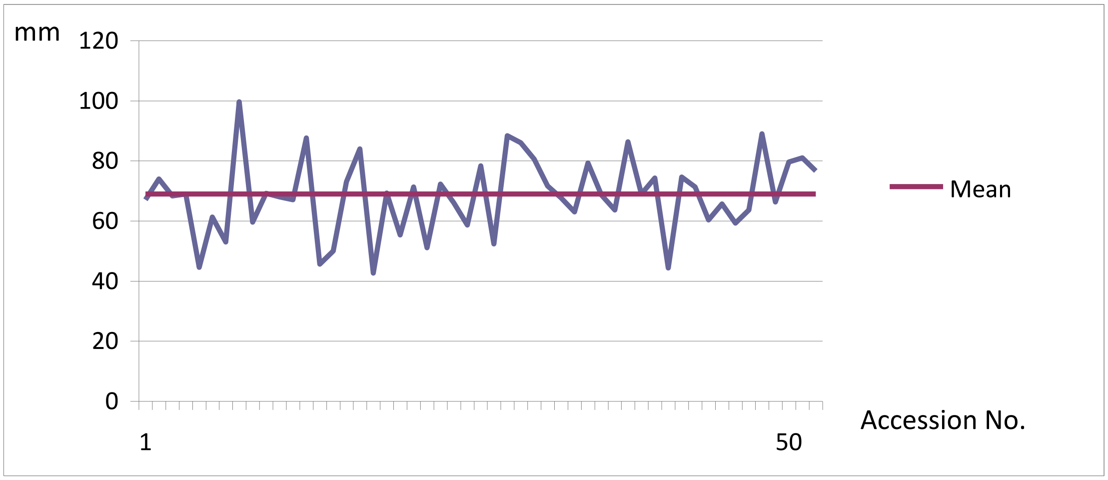


Figure 18. Frequency distribution of terminal leaflet width in collected yardlong bean and cowpea accessions.

1.2.4 Pod length

Mean pod length of each accession was shown in Table 3 . Pod length is one of the necessary agronomic quantitative traits for the studied accessions. Cowpea accession no. 5b (IT82E-9 indeterminate) had the lowest value for the pod length (12.2 cm), while the accession no. 1 (yardlong bean Samchook) had the highest values for the observed characteristic (63.2 cm). Most accessions showed significant deviations, both positive and negative in relation to the average value for the pod length (Table 3 and Figure 19). Yardlong bean accessions no.1 (Samchook), 2 (KU-20), 3 (variety Selected- PSU), 8 (Malaysia 308), 15, 19, 23 and 45 (unknown), 16 (Trang 1), 26 (Dang), 43 (Trang 3), 47, (Ranong) and 49 (Pattani 2) showed significantly positive deviation compared to average value, while four cowpea accessions no. 5a and 5b (IT82E-9), 6 (IT82E-16) and 14 (VIG 009) showed significantly negative deviation to average value . Negative deviation to the average pod length was also observed on the following yardlong bean accessions: accession no. 10 (Yumi), 12 (Suranaree), 20 (Foundsai) and 21 (Teenman), 36 (unknown), 39 (line), 40 (Pran) and 44 (Dang).

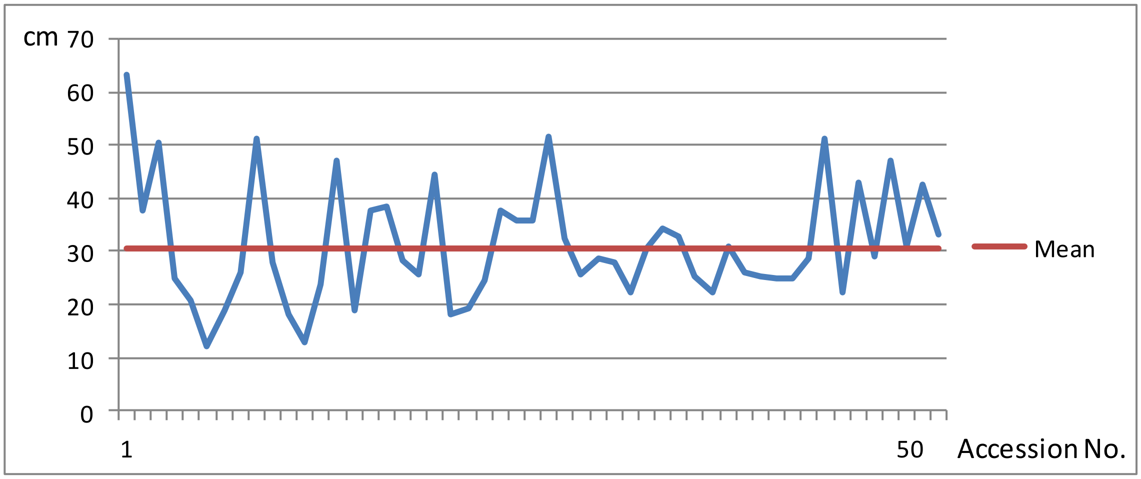


Figure 19. Frequency distribution of pod length in collected yardlong bean and cowpea accessions

1.2.5 Pod weight

The results for the pod weight are presented in Figure 20. The highest and lowest values of the pod weight were recorded in has accession no. 5b (cowpea IT82E-9 indeterminate) (3.4 g) and Samchook (29.2 g), respectively. Weight of pods, in most accessions, was in positive correlated with the pod length. Mean value for pod weight was 12.95 g. F- test shown that there were high significant differences between the accessions for this trait (Table 3).

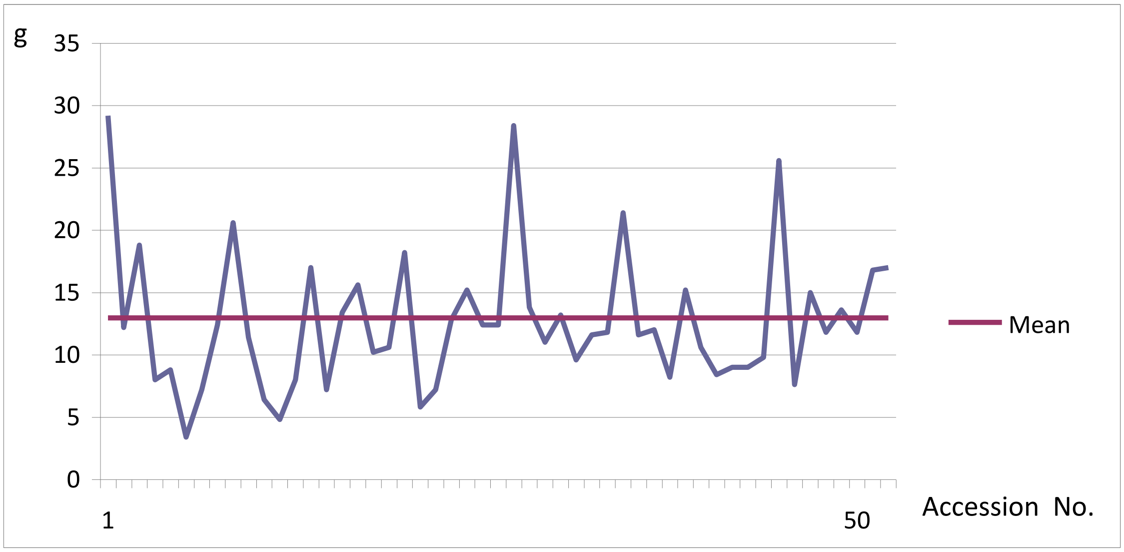


Figure 20. Frequency distribution of pod weight in collected yardlong bean and cowpea accessions

1.2.6 Seed length

The result for seed length of the studied accessions was presented in Figure 21. Seed length very varied positively or negatively in relation to the mean value. Accession no. 1 (Samchook) has a lowest value for seed length trait (7.02 mm). Accession no. 19 unknown from nakorn-sithammarat and no.43 (Trang3) had the highest value for seed length (12.62 mm). Seventeen accessions had significant positive differences compared with average value (accession no. 2, 8, 9, 11, 15, 16,19, 26, 27, 37, 43, 45, 46, 47, 48, , 49 and 50). Negative deviations from the average value (10.57 mm) are as the followings; accessions no. 5a (IT82E-9 determinate) and 5b (IT82E-9 indeterminate), 6 (IT82E-16), 14 (VIG 009). Yardlong bean accessions with negative deviation looking on the average value were accession no. 7 (Tahanpran), 10 (Yumi), 18, 29,30,36,38 (unknown), 20 (Foundsai), 21 (Teenman), and accession no. 44 (Dang) (Table 3).

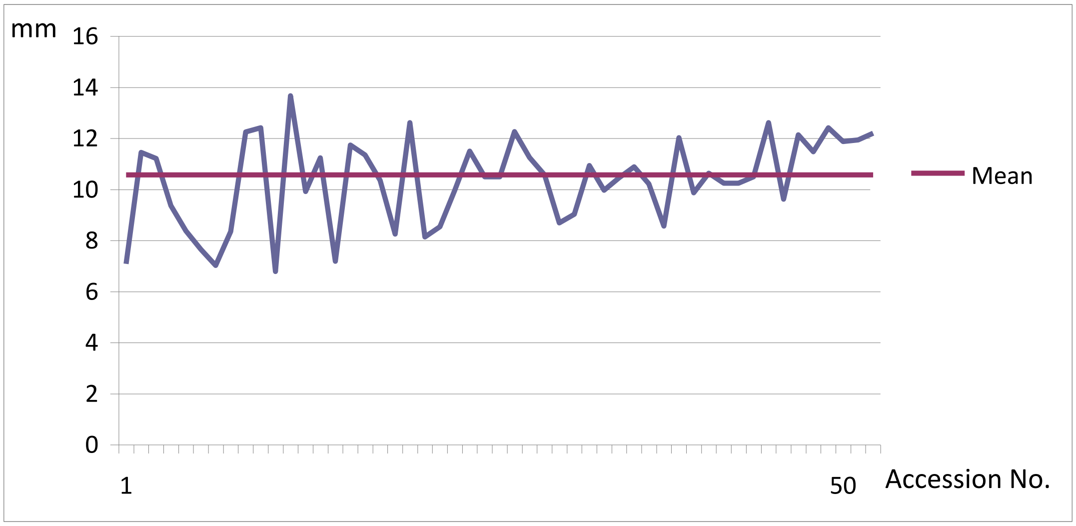


Figure 21 . Frequency distribution of seed length in collected yardlong bean and cowpea accessions

1.2.7 Seed width

Figure 22 showed seed width data for accession studied. Seed width was the highest in accession no. 4 (Chia Tai). The lowest value was recorded on accession no. 5a (IT82E-9 determinate). Significant positive variability related to the mean value (5.84 mm) was recorded for cowpea accessions number 5a (IT82E-9 determinate) and 6 (IT82T), yardlong bean accessions no. 31 (Kampong) 35, 38 (unknown) and 49 (Pattani 2). Negative significant differences were found in yardlong bean accessions no. 23,30, 33,34,36 (unknown), 32 (Founsai), and 44 (Dang). Mean value for seed width was 5.84 mm. F- test showed high significant value for studied accessions for this trait. (Table 3).

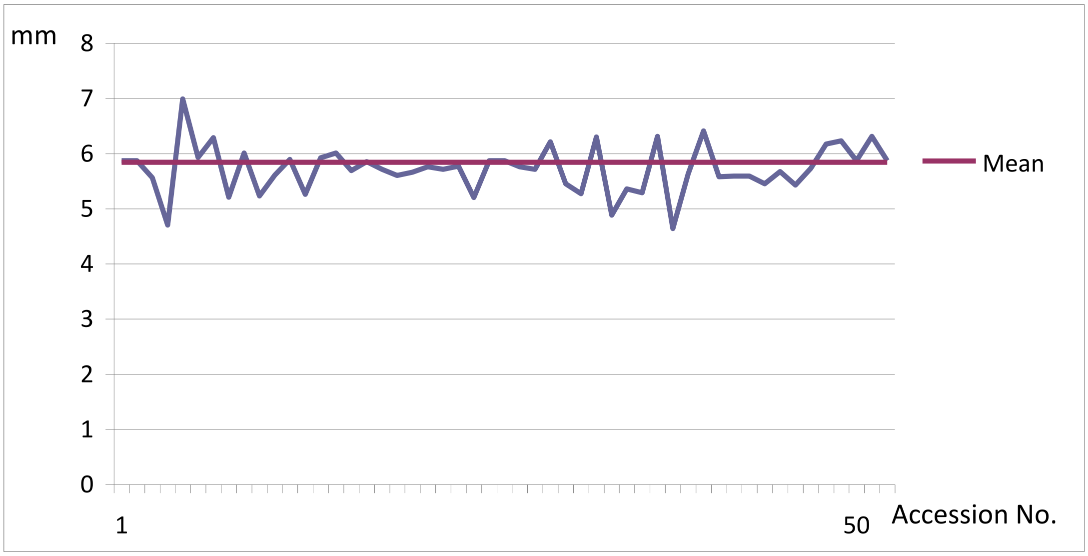


Figure 22. Frequency distribution of seed width in collected yardlong bean and cowpea accessions

2. Screening of BICMV resistant sources in yardlong bean and cowpea

Collected accessions were tested for resistant on predominant virus in this part of the world on yardlong bean and cowpea, on BICMV using ELISA method. Study results were inspected looking on different origin, different genetic constitution, of collected accessions. Table 4 showed the results of plant symptom and results of ELISA test in artificially infected plants of studied accessions. Verified results very varied from accessions to accessions which showed high level of infection in the following accessions: cowpea accession no. 6 (TT82E-16), 8 (Malaysia 308), 14 (VIG 009), yardlong bean accessions no.19, 20, 34, 36, 46 (unknown), 21 (Teenman), 31 (kampong), 37 (Taidang), 39 (line), 40 (Pran), 43 (Trang 3), 44 (Dang), 48 (Pattani 1) and 50 (Pattan 3). The eight following accessions showed no symptom of BICMV but results from ELISA were positive: accession no. 4 (Chai-tai), 9 (Khao-hinson), 13 (Cameron), 18 (unknown from Nakorn-sithammarat), 26 (Dang), 30 (unknown from Trang), 33 (unknown from Nakorn-sithammarat) and 38 (unknown from Khlong Hoi Khong). There were only 2 accessions with no symptom developed and revealed negative ELISA reactions; accession no.16 (Trang 1) and 42 (Taitor). These accessions can be used as sources of BICMV resistance for yardlong bean and cowpea.

Table 4. Evaluation of resistant to BICMV in yardlong bean and cowpea accessions based on visual symptom and ELISA test.

Accessions number	Average absorbency value	ELISA test/symptom
1	0.4955	PS
2	0.4818	PS
3	0.3246	PS
4	0.2685	P
5a	0.4388	PS
5b	0.3822	PS
6	0.5611	PS
7	0.3469	PS
8	0.2962	PS
9	0.1620	P
10	0.2639	PS
11	*	*
12	0.6934	PS
13	0.5167	P
14	0.8393	PS
15	0.3540	PS
16	0.1470	N
17	0.3407	PS
18	0.3435	P
19	0.4333	PS
20	0.6125	PS
21	0.6800	PS
22	0.2498	PS

Table 4 (cont.) Evaluation of resistant to BICMV in yardlong bean and cowpea accessions based on visual symptom and ELISA test

Accessions number	Average absorbency value	ELISA test/ Symptoms
23	0.3390	PS
24	0.2084	PS
25	*	*
26	0.5340	P
27	0.2277	PS
28	0.5007	PS
29	0.3008	PS
30	0.3835	P
31	1.2798	PS
32	0.4659	PS
33	0.4346	P
34	0.4192	PS
35	0.3810	PS
36	0.4763	PS
37	0.5367	PS
38	0.2855	P
39	0.4712	PS
40	0.8083	PS
41	*	*
42	0.1960	N
43	0.3135	PS
44	0.6638	PS
45	0.6385	PS

Table 4. Evaluation of resistant to BICMV in yardlong bean and cowpea accessions based on visual symptom and ELISA test

Accessions number	Average absorbency value	ELISA test/ Symptoms
46	0.4238	PS
47	0.4128	PS
48	0.6237	PS
49	0.6674	PS
50	0.8826	PS

* not determined

P- plant showed no symptom but positive for ELISA test

N- plant showed no symptom and negative for ELISA test

PS- plant showed symptom and positive for ELISA test

Chapter 4

Discussion

Successful conservation of germplasm largely depends on the understanding of the diversity within the species (Karuri *et al*., 2010). Germplasm with broad genetic base provides buffer and resilience against climatic and other environmental changes and ensures sustainable food security. The presence of genetic variability among crop genotypes depicts the richness of the gene pool to assures plant breeders of the possibilities of combating subsequent food security crisis. Understanding the similarity and distance of genotypes within the same species is an important primary knowledge to guide selection and improvement in breeding programs (Adewale *et al.,* 2011). Germplasm or cultivar evaluation is usually based on morphological characters. However, there are several disadvantages of using morphology as a genetic marker: 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 (Nualsri and Konlasuk, 2000). However, morphological characters obtained from field experiment are important information in breeding program.

Accessions of two *Vigna unguiculata* representatives, yardlong bean and cowpea, were studied using morphological markers. Morphological markers have traditionally been employed in establishing phylogenic relationships among genotypes between and within species and for various other purposes including identification of duplicates, studies of genetic patterns, and correlation of characteristics of agronomic importance. Obute (2001) used morphological traits (plant height, number of leaves, leaf length, the number of pods per plant, pod length and number of seeds per pod) to characterize an aneuploid *V. unguiculata* from the other cytotypes. Pasquet (1993) carried out an infraspecific classification on *V. unguiculata* using their morphological traits. Morphological traits of plants can be grouped as either quantitative or qualitative (Magloire, 2005), as it is prescribed in our data. The total of 24 varieties of yardlong bean and 13 varieties of cowpea were characterized for morphological characters and pod yield potential, consuming qualities and pest resistant in field experiment (Sarutayophat *et al*., 2007). But Liu (1999) thought that quantitatives traits express strong environmental effects, and often also genotype with environment interaction. Liu and Furnier (1993) emphasized the fact that many of the morphological traits are also difficult to analyze because they do not have the simple genetic control assumed by many in genetic models.

In the present study, genetic diversity of collected accessions of yardlong bean and cowpea using both qualitative and quantitative characteristics. An overall, relatively high level of dissimilarity was observed among the accessions for most of the morphological characteristics analyzed in our studies. The similar investigation was done by Adewale *et al.* (2010). They concluded that there was high variability among yardlong bean and cowpea genotypes based on phenotypic characters. The same authors concluded that specific variation which differentiate genotypes with respect to some phenotypic characters may have ensured from natural and environmental mutations of the phenotypic traits.

Qualitative characteristics

In our study, some qualitative characteristics varied slightly, like growth habit which were determinate and indeterminate (Figure 6). All cowpea accessions, except accession no. 5b (IT82E-9 indeterminate) have determinate growth habit, while yardlong bean have mostly indeterminate growth habit. Accession 5 was divided for this reason. Plants showed the mixture of both determinate and indeterminate growth habit. Reason can be in not well done seed production of this variety resulted in cross contamination with other indeterminate types, or mechanical mixture in a process of manipulation with accessions. Cobbinah *et al*. (2011) found that growth habit is very important in a cropping system and affected on harvesting of yardlong bean and cowpea. Timko *et al*. (2007) stated that most cowpea accessions have determinate stem growth. Tantasawat *et al*. (2010) looking on qualitative characteristics and found that indeterminate growth was dominant over determinate growth habit.

Plant pigmentation is genetically conditioned. In our study, most of the plants have no pigmentation, that mean they were green. Some accessions have been complete pigmented and all of them were yardlong bean (Figure 7 and Table 2). Most of the studies involved pigmentation of one or the other plant parts at a time, it seems to be several gene expressed for the same trait or similar traits. The pigmentation of whole stem, petiole, peduncle, and pod are independent of the pigmentation on the joints. It has also been observed that whenever the calyx is pigmented, the pod tips are also pigmented and this is independent of other pigmentation (Singh *et al*., 1997). Fery (1985) showed that anthocyanin and melanin like substances are responsible for color in cowpea and the expression of any pigment on the plant is the result of the interaction between several pigment genes and a general color factor. Monogenic control for color expression was found for leaf node pigmentation, flower (petal) color, immature pod color, seed coat color, seed eye color and seed eye color pattern.

Some of qualitative characteristics, like flower color, immature and mature pod pigmentation (Figure 8, 10, 11) showed a high level of variation in the present study. A large percentage of accessions with a violet flower color can be partly explained from genetic point of view, by dominant violet color as inheritance of this trait (Sangwan and Lodhi, 1998). Branca and La Malfag (2008) also found that flower color varies from white to rose and lilac, grouped in two or three on long pedicels. Agbicodo (2009) describe yardlong bean and cowpea flowers as conspicuous, mostly self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple in color. Figure 6 shows that our accessions had violet, white/violet and white color. Singh *et al*. (1997) published in their study that flower color of *Vigna* range from white, to cream, yellow, mauve or purple.

There is no emphasis in breeding cowpeas for the shape of their leaves, leaf shape is important for classifying and distinguishing cowpea varieties. The shape of the leaves may also be potentially useful as a morphological or physical marker used during the selection process if it is closely linked with an agronomic trait of interest. Interestingly, many wild cowpea relatives have the narrow or hastate leaf shape whereas most cultivated varieties of cowpea have the more common ovate or subglobose leaf shape (Potorff *et al*., 2012). Terminal leaflet shape has four forms in our investigation; hastate, sub-hastate, ovate and sub-ovate (Figure 9 and Table 2). Morphological plasticity as reported by Austin (1997), cited by Karuri *et al*. (2010) stated that the variation in the morphological characters of genotypes which are phenotypically similar is due to genetic differentiation especially in the presence of varying conditions and parallel evolution. The USDA, which houses cowpea accessions from 50 countries, classified cowpea leaf shapes into five categories; globose, hastate, sub-globose, sub-hastate, strip and ovate-lanceolate (Pottorff *et al*., 2012).

Pods of yardlong bean and cowpea containing seeds vary in size, shape, color and texture. Pods are cylindrical and may be curved or straight, various colors including white, cream, green, buff, red, brown, and black (Timko *et al*., 2007). Pod curvature may be of beak weak, medium, strong. In our study, we recovered three types of pod curvature: strait, slightly curved and curved. Duke (1987) refered that pod can be curved, straight or coiled, which is consistent with our results. The cyclindrical pods are often curved, 20 - 35 cm long and pale green according Branca and La Malfag (2008).

Immature pod pigmentation showed high level of variability (Figure 11). Approximately more than a half of accessions have green color immature pod (no pigmentation) while 21% of accessions were uniformly pigmented pod (violet or purple). The rest of accessions have pigmented valves, splashes of pigmented, pigmented tip and pigmented satures immature pods. Similar investigation was done by Cobbinah *et al*. (2011). Their results are in accordance with results showed in Figure 11. The general trend, of their results is that 50.7% showed no pigmentation, 14.2% of the immature pods were pigmented at the tips while 9% of the accessions were uniformly pigmented. Fourteen percent of accessions have immature pods with pigmented at the valves as well as those with pigmented suture and splashes of pigmented. Mustapha and Singh (2008) revealed that pigmentation is dominant over non-pigmentation.

Seed shape is a major characteristic correlated with seed development in the pod. Seeds develop a kidney shape if not restricted within the pod. When seed growth is restricted by the pod, the seed becomes progressively more globular (Davis *et al*., 1991). Seeds can be kidney or egg shaped, spherical or rhomboid. Seed may also be speckled or patterned looking on literature data. In collected accessions, in our study, predominant was kidney seed shape, than ovoid, rhomboid and globose. Only cowpea accessions have rhomboid seed shape (Figure 14). According to Rao *et al*. (2006) seeds are variable in shape, globular to kidney-shaped.

Eye color in collected accessions was mostly brown and black. Just three accessions have tan brown eye color (Figure 13). Timko *et al*. (2007) observed that seeds of well-known cowpea types, such as “blackeye pea” and “pinkeye,” are white with a round irregular-shaped black or red pigmented area encircling the hilum, giving the seed the appearance of an eye. Eye color is qualitative trait which is genetically controlled, by major genes (Quinn and Myers, 2002). Presence of pigment was dominant over absence of pigment and the black seed eye was dominant over brown eye. For seed eye pattern however, partial dominance of the very small eye type over the Holstein eye type was observed (Padi, 2003). These results are in agreement with analysis results in collected accessions. Drabo *et al*. (1988) reported that three (W, H, O) and five (R, P, B, M, N) major genes control eye pattern and seed coat color, respectively in *Vigna unguiculata.*

Quantitative characteristics

The study of relationships among quantitative characteristics is important for assessing the feasibility of joint selection of two or more traits and hence for evaluating the effect of selection for secondary traits on genetic gain for the primary trait under consideration. A positive genetic correlation between two desirable traits makes selection easy for improving both traits simultaneously. A negative correlation between two desirable traits makes it impossible to achieve significant improvement in both traits (Ezeaku and Mohammed, 2006).

Results of our study link with earliest characteristic showed that accession need 39 days to flowering, and latest 69 days. Average value was 51 days (Figure 16). Results we got showed highly singnificant differences compared with the average value. Cowpea accession number 14 (VIG 009 from Serbia) had a short period for flowering in Serbia (Mikic *et al*., 2010). However, in the present study, this accession took more days to flower. It can be explained by the influence of geographic location of Thailand compared to Serbia. A day to flowering is a component of the adaptation of a variety to a particular environment and it also determines pod set and crop yield (Ishiyaku *et al*., 2005). Plant growth and development, especially flowering, is dependent on the interaction of many complex processes which are influenced by both genetic and environmental factors (Uarrota, 2010). Mukhtar and Singh (2006) reported that in West and Central Africa, photoperiod is the most important environmental variable affecting time of flowering and that most cowpea varieties under cultivation are unimproved, local types which are photoperiod sensitive. Days to flowering is very important trait because it is in positive correlated with maturity date (r=0.52) (Negri *et al*., 2000). Adeyanju and Ishiyaku (2007) have the same opinion and they indicated earliness in cowpea (*Vigna unguiculata* (L.) Walp) as an important agronomic trait since it has been reported to yield the dividends of opening the possibility of successful sole cropping in areas with short rainy season, double/triple cropping in rice and/or wheat based systems. If the genetic basis of early and continuous flowering is understood, it can be exploited in the development of cowpea varieties that can flower and pod continually thereby ensuring all year round availability of cowpea for the teaming population in sub Saharan Africa (Manggoel and Uguru, 2012). Looking on Mahalakshmi *et al*., (2006) data, early flowering was associated with small leflets, fewer pods and fewer seeds per pod, and this association was retained in the core but the magnitude was lower. These data are in agreement with results obtained in our research, looking on terminal leaflet length and width, pod weight traits.

Terminal leaflet length and terminal leaflet width were the characteristics with the lowest variation between collected accessions in our study. Commonly, the terminal leaflet is longer and larger than the lateral leaflets (Davis *et al*., 1991). Peksen *et al*. (2005) concluded that leaf shape is useful in classification and identification. Size of terminal leaflet is important for perform photosynthesis. If they have larger area possibility for higher value for photosynthesis are better. Assimilates from photosynthesis are the main source of C from N_2 fixation in grain legumes and reduction in assimilated C causes a reduction in N_2 fixation. It is possible that the reduction in the net photosynthetic rate caused the 37% reduction in N and the 21%reduction in total plant N, since greater decreases in N_2-fixation can occur with small decreases in assimilation of CO_2 in soybean (Abdelhamid *et al*., 2011).

Yardlong bean, which is grown in Southern Thailand, produced a relatively low pod yield because of unfavorable environmental conditions, such as heavy rainfall. Therefore, it is desirable to improve new varieties with high adaptability to such unfavorable environmental conditions. Sarutayophat and Nualsri (2010) concluded that for yield components, significant differences were found in almost all characters in the 4,501 population, notably the number of pods/ plant, pod weight, pod length, pod diameter and the number of inflorescence/plant. Nualsri and Benchasri (2009) showed that highly differences were founded in the following characters; pod length, number of pods/plant and pod yield/plant. In our study pod length and pod weight were quantitative characteristics with highest variability, beside days to flowering.

Yardlong bean is characterized by its very long and succulent pods (30 – 90 cm in length), where a cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*, *unguiculata* Group and Biflora Group) has shorter pods (20 - 30 cm in length) (Verdcourt, 1970). Average pod length in studied accessions was 30.4 cm, which is in accordance with Benchasri and Bairaman (2010). They found that variety Selected- PSU have significant longest pods (53 cm), our result was 50.6 cm. Sarutayophat *et al*. (2007) also got pod length for variety Selected- PSU 57.1 cm, and also reported pod length for IT82E-16 was 15.9 cm. In our experiment, pod lenght of IT82E-16 was 19.0 cm. Negri *et al*. (2000) estimated the inheritance and the correlation of quantitative characters and analyzed in 24 genetypes of yardlong bean (*V. sesquipedalis* (L.) Fruw.). They found that yield per plant was positively correlated with number of pods per plant (r= 0.70) and pod length (r= 0.59). In

Babaji *et al.* (2011) investigation, pod length and pod yield/ha were influenced by variety and residual of rates of farmyard manure. Environmental conditions had little or no effect on pod length in cowpeas (Addo-Quaye *et al*., 2011). Fery (1985) showed that pod length was highly heritable with average heritability estimate of 75.2 %. High heritability and genetic advance for this character is very much fruitful during selection program. Dharmalingam and Kadambavanasundaram (1989) reported high variability in pod length. Some reports indicated that high estimates of both heritability and genetic advance.

Yield of vegetable pods per plant recorded the highest phenotypic and genotypic coefficients of variation followed by number of pods per plant and pod weight. These information suggested the scope for improvement of these characters through selection (Vidiya *et al*., 2002). Pod weight was inherited quantitatively, and in the present study, this characteristics investigated as an important indicator of genetic variability. Pod weight ranged from 8.0 to 28.4 g in our studies. These data showed a large variability between studied accessions. There was high significant correlation between pod length and pod weight. Pod weight exerted positive indirect effect pod length and pod girth and negative indirect effect via number of pods per plant. Both pod weight and number of pods per plant had high direct effect along with high genotypic correlation (Hazra *et al*., 1994). Several studies identified pod weight as one of the major contributors to pod yield (Sobha, 1994; Chattopadhyay *et al*., 1997, Resmi, 1998). The study of Vidya and Oommen (2000) indicated that number of pods per plant and pod weight should be given due importance in selection programs for yield improvement in yardlong bean since these characters recorded significant genotypic correlation with high direct effect on pod yield.

Cowpea has relatively small dicotyledonous ranging in size 2 -28 g per 100 seeds. The dimensions of cowpea seed range from 2- 12 mm in lenght, 6.6 mm in width and 4.4 – 4.9 mm in thickness (Olubusayo, 2010). In our study, seed length varied from 7.08 mm on accession number 1(Samchook) to 12.62 mm on accession number 19 (unknown from Nakorn-sithammarat) and 43 (Trang 3). This characteristics was one of the highest variability in quantitative characteristics in our study. Seed width ranged from 4.7 mm in accession number 36 (unknown from Nakorn-sithammarat) to 6.9 mm, in accession no. 5a (IT82E-9 determinate). There was no large variability for this characteristic. Seed weight was inherited quantitatively and small seed was partially dominant to large seed size. Gene action was predominantly additive, but dominance and additive × additive epistatic effects were also significant (Drabo *et al*., 1984).

Screening of Blackeye Cowpea Mosaic Virus* (BICMV) *resistant sources in yardlong bean and cowpea

Breeding program to provide resistance against BICMV is essential for the production of both yardlong bean and cowpea. The source of the BICMV resistant genes from *Vigna unguiculata* germplasm, or from native specices must be evaluated. This study aims to evaluate the BICMV resistance in yardlong bean and cowpea that are collected from various sources in southern Thailand and will be served as parental lines for resistance to BICMV in yardlong bean breeding. In our investigation, we evaluated BICMV infection by symptoms and ELISA. ELISA technique is currently popular because it is easy, quick, precise detection and can be used to determine the large amounts of samples in short period (Katoch *et al*., 2003). ELISA method can be used both mono- and polyclonal antibodies that specific to viral disease. (Porta *et al*., 1989; Wahyuni *et al*., 1992; Hsu *et al*., 2000; Haggag *et al*., 2009). Eventhough, monoclone antibody has more specific to virus higher than polyclonal antibody. However, the large quantities of polyclonal antibody are relative quick and inexpensive to produce compared to monoclonal antibody. Since polyclonal are non specific in that they are capable of recognizing multiple epitopes on any one antigen. In the present study, polyclonal antibody was used.

Individual plant was considered susceptible if it developed BICMV symptom and gave positive ELISA reaction or no symptom of BICMV developed but ELISA tests indicated the presence of BICMV. If no symptom developed and ELISA reaction was negative, plant was considered as resistance. Results from ELISA were not correlated with the visual symptom rating. Many of plants did not show symptoms but gave positive ELISA reaction even in plants belong to set of controls (plants with no inoculation) such as accession no. 4, 9, 13, 18, 26, 30, 33 and 38. It may be explained by aphid or seed transmission. BICMV can be transmitted through many aphid species in a non-persistent manner, and through the seed of cowpea or yardlong bean (Atiri *et al*., 1986). Although all plants have grown under the screenhouse, but some aphids were observed on plants. Symptoms and ELISA in controls were absolutely negative in the following; accession no. 6 (IT82E-16), 9 (Khao-hinson), 30 (unknown from Nakorn-sithammarat) and 6 (Trang). Only in cowpea IT82E-16, artificially infected plants have high level of infection. Those are in accordance with Puttaraju and Santhosn (2000) results. They found that cowpea varieties inoculated with BICMV at the primary leafs showed 92 - 100% infection at the first trifoliate leaves.

Results from the present experiment showed that from 50 accessions of yardlong bean and cowpea evaluated for BICMV resistance, only accessions no. 16 (Trang 1) and no. 42 (Taitor) were resistance to BICMV. Bashir *et al.* (2002a) evaluated cowpea germplasm accessions (local and exotic) for BICMV resistance under greenhouse conditions by sap inoculation. They reported that out of 134 accessions, only two were found to have resistance to BICMV. Results from the present studies indicated two resistant accessions (Trang1 and Taitor) can be used as sources for further breeding yardlong bean and cowpea resistant to BICMV.

Chapter 5

Conclusions

Observed morphological makers of 50 accessions of yardlong bean and cowpea collected mostly in southern Thailand showed large differences in morphological, both qualitative and quantitative characteristics, as well as some of that resistance or susceptibility on BICMV.

In qualitative characteristics, the high variability was observed on immature and mature pod pigmentation characteristics. The lowest variability was found on growth habit. Moderate variability, compared to the previous two, shown on terminal leaflet shape, plant pigmentation, flower color, eye color, pod curvatureand seed shape. It is not possible to mark any of studied accessions as sutitable for some breeding program. It is depended on characteristic design by breeders and their objectives. As a positive result we can offer accessions with high variability in qualitative characteristics.

Quantitative characteristics are linked with yield, and from that point of view result we got have high value. Looking on studied characteristics, high variability was observed on the following characteristics; days to flowering, pod length, pod weight and seed length. The terminal leaflet length and terminal leaflet width showed low level of variability.

Verified results we have got by artificial infection of young plants with BICMV very varied from accession to accession. Based on visual symptoms and ELISA test, all accessions were susceptible to BICMV except 2 accessions, Trang 1 and Taitor that showed high resistance to BICMV. These two resistant accessions will be used for as sources for breeding yardlong bean and cowpea resistant to BICMV.

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