Evaluation of Germplasm and Comparison between Pedigree and Single Seed Descent Methods in Yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc)

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ชื่อวิทยานิพนธ์	การประเมินเชื้อพันธุกรรมและเปรียบเทียบวิธีการคัดเลือกพันธุ์ระหว่างวิธีสืบประวัติและหนึ่งเมล็ดต่อต้นในถั่วฝักยาว
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บทคัดย่อ

การทดลองเพื่อศึกษาลักษณะสัณฐาน ศักยภาพการให้ผลผลิตและความสัมพันธ์ทางพันธุกรรมระหว่างถั่วฝักยาวและถั่วพุ่ม จำนวน 37 พันธุ์ เพื่อกำหนดพันธุ์พ่อแม่และเปรียบเทียบประสิทธิภาพการคัดเลือกพันธุ์ระหว่างวิธีสืบประวัติและหนึ่งเมล็ดต่อต้น สำหรับการปรับปรุงพันธุ์ถั่วฝักยาวโดยปลูกถั่วฝักยาว จำนวน 24 พันธุ์ และถั่วพุ่มจำนวน 13 พันธุ์ ในแปลงทดลอง ใช้แผนการทดลองแบบสุ่มภายในบล็อก (RCBD) จำนวน 2 ซ้ำ จดบันทึกลักษณะต่าง ๆ เช่น ลักษณะการเจริญเติบโต อายุออกดอก ผลผลิต องค์ประกอบผลผลิต คุณภาพการบริโภค และความทนทานต่อแมลงศัตรู เป็นต้น ผลการทดลองพบว่าถั่วฝักยาวและถั่วพุ่มที่ใช้ในการทดลองให้ผลผลิตฝักสดต่อต้น จำนวนฝักต่อต้น และความยาวฝักแตกต่างกันทางสถิติที่ระดับความเชื่อมั่น 99% และพบว่าถั่วฝักยาวจำนวน 22 จาก 24 พันธุ์ มีการเจริญเติบโตแบบทอดยอด ในขณะที่ถั่วพุ่มจำนวน 10 จาก 13 พันธุ์ มีการเจริญเติบโตแบบไม่ทอดยอด เดนโดรแกรม (dendrogram) ที่สร้างจากแถบดีเอ็นเอที่มีความแตกต่างกันจำนวน 23 แถบ โดยใช้เทคนิค RAPD จากไพรเมอร์ 5 ชนิด (OPC-06, OPR-12, OPZ-03, OPZ-08 และ OPZ-13) สามารถแยกกลุ่มความสัมพันธ์ระหว่างถั่วฝักยาวและถั่วพุ่มออกจากกันได้ ข้อมูลที่ได้จากการปลูกทดสอบในแปลงและจากผลการวิเคราะห์ความสัมพันธ์ทางพันธุกรรม คณะผู้วิจัยได้คัดเลือกถั่วฝักยาวพันธุ์ VU162 เป็นพันธุ์แม่ และ VU171 และ VU189 เป็นพันธุ์พ่อแล้วผสมพันธุ์สร้างลูกผสมชั่วแรก จำนวน 2 คู่ คือ คู่ผสม 4501 (VU162 x VU189) และคู่ผสม 4502 (VU162 × VU171) ปลูกลูกผสมชั่วแรกให้ผสมตัวเอง 1 ครั้ง ได้ลูกผสมชั่วที่ 2 จำนวน 2 ประชากร เพื่อใช้เป็นแหล่งความแปรปรวนสำหรับการคัดเลือกพันธุ์และเปรียบเทียบประสิทธิภาพการคัดเลือกระหว่างวิธีสืบประวัติและหนึ่งเมล็ดต่อต้น หลังจากการคัดเลือกจำนวน 2 ชั่วรุ่นแล้วจึงนำลูกชั่วที่ 4 (F_4 progenies) จำนวน 30 สายพันธุ์ต่อประชากร (จำนวน 15 สายพันธุ์ต่อวิธีการคัดเลือก) ไปปลูกทดสอบผลผลิตที่ศูนย์วิจัยพืชไร่สงขลา จังหวัดสงขลา ในปี พ.ศ. 2547 โดยใช้แผนการทดลองแบบสุ่มภายในบล็อก จำนวน 3 ซ้ำ ผลการทดลองพบว่าสายพันธุ์ชั่วที่ 4 ที่ได้จากการคัดเลือกโดยวิธีสืบ

ประวัติและหนึ่งเมล็ดต่อต้นให้ผลผลิตและองค์ประกอบผลผลิตไม่แตกต่างกันทั้ง 2 ประชากร คือ พบว่า วิธีการคัดเลือก 2 วิธีดังกล่าวมีประสิทธิภาพในการปรับปรุงผลผลิตถั่วฝักยาวไม่แตกต่างกัน ดังนั้นวิธีหนึ่งเมล็ดต่อต้นจึงเป็นวิธีคัดเลือกพันธุ์ถั่วฝักยาวที่เหมาะสมกว่าวิธีสืบประวัติ เพราะว่าวิธีหนึ่งเมล็ดต่อต้นสามารถประหยัดเวลาและค่าใช้จ่ายได้มากกว่า เนื่องจากถั่วฝักยาวเป็นพืชที่ทยอยออกดอกและฝักทยอยสุกแก่ ดังนั้น การเก็บเมล็ดจากเฉพาะฝักแรกของแต่ละต้นในวิธีหนึ่งเมล็ดต่อต้นโดยไม่ต้องรอจนกระทั่งฝักส่วนใหญ่ในแต่ละต้นสุกแก่ จึงเป็นวิธีที่เหมาะสมกว่า การทดสอบความก้าวหน้าจากการคัดเลือกพันธุ์โดยทดสอบสายพันธุ์ชั่วที่ 4 อาจไม่ได้ผล เนื่องจากสายพันธุ์ชั่วที่ 4 ยังไม่คงตัวทางพันธุกรรม ควรคัดเลือกสายพันธุ์ไปจนถึงชั่วที่ 6 หรือมากกว่านั้น แล้วจึงค่อยทดสอบความสามารถของสายพันธุ์จะดีกว่า แต่หากจำเป็นต้องทดสอบเพื่อค้นหาสายพันธุ์ดีเด่นในชั่วแรก ๆ ควรเพิ่มการทดสอบให้มากกว่าปกติ อย่างไรก็ตามการทดลองในครั้งนี้พบว่าสายพันธุ์ชั่วที่ 4 ที่ดีที่สุดและที่ดีที่สุด 3 สายพันธุ์แรก (the three top) ที่ได้จากการคัดเลือกโดยวิธีสืบประวัติและหนึ่งเมล็ดต่อต้นจากทั้ง 2 ประชากรให้ผลผลิตฝักสดสูงกว่าค่าเฉลี่ยของพ่อแม่และพันธุ์เปรียบเทียบ ผลการวิเคราะห์สหสัมพันธ์ระหว่างลักษณะพบว่า จำนวนฝักต่อต้นมีสหสัมพันธ์ทางบวกกับผลผลิตฝักสดมากที่สุดทั้ง 2 ประชากร คือ มีค่าสหสัมพันธ์ระหว่างกันเท่ากับ 0.7540** และ 0.9229** ในคู่ผสม 4501 และ 4502 ตามลำดับ และพบว่าอัตราพันธุกรรมอย่างแคบสำหรับผลผลิตฝักสดในทั้ง 2 ประชากรค่อนข้างต่ำมาก คือ เท่ากับ 2.64 และ 1.69% ในคู่ผสม 4501 และ 4502 ตามลำดับ

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ABSTRACT

This study was conducted to characterize morphological traits, yielding potential, investigate genetic relatedness among 24 yardlong bean and 13 cowpea accessions for parental varieties and to compare the effectiveness between pedigree (PS) and single seed descent (SSD) methods for yardlong bean improvement. Twenty four yardlong bean and 13 cowpea accessions were planted in the field with two replications in a randomized complete block design (RCBD). Growth habit, days to flowering, pod color, pod length, number of pods/plant, yield/plant, consumption quality and tolerance to insect pests were recorded. The results showed that highly differences were found in the following characters; pod length, number of pods/plant and pod yield/plant. Twenty two of 24 yardlong bean accessions exhibited indeterminate growth habit while 10 of 13 cowpea accessions had determinate growth habit. A dendrogram based on 23 RAPD polymorphic fragments obtained from 5 primers (OPC-06, OPR-12, OPZ-03, OPZ-08 and OPZ-13) revealed fairly good separation of groups between yardlong bean and cowpea. Based on morphological characters and genetic relatedness, VU162 was chosen as a female parent while VU171 and VU189 were used as male parents. Crossing were made between VU162 × UV189 (cross no. 4501) and VU162 x VU171 (cross no. 4502) to produce two F_1 hybrids. F_1 hybrids were self-pollinated and two segregated F_2 populations were used as sources for yardlong bean improvement. The effectiveness between 2 selection methods; pedigree and single seed descent was studied. Thirty F_4 progenies and the parents of each population were tested in separately experiments with two check cultivars in 2004 at the Songkhla Field Crop Research Station, Songkhla Province. The RCBD with three replications were used. Results indicated that no

significant difference was found between PS and SSD for pod yield and yield components in two crosses of yardlong bean. This study revealed equally effective of SSD and PS methods for pod yield improvement in yardlong bean. However, the SSD was preferred since it was economical for time required and cost effectiveness in handling segregating generations. The SSD method need less time and cost to achieve homozygous lines. Since yardlong bean is indeterminate growth habit, harvesting only the first pod from each plant without waiting for complete pod maturity would be a very benefit method. The results obtained from this study indicate that genetic advances in yield and yield components of F_4-based yardlong bean progenies from PS and SSD method were not effective. F_4 is still very heterogenic and will be segregating in the subsequent generations, at least F_6 or more generation should be performed. Early generation testing is effective in identifying superior pure lines, but requires extra yield testing. However, the best and the three top F_4 progenies derived by PS and SSD of both populations produced higher pod yield than the mean parent and check cultivars. The number of pods per plant showed the highest positive correlation with pod yield in both populations with correlation coefficients (r) of 0.7540** and 0.9229**, respectively. Low narrow-sense heritability for pod yield was recorded in the 4501 and 4502 populations (2.64 and 1.69%, respectively).

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Teerawat Sarutayophat

CONTENTS

LIST OF TABLES

LIST O F TABLES (CONT' D)

LIST OF FIGURES

INTRODUCTION

Yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc.) is known as vegetable cowpea, asparagus bean, string bean, snake bean, snake pea, snap pea, bodi, bora and sitao. Its origin is possibly in the Middle West Africa or in Southern China. Yardlong bean is widely grown in Southeast Asia, South China and West Africa for immature pods which are used as a vegatable. Yardlong bean is one of the economically important vegetable crops in Thailand. Production area of yardlong bean in Thailand is about 18,560-20,160 ha annually. Cowpea (*Vigna unguiculata* ssp. *sinensis*) is one of the important food grain legumes in all tropical areas, particularly in Africa. It is cultivated on at least 12.5 million hectares, with an annual production of over 3.0 million tons worldwide (Fana *et al*., 2004). It is usually grown intercropped with sorghum or millet and also in rotation cropping system. In Thailand, yardlong bean is considered have relatively low pod yield productivity and stability. It is quite sensitive to unfavorable environmental conditions such as high temperature and dry weather, too cloudy sky or heavy rain, and susceptible to various diseases and insect pests. Therefore, it is desirable to develop a new better adaptable and productive variety.

Evaluation of collected germplasm is the first important procedure in breeding programs. Morphological information from evaluated trials is useful for utilization of germplasm. However, there is concern that plants developed using such information will be further affected by environmental modifications (Dijkhuizen *et al*., 1996; Nualsri and Konlasuk, 2001). Therefore, molecular markers are used to enhance utilization of germplasm collections. Many molecular markers may provide useful information in utilization of germplasm. Among these markers, random amplified polymorphic DNA (RAPD) offers several advantages for identification of genetic variability at the DNA level (Liu, 1996; Nualsri and Konlasuk, 2000).

Selection after hybridization or induced genetic variation is also important procedure in breeding programs. There are many selection methods for self-pollinated crops, such as bulk, pedigree, single seed descent, early generation testing etc,. One method may have advantages and disadvantages compared to the others. Pedigree selection method has been widely used for handling selected superior progenies at each segregating generation. Obviously genetic variability during succeeding generations by this method is reduced so the breeding potential of

the parents on stable progenies may not be fully exploited. The single seed descent is a method of handling a segregating population without selection until the desired level of homozygosity is achieved, then, lines selection is begun. However, selection on a single plant basis can be practiced during any generation of single seed descent. The effectiveness of selection methods in any particular trial are considered based on genetic variability and heritability of characters. Therefore, breeders must evaluate the effectiveness of different selection methods when they choose selection procedures for particular crops.

The objectives of this study were; 1.) to characterize agronomic traits and yielding ability and to determine the degree of genetic similarity among a total of 37 yardlong bean/cowpea accessions using RAPD markers and choose the superior to use as parental varieties/lines, 2.) to compare the effectiveness of two selection methods, single seed descent and pedigree selection for yield and yield components of two yardlong bean populations, 3.) to estimate heritability and correlation coefficient among yield and yield components, and 4.) to produce and test superior F_4 progenies for future breeding program.

LITERATURE REVIEW

Yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis* (L.) Verdc) (Tindall, 1983; Kulkarni and Birari, 1999; Ponce and Casanova, 1999) belongs to the Leguminosae family. It is one of three subspecies of cowpea, the other two subspecies are cowpea or common cowpea (*Vigna unguiculata* ssp. *sinensis*) and catjian cowpea (*Vigna unguiculata* ssp. *unguiculata*) (Tindall, 1983; Lerd-anantragul, 1979; Santhadphanich, 1987). All the subspecies have the same chromosome number (2n = 2X = 22) (Knott and Deanon, 1967; Bounnhong, 1997). Yardlong bean is also known as asparagus bean, string bean, snake bean or vegetable cowpea (Purseglove, 1977; Quan, 1996). The origin of yardlong bean is possibly in the Middle West of Africa or in the Northeastern part of Yuanan Province in Southern China (Purseglove, 1977; Quan, 1996; Bounnhong, 1997). It is a highly self-pollinating annual crop with a climbing vine. Natural crossing between plants in a row is less than 1% (Sitathani, 1977). Yardlong bean is widely grown in Southeast Asia, South China, Central and West Africa for the immature pods which are used as vegetables. Pod quality is judged on the basis of pod colour and length; desirable qualities differ in different markets. For instance, Thailand and Hong Kong prefer light green and extra long pods, Brunei prefers dark green, short pods, while European and Canadian markets prefer dark green, and medium pod length (Bounnhong, 1997). It exhibits vigorous growth in a warm climate. Optimum average temperature during the growing period is 20 °C to 30 °C (Santipracha and Santipracha, 1994). It prefers full sunshine during growth and development, whereas cloudy and rainy weather cause low yield due to flowers and young pods dropping. It can be grown in various soil types, from sandy loam to clay, but loam and sandy loam with pH 6.2-7.0 are the best for yardlong bean production (Bounnhong, 1997).

1.1 Germplasm evaluation

As other crops, yardlong bean breeding program comprises of four important procedures. For the first step, breeders must collect and evaluate germplasm materials to select the parental lines or cultivars. Germplasm relationships can be evaluated base on morphological characters or molecular markers. Second step, making crosses between parental lines to produce

genetic variability population as materials for selection. Third step, selection of desirable recombinant genetic lines. Breeder must consider which effective selection procedure should be used in their breeding program. Evaluation of selected lines finding for best lines use as new elite varieties was the fourth or last step in any breeding program.

1.1.1 Germplasm evaluation by morphological characters

Germplasm or cultivar evaluation is usually based on morphological characters. However, there are several disadvantages of using morphology as 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 (Dijkhuizen *et al*., 1996; Nualsri and Konlasuk, 2000).

1.1.2 Germplasm evaluation by molecular markers

In recent year, many types of molecular markers have been used to observe variation directly at the DNA level (Nualsri and Konlasuk, 2000). Their application includes the analysis of segregating populations, multiple traits screening, selection for resistance to pest and disease, cultivar identification, germplasm characterization and estimation of genetic relatedness etc. (Langridge *et al*., 1999).

There are two basic techniques used in molecular marking, the Southern blot analysis and the polymerase chain reaction (PCR). The PCR-based technique is also the basis of several other techniques via some modification, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeated (SSR) or microsatellite, simple sequence length polymorphism (SSLP), etc. These various molecular markers have different advantages and disadvantages based on many factors such as cost, reliability, simplicity and time requirement.

RAPD markers have demonstrated their usefulness as genetic markers for a variety of eukaryotic organisms. RAPD are polymorphic DNA sequences separated by gel electrophoresis after polymerase chain reaction (PCR) amplification using random oligonucleotide primers. If these priming sites are within an amplifiable distance of each other, a discrete DNA product is produced through thermocyclic amplification (Nualsri and Konlasuk, 2000). RAPDs

are generally dominant markers and are difficult to transfer between species due to their random nature and short primer length. (Langridge *et al*., 1999; Tragoonrung *et al*., 2001). However, RAPD analysis offers several advantages such as 1) only small amounts of DNA are required, 2) no prior DNA sequences are needed, 3) no radioactive involvement and 4) the technique is simple and not too expensive. The technique has been used as a tool to generate molecular markers for several species including yardlong bean (Phansak *et al*., 2001), *Lansium domesticum.* (Konlasuk *et al*., 2001), broccoli and cauliflower (Hu and Quiros, 1991), sago palm (Hisajima and Boonsermsuk, 1997).

1.2 Genetic studies in yardlong bean and cowpea

Today yardlong bean is widely cultivated in tropical Asia, especially in Southeast Asia and South China. Immature pods are produced in Southeast Asia, particularly in Indonesia, Malaysia, Philippines and Thailand, yielding approximately 3.5, 15.0, 1.1 and 12.0 t/ha, respectively (AVRDC, 1993). In China, immature pod yield varies from 9.0 t/ha to 30.0 t/ha (Quan, 1996). Yardlong bean is quite sensitive to unfavorable environmental conditions such as too hot and dry, too cloudy or heavy rain, and susceptible to various diseases and insect pests. Seedborne diseases are usually a serious problem in yardlong bean/cowpea production. Among seedborne diseases, cowpea aphid-borne mosaic viruses (CABMV) such as the cucumber mosaic virus (CMV) and blackeye cowpea mosaic virus (BLCMV), which can be transmitted by mechanical injury or aphid vector (*Aphis craccivora* Koch), are the important seedborne diseases (Chang *et al*., 2002; Anthony *et al*., 2003). Therefore, it is desirable to improve and release new varieties which can increase pod yield productivity.

Generally, before starting a breeding program, scientists should study the genetic background and related characteristics of the crop.

1.2.1 Genetic studies for yield and its components

Yield and its components are classified as quantitative traits, and affected by environmental conditions. There are several studies on quantitative traits and genetic inheritance in yardlong bean and cowpea.

Genetics of pod yield and its components in yardlong bean were studied by Umaharan *et al*. (1997). The results showed that broad-sense heritability for pod weight was 84% and narrow-sense heritability was 75%. The additive effect was positive and larger than dominant component suggested that selection focused on average pod weight and clusters per plant could be effective in early generation. Damarany (1994) studied genetic variability and heritability in cowpea, and found that broad-sense heritability for pods per plant was 85.9%, indicating that selection would be effective to improve this trait.

Inheritance and correlation of economic characters of yardlong bean have been previously studied in Thailand (Santhadphanich, 1987; Ratanapitak, 1992; Pornsuriya, 1994). Santhadphanich (1987) studied the inheritance of some interesting characters in four crosses of yardlong bean. She found that immature pod yield per plant has low heritability of 4.03-25.30% indicating that it would be difficult to improve pod yield per plant. She also found that the number of pods per plant is highly positive correlated with pod yield per plant, with a correlation coefficient (r) ranging from 0.899 to 0.947, suggesting that selection based on number of pods per plant would be effective for improvement of pod yield per plant. Ratanapitak (1992) studied the genetic inheritance of yield and yield components of yardlong bean by crossing yardlong bean with cowpea and yardlong bean with yardlong bean. She found additive gene actions in the cross between yardlong bean and cowpea played an important role in pod length and pod yield per plant, suggesting that selection focusing on pod length and pod yield per plant could be effective. The additive gene action in the cross between yardlong bean and yardlong bean played a great role in total pod number per plant and pod length. She also found that total pod number per plant was significant positively correlated with pod yield per plant with a correlation coefficient (r) ranging from 0.871 to 0.942. Pornsuriya (1994) also studied the genetic inheritance of crossing yardlong bean and cowpea. He found heterosis in the number of pods per plant and pod yield per plant, and also found that narrow-sense heritability for pod length, pod numbers per plant and pod yield per plant were rather high. These results suggest that it is possible to increase pod yield by selection after making a cross between yardlong bean and cowpea.

1.2.2 Genetic studies for some important traits

Yardlong bean/cowpea are variable species composed of wild perennials, wild annuals and cultivated forms, and genetic studies on an important traits among these variable forms has been useful. Kar *et al*. (2000) studied the relationship between protein content and pod yield in three groups of cowpea (unguiculata, biflora, and sesquipedalis). The sesquipedalis group had the highest protein content of pod and seed, and pod yield. However, correlation between protein content of pod and seed, and pod yield was not significant.

Thirty-two accessions of cultivated and wild cowpeas were analyzed for phenolic content by Cardinali *et al*. (1995). The cultivated cowpea always contained three flavonoid aglycones: quercetin, kaempferol and isorhamnetin. They also observed that resistance to aphid in cultivated cowpea was related to high flavonoid levels. The inheritance of pod dehiscence in crosses involving wild, weedy and cultivated varieties of cowpea was studied by Aliboh *et al*. (1996) who found that pod dehiscence is inherited by dominant monogenic allele.

1.2.3 Genetic studies for disease/insect pest resistance

Diseases and insect pests, especially seedborne diseases such as cowpea aphid-borne mosaic virus or bacterial blight caused by *Xanthomonas campestris* pv. vinicola (Anthony *et al*., 2003) are particularly important in terms of their effect on yardlong bean/cowpea production. Singh (2002) reviewed in several reports on the inheritance of diseases resistance in cowpea, he reported that resistance to cowpea severe mosaic comovirus (CSMCV) and blackeye cowpea mosaic virus are controlled by single recessive genes. Ryerson and Heath (1996) studied the inheritance of resistance to rust (*Uromyces vignae*) in cowpea cultivar Calico Crowder. The segregation pattern in F_2 and subsequent generation suggested that multiple genes controlled the rust resistance.

Roberts *et al*. (1996) identified IT84S-2049 cowpea line from IITA (International Institute of Tropical Agriculture) to be completely resistant to diverse populations of the root-knot nematodes (*Meloidogyne incognita* and *M. javanica)*. Systematic genetic studies indicated that the resistance in IT84S-2049 was conferred by a single dominant gene, designated as *Rk*2.

1.3 Breeding self-pollinated species

Genetic variability is an important component in breeding programs. Inducing genetic variability is necessary for a self-pollinated breeding program because individual self-pollinated species such as yardlong bean are homozygous and their genetics are stable over generations unless a natural mutation occurs. Such natural spontaneous mutations occur at a very low rate and in a haphazard manner. Cultivar improvement in self-pollinated species is accomplished by inducing genetic variability and then recombining desirable genes that are found in two or more different parents in a single genotype. Plant breeders must consider various factors, such as yield, adaptability, pest or disease reactions, knowledge of genetic control of these characters, etc., before deciding which breeding program to use. Various types of crossing can be made in a breeding program such as single cross, single cross followed by backcross, three-way cross, modified backcross and multi-parental crosses. Breeders have flexibility in deciding which crossing design to use, depending on the characteristics of the parental materials.

1.4 Methods of selection

Selection is one of the oldest breeding procedures and is the basis of all crop improvements. It has been practiced since the earliest times that man began to cultivate crops. Selection is a process based on selecting individuals or groups of plants from mixed populations, either naturally or artificially. Desirable superior lines are selected during succeeding generations by inbreeding to increase homozygosity. There are many methods of selection after hybridization, but three classic and most widely used in self-pollinating crops are pedigree, bulk and single seed descent (Ferraz de Toledo *et al.*, 1982). The efficiency of the selection depends on the presence of genetic variability and heritability of characters.

Heritability is the degree of genetic variability which may be transmitted to the progeny. Heritability may also be defined as the proportion of variation in a progeny which results from genetic factors from the total variation. Agronomic characters differ in their degree of heritability. A character such as yielding ability is so greatly influenced by environmental conditions that it will have a low heritability, while characters that are not greatly influenced by

environmental conditions will have a higher heritability. Selection for characters with low heritability are usually not effective, especially in early generations.

Sitathani (1977) tried to improve yardlong bean varieties through selection by a pure line method from three segregated original varieties. The selected lines showed more uniformity in growth habit, but yields were not significantly different from the original varieties, showing limited scope for yield improvement.

Ntare *et al.* (1984) studied the effectiveness of selection for yield from two cowpea crosses in Nigeria. They found that the differences in yielding ability of F_3 lines persisted over generations, indicating that selection was effective, and confirmed by the highly significant correlation between F_3 yields and those of later generations, which ranged from 0.51 to 0.85. A significant linear correlation between the visual rating of the F_3 and F_6 yields with actual yields from yield tests indicated that it was possible to identify promising lines of cowpea visually.

Mehta and Zaveri (1997) comparatively studied different breeding methods for cowpea. They found that the mean performance of F_3 progenies derived from single seed descent method was better than that of progenies developed via single plant selection for yield and yield components. Also, the broad-sense heritability was higher in populations developed through the single seed descent method.

1.4.1 Pedigree method

The pedigree selection method is the conventional method of accumulating genetic recombination in each generation. It was first established in the year 1890 or earlier (Jensen, 1988). Every F_1- hybrid derived from hybridization is expected to segregate for a large number of gene combinations in the F_2 - population, and every F_2 individual will differ from every other individuals. There are many methods to select desirable individuals from segregated populations. Among these methods, pedigree is the method most widely used by modern-day plant breeders.

Pedigree selection begins in the F_2 population and continues through successive segregated generations until homogeneous lines are developed. Desirable superior individual plants are selected and relationships recorded between parents and progenies. Pedigree records are also included with the distinguishing features of families and important characteristics

recorded. These records are useful to breeder to decide which families will be continued and which ones will be discarded. Repeated pedigree selection can increase homozygosity, but many generation cycles are required to reach homozygosity in loci associated with agronomic traits (Inagaki *et al*.,1998). In the F_3 and F_4 generations, many loci become homozygous, however many are still heterozygous so that plants within a family are likely different genetically from one another. In these generations (F_3 and F_4), progenies are selected within the superior families. By the F_5 or F_6 generation, most families can be expected to be homozygous at most loci. In these homozygous generations, selections are practiced among families.

Several cowpea cultivars have been developed by pedigree method such as Mouride, Melakh, Ein El Gazal, Lori Nie'be', CRSP Nie'be', etc., which were developed for rainfed production in the tropical Sahelian zone of Africa, and selected for ability to cope with drought and resistance to local severe insects pest and diseases (Anthony *et al*., 2003). Also, several cowpea cultivars for subtropical America, such as California Blackeye 27, Better green, Charleston Greenpack, Coronet were also developed by the pedigree method (Anthony *et al*., 2003).

Pedigree has also been used for cultivar development in other legumes such as soybean cultivar Sukhothai 3, S.J.5, etc., (Department of Agriculture, 2001).

1.4.2 Single seed descent method

The concept of a single seed descent method (SSD) was first proposed by Goulden in 1941 (Walter, 1987) and later modified by Grafius (1965) and Brim (1966) (Tee and Qualset, 1975). Single seed descent method consists of advancing hybrid populations by taking a single seed (or one to three seed) from each plant and compositing the seeds to perpetuate the next generation. The procedure is repeated until the desired level of inbreeding is achieved, after which superior lines are selected and evaluated for desirable characters. However, selection on a single plant basis can be practiced during any generation of single seed descent. This method minimizes natural selection without eliminating it. Thus, if population size is limiting, it is expected that the SSD method will maintain more genetic variability than will the others.

INCA and INCA-LD are two relatively new yardlong bean cultivars which were developed via the single seed descent method (Ponce and Casanova, 1999). Other legume species, such as soybean variety Chiang Mai 2 and mungbean variety Chai Nat 72, were also developed by single seed descent in the F_2-F_4 generation followed by single plant selection in F_5- F_6 generation. Nowadays, single seed descent is widely used throughout the world in soybean cultivar improvement (Srinives, 1985).

MATERIALS AND METHODS

Materials

1.1 Plant materials

A total of 24 accessions of yardlong bean and 13 accessions of common cowpea were collected from the Tropical Vegetable Research Center (TVRC) of Kasetsart University, Royal Project at Chachoensao, Field Crops Research Center at Ubon Ratchathani and some local markets. The accession numbers, varieties/lines and domestic data are shown in Table 1.

Table 1 Sources of yardlong bean and common cowpea germplasm used in this study

Accessions	Variety/Name	Source	Original sources	
1 VU 012*	Thuapee	TVRC	Mukdahan,	Thailand
2 VU 041-A*	Thuafakyaao	TVRC	Narathiwat,	Thailand
3 VU 051*	Thuafakyaao	TVRC	Singburi,	Thailand
4 VU 054*	Thuadoung	TVRC	Chainat,	Thailand
5 VU 063*	-	TVRC	-	-
6 VU 136*	Khoewdoke#2	TVRC	Nonthaburi,	Thailand
7 VU 144*	PS#1	TVRC	-	Thailand
8 VU 163*	CSL-14	TVRC	Univ. of Philippines,	Philippines
9 VU 124*	-	TVRC	-	Thailand
10 VU 135*	RW#24	TVRC	-	Thailand
11 VU 146*	Rajburi	TVRC	Rajburi,	Thailand
12 VU 162*	-	TVRC	Songkhla,	Thailand
13 VU 171*	Green arrow 692	TVRC	Chiangmai,	Thailand
14 NR001*	Kaohinsornt	Royal Project	-	Thailand
15 NR002*	Pranomsarakram	Royal Project	-	Thailand
16 NR003*	Evergreen	Local market	Songkhla,	Thailand
17 KU#20*	KU#20	KU	Nakornpathom,	Thailand
18 NR005*	Shaipin#1	Local market	Songkhla,	Thailand

Note * yardlong bean accession

- no information

Table 1 (Cont'd) Sources of yardlong bean and common cowpea germplasm used in this study

Accessions	Variety/Name	Source	Original sources	
19 NR006*	Big-1	Local market	Songkhla,	Thailand
20 NR 007*	-	Farmer	Chaiyapum,	Thailand
21 SR00-0274*	-	TVRC	-	-
22 Selected PSU #1*	Selected PSU #1	PSU	Songkhla,	Thailand
23 VU 189*	-	TVRC	-	China
24 VU 174	F_7 18-1-2-4	TVRC	-	Bangladesh
25 VU 176	F_7 18-1-1-1	TVRC	-	Bangladesh
26 VU 173	F_7 18-1-4-1	TVRC	-	Bangladesh
27 VU 178	F_7 18-1-1-1-2	TVRC	-	Bangladesh
28 VU 179	F_7 13-1-1-3	TVRC	-	Bangladesh
29 SR 00-379	-	TVRC	-	Sri Lanka
30 SR 00-379A	-	TVRC	-	Sri Lanka
31 SR 00-863	-	TVRC	-	Sri Lanka
32 SR 00-1139	-	TVRC	-	Sri Lanka
33 SR 01-0402	-	TVRC	-	Sri Lanka
34 SR 99-334*	-	TVRC	-	Sri Lanka
35 IT 82E-9	-	Field Crops R.C.	Ubon Ratchathani,	Thailand
36 IT 82E-16	-	Field Crops R.C.	Ubon Ratchathani,	Thailand
37 IT 84D-666	-	Field Crops R.C.	Ubon Ratchathani	Thailand

Note * yardlong bean accession

- no information

1.2 Agricultural materials

- fertilizers (formular 15-15-15 and 46-0-0)
- insecticide
- fungicide
- bamboo stake for vine climbing

2. Laboratory materials

2.1 Chemicals

- PVP-40 (Polyvinylpyrrolidone)
- NaCl
- Na_2EDTA (Disodiumethelenediaminetetraacetate)
- Tris-HCl
- CTAB (Hexadecyltrimethyammonium bromide)
- β-mercaptoethanol
- isopropanol
- ethanol
- dNTP (dATP, dTTP, dGTP and dCTP) (Promega, U.S.A)
- primers
- $MgCl_2$
- Taq Polymerase (Promega, U.S.A)
- 10 x Taq buffer
- LE agarose
- SeaKem agarose (FMC Bioproduct: U.S.A)
- Tris Base
- boric acid
- ethidium bromide
- DNA ladder (100 bp and 500 bp: Operon, U.S.A)
- glacial acetic acid
- λDNA
- ethanol
- chloroform

2.2 Laboratory equipments

- PCR Machine (Hybaid, UK)
- electrophoresis equipment
- micro centrifuge
- vortex
- autoclave
- UV-transilluminater
- mortar and pestle
- micro pipette and tips
- microwave
- gel documentation

Methods

1. Evaluation of yardlong bean and cowpea germplasm for parental varieties

1.1 Evaluation by morphological characters

A total of 24 accessions of yardlong bean and 13 accessions of cowpea were evaluated for parental selection using Randomized Complete Block Design (RCBD) with two replications. Agronomics data were collected from twenty plants per plot. Variance were analyzed by SAS program (SAS, 1985), and the following characters were recorded:

1) growth habit, related characters
2) days to 50% flowering
3) pod length
4) pod diameter
5) pod weight
6) pod color
7) number of pods per plant
8) pod yield per plant
9) qualities for consumption (1.0-5.0 score; 1.0 is very poor, 5.0 is the best)

10) resistant to aphid (0.0-4.0 score; 0.0 is very susceptible, 4.0 is highly resistance)

Consumption quality was evaluated by trained taste panels with a consumption score of 1.0-5.0. Tasting score was based on pod structure and components, viz., sweetness, bean aroma, wall tenderness. Preference quality was a little sweetness taste with bean aroma and tender pod wall. A score of 1.0 is very poor and 5.0 is the best.

Aphid (*Aphis craccivora* Koch) is very important insect pest for bean/pea cultivation. It not only directly damaged to young parts of plant but also being a vector of cowpea aphid-borne mosaic virus (CABMV) which cause seed-borne disease. Aphid resistant was evaluated and scored by breeder with a score of 0.0-4.0. A score of 0.0 is very susceptible and 4.0 is no aphid appearance.

1.2 Genetic analysis by molecular markers

Genetic analysis of 36 accessions of yardlong bean and cowpea was investigated using Random Amplified Polymorphic DNA (RAPD) markers for characterization of each individual and estimated for relatedness among and within yardlong bean/cowpea accessions. The procedures of RAPD analysis are as following:

1.2.1 DNA extraction and RAPD amplification procedures

DNA was extracted from approximately 200 mg of young clean fresh leaves of 36 accessions. Procedure was modified from Doyle and Doyle (1990) as described by Nualsri and Konlasuk (2000). The amount of DNA was estimated by electrophoresis and known amount of λDNA was used as standard. One hundred and twenty 10-base oligonucleotide primers Kit A, B, C, R, T and Z from Operon, Alameda, U.S.A. were used for the first step screening. The amplification reaction was performed in a reaction volumn of 25 μl, containing 2.5 μl of 10 × buffer, 3.0 μl of 25 mM $MgCl_2$, 200 μM of each dNTP, 0.3 μM of primer, 0.2 μl (1.0 unit) of Taq DNA polymerase and 1.0 μl of 100 ng template DNA. The thermal profile for PCR was as described by Phansak *et al*. (2001), started from 35 cycles of 94 °C for 30 sec, 37 °C for 30 sec, 72 °C for 1 min and finally 72 °C for 5 min. After amplification, 10μl of PCR products were separated by electrophoresis at 50 V for 2 h and 30 min on 1.75% LE agarose

(Promega, Medison, U.S.A.) using TBE buffer. The gel was stained with 0.5 μg/ml of ethidium bromide for 30 min and washed by soaking in double deionized water for 20 min and photographed using gel documentation. Seventeen primers, which produced a clear polymorphic banding (OPA-09, OPB-04, OPB-07, OPB-08, OPB-17, OPC-06, OPC-07, OPC-10, OPC-14, OPR-02, OPR-08, OPR-12, OPZ-03, OPZ-07, OPZ-09, OPZ-12 and OPZ-13) were selected to re-screen for polymorphic fragments and only fives primers were chosen for further studies (Table 6).

1.2.2 RAPD analysis

Tolal of a clear amplified fragments from those 5 selected primers were scored. Each band was treated as a separate putative locus and scored as present (1) or absent (0) in each accession. Hierarchical Cluster Analysis was performed using the SPSS programs (Vanichbancha, 2003) to identify genetic relatedness among accessions. Similarity coefficient was generated by Jaccard's method (Jaccard, 1908; Teknomo, 2008). Jaccard's coefficient which used to measure similarity on binary variables by simple matching coefficient (Teknomo, 2008) as;

$$\text{Similarity coefficient} \quad S_{ij} = \frac{p}{p+q+r}$$

Where:

S_{ij} = similarity coefficient between the i^{th} accessions and the j^{th} accessions

p = number of markers that positive for both accessions

q = number of markers that positive for the i^{th} accessions and negative for the j^{th} accessions

r = number of markers that positive for the i^{th} accessions and negative for the j^{th} accessions

These similarity coefficients were used to construct a dendrogram using unweighted pair-group method using arithmetic average (UPGMA).

2. Selection criteria for parental varieties

Evaluation of germplasm for parental varieties focused on pod yield per plant, pod length, pod color, qualities for consumption and resistant to important insect pest. Also, genetic relatedness realized by RAPD markers was very important criteria selection for parental varieties. The selected varieties based on the following characters compared to Selected PSU#1 as a check variety:

1) green to darkgreen color
2) pod length $\geq$ 30.0 cm.
3) pod yield/plant > check variety
4) qualities for consumption score $\geq$ 4.0
5) aphid resistant by visual score $\geq$ 3.0

Superior varieties which exhibited a wide genetic related to the other were preference to select for parental varieties.

3. Hybridization of selected varieties

Based on morphological characters and RAPD markers analysis, two superior varieties “VU171” and “VU189” were chosen as male parents and the one best local variety “VU162” was chosen as female parent. The seeds of these selected varieties were collected, grown and crossing were made to produce two single crosses (cross 4501; VU162 × VU189 and cross 4502; VU162×VU171). Anther of bisexual flower of female parent were eliminated for 12-24 hours before receptive and covered with glassine bag to protect undesirable hybridization. Viable and active pollen of male parent were collected and pollinated to female stigma manually in early morning of the next day and recovered glassine bag immediately. For three-four days, successful hybridized flower developed newly pod with 2.0-3.0 cm length. Two F_1- hybrids were grown to multiply F_2- seeds.

4. Selection by different methods

Two F_2 populations were used as segregated materials for selection by the two different methods, pedigree and single seed descent. The procedure, used for each method were as follows:

4.1 Pedigree method

In the pedigree method, selection was first made in the F_2 population. Individual plants were selected based on visual evaluation, from among hundreds of individuals by the following procedure (Figure 1):

F_2: Approximately 800 F_2 plants were grown in the field for each cross. Desirable individuals F_2 plants were selected, necessary details recorded, and seeds collected from individuals separately. The 30 plants were selected to produce F_3 generation.

F_3: Thirty F_3 progenies were space-planted in a row. The desirable rows were selected, then fifteen desirable F_3 plants within those rows were selected to produce F_4 generation and seeds collected separately.

F_4: Fifteen F_4 progenies were planted for yield testing with those lines derived from the single seed descent method with nine F_3 lines which produced desirable-selected F_4 progenies, F_3, F_2 population, two check cultivars, and parents.

4.2 Single seed descent method

The single seed descent procedure was performed by harvesting two seeds from each plant. The procedure was started in F_2 generation. In this experiment, the same F_2 population used for the pedigree method was also used for the single seed descent procedure, which was described following (Figure 1).

F_2: Approximately 800 F_2 of each population were space planted. Two seeds per plant from each individual were harvested. A separate reserve sample of two seeds per plant was harvested from the population to ensure that procedure would going on, although unexpected situation such as flooding expression.

F_3: The F_3 seeds from the F_2 generation were space planted. Desirable fifteen F_3 plants were selected based on visual selection and seeds collected from each plant separately.

F_4: Fifteen F_4 progenies were planted for yield testing with those progenies derived from the pedigree method.

5. Criteria for selection

Desirable F_2 and F_3 individuals from the pedigree method and desirable F_3 individuals from the single seed descent method were selected base on the following criteria:

5.1 high potential yield; marketable pod more than 20 pods per plant

5.2 pod length > 40.0 cm with green to dark-green color

5.3 vigorous growth; fast establishment at early stage and have no leaf diseases

5.4 high quality for fresh consumption with range of good - very good consumption (score 4.0-5.0, respectively)

5.5 aphid resistance rating score 3.0-4.0

6. Evaluation of derived progenies

Separate populations of the two hybrids from each cross (4501 and 4502) were used for evaluation of response to selection in the same field experiment. Each population consist of F_2, F_3 selected F_4 progenies, parental varieties and two check cultivars; national check (VU135) and local check cultivar (selected PSU # 1). RCBD with 3 replications were used for both experiments. Each progeny/cultivar was grown 2 rows per plot, 12 plants per row. The agronomical characteristics as described above and yield were recorded. Data collected from 20 plants per plot were analyzed.

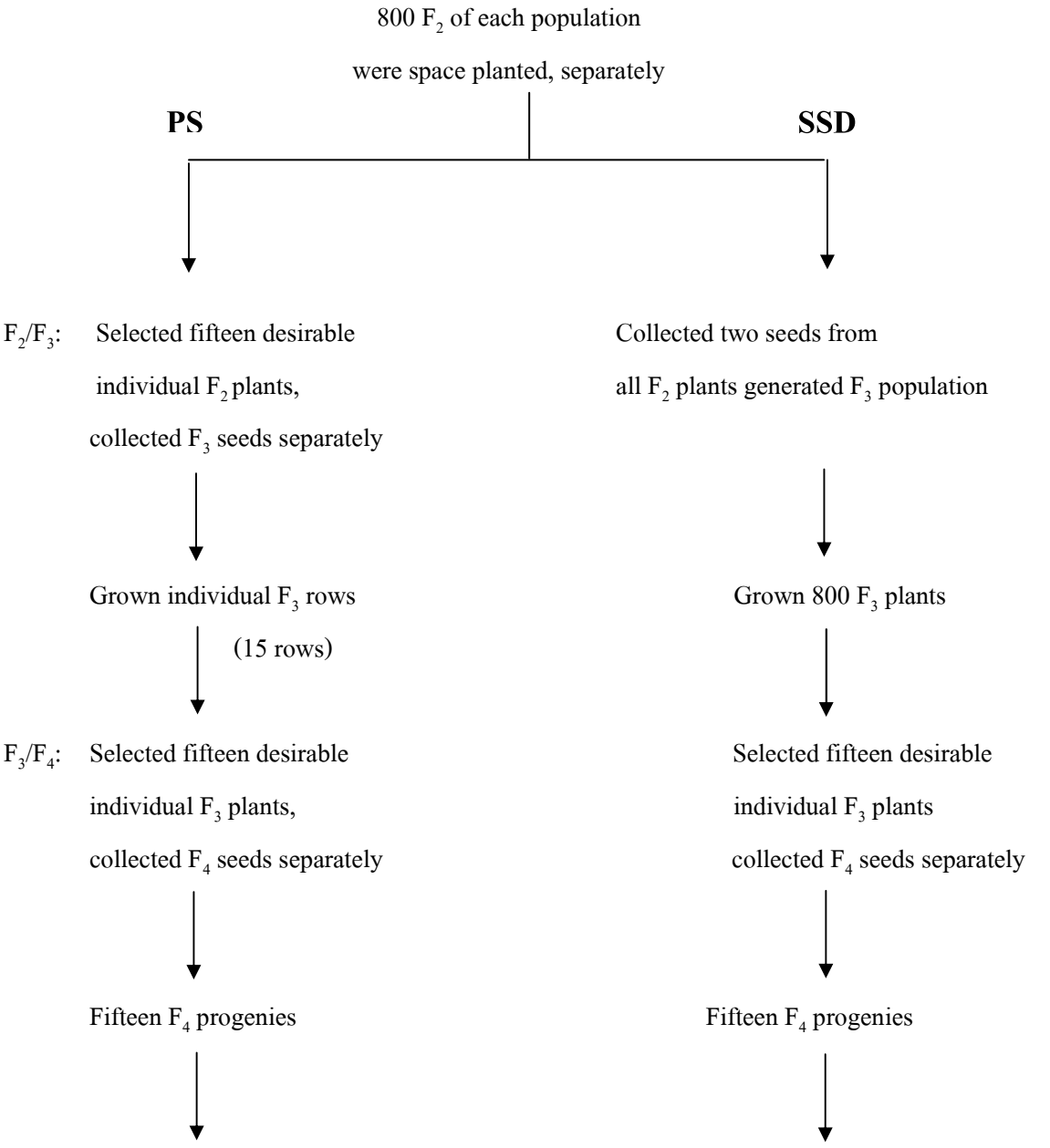


Figure 1 Diagrammatic illustration of pedigree and single seed descent methods for each yardlong bean population.

7. Statistical analysis

Data from experiment I and experiment II were separately analyzed to estimate the variance components, heritability and correlation coefficient. The statistical model for randomized complete block was;

$$Y_{ij} = \mu + L_i + R_j + E_{ij}$$

Where:

Y_{ij} = Observation of the i^{th} progeny in the j^{th} replication

μ = Population mean

L_i = Effect of the i^{th} progeny

R_i = Effect of the j^{th} replication

E_{ij} = Random error

7.1 Analysis of variance

The analysis of variance components were calculated based on Falconer (1981) and functional analysis which used to compare lines means were computed according to Uppraditsakul (1983). The format of analysis is presented in Table 2.

7.2 Estimation of heritability

Heritability were estimated in narrow-sense (h^2) by the regression of F_4 progenies on the F_3 parental lines (of pedigree lines) (Smith and Kinman, 1965) as;

$$\text{Heritability } (h^2) = b_{yx} / 2r_{xy}$$
$$= (4/7)b_{yx}$$

Where :

b_{yx} = is the regression of y_i offspring on x_i parent

X_i = is mean of i^{th} F_3 parent

Y_i = is mean of i^{th} F_4 offspring of i^{th} F_3 parent

Table 2 Form of variance analysis in RCBD, functional analysis and mean square

Source	d.f.	M.S.
Replications	r-1	M_1
Treatments	t-1	M_2
C_1; best (SSD) vs best (PS)	1	M_3
C_2; 3 top (SSD) vs 3 top (PS)	1	M_4
C_3; all 15 (SSD) vs all 15 (PS)	1	M_5
Error	(t-1) (r-1)	M_6
Total	tr-1	M_7

Where:

M_6 = Error variance

M_2 = Genotypic variance among progenies

M_3 = Genetic variance of the best progeny from two selection methods

M_4 = Genetic variance of three top progenies from two selection methods

M_5 = Genetic variance of all 15 progenies from two selection methods

t = Number of progenies

7.3 Estimation of correlation coefficient

Correlation coefficients were calculated for each pair of agronomical traits to estimate the degree of association. The simple correlation coefficient was computed as described by Uppraditsakul (1983);

$$\text{Correlation coefficient (r)} = \frac{\sum\left(X_i - \overline{X}\right)\left(Y_j - \overline{Y}\right)}{\sqrt{\left[\sum\left(X_i - \overline{X}\right)^2\right]\left[\sum\left(Y_j - \overline{Y}\right)^2\right]}}$$

Where:

X_i and Y_j are observations of i^{th} and j^{th} traits among F_4 progenies, (i, j = 1, 2, …,6)

RESULTS

1. Evaluation of yardlong bean and cowpea germplasm for parental varieties

1.1 Morphological and yield of collected germplasm

Yardlong bean and cowpea accessions from field experiment showed various morphological characters. Twenty–two of 24 yardlong bean accessions were of indeterminate growth habit, except VU189 and Kaohinsornt were determinate. Ten of 13 cowpea were determinate while other three accessions (SR00-863, VU174, SR00-1139) had semi-determinate growth habit. Most yardlong bean had green to darkgreen pod color with the average scored for consumption qualities of 3.96. Two yardlong bean accessions (VU144 and SR00-0402) were blue-green in pod color while KU20 had purple-red pod with the consuming quality ranging from 1.0 to 2.0. Pod color of most cowpea was light-green to darkgreen, except that of SR00-1139 which was gray-green. Most of yardlong bean accessions usually had higher pod qualities than cowpea. Overall, mean consuming quality score of yardlong bean was 3.65 compared of 1.65 in cowpea. It was noted that among yardlong bean accessions, pod that were green to darkgreen in color had higher consuming quality compared to blue-green or purple-red pod (Table 3).

Pod length, number of pods per plant and pod yield (g/plant) of all 37 accessions were highly significantly different. The top three highest pod yield were found in SR99-334, VU163 and VU171. They produced 360.6, 346.5 and 306.9 g fresh weight/plant, respectively (Table 5). Mean pod yield of 24 yardlong bean was 212.1 g/plant while that of 13 cowpea was 117.4 g/plant, or only 45.4 % compared to yardlong bean. Pod length of 37 accessions varied from 14.9 to 58.3 cm. Mean pod length of 13 cowpea was 21.3 cm while that of yardlong bean was 48.7 cm (Table 4).

VU189 and Kaohinsornt which were classified as yardlong bean exhibited some morphological difference from other yardlong bean accessions. These two accessions had determinate growth habit with pod length of 34.0-34.9 cm. VU189 and Kaohinsornt might be improved varieties derived from yardlong bean and cowpea cross followed by selection or backcrossing.

Based on pod yield and morphological characters obtained from field experiment, five superior varieties were the first round selected for parental varieties. VU162 was the best among domestic germplasm. It exhibited relative high pod yield with high consuming qualities and resistant to aphid. It was expected to be the most suitable female parent to make cross with others to produce a segregated populations. Four list of interest superior varieties for used as male parents were VU163, VU171, VU135 and VU189. These four varieties exhibited superior performance although its yielding were less than of SR99-334.

Table 3 Morphological characters of 37 yardlong bean and cowpea accessions

Accession	Date to 50% flowering	Consumed 1/ qualities	Growth habit	Pod color
1 SR99-334*	41	4.0	indeterminate	green- darkgreen
2 VU 163*	40	4.5	indeterminate	green
3 VU 171*	37	4.5	indeterminate	green-darkgreen
4 VU 012*	37	3.0	indeterminate	green
5 VU 162*	40	4.0	indeterminate	green
6 VU 124*	42	3.0	indeterminate	green
7 VU 041-A*	40	4.0	indeterminate	green
8 Selected PSU #1*	39	4.0	indeterminate	green
9 VU 146*	40	4.0	indeterminate	green
10 VU 135*	42	5.0	indeterminate	green
11 VU 144*	39	1.0	indeterminate	blue-green
12 Evergreen*	38	4.5	indeterminate	green
13 VU 136*	39	1.5	indeterminate	green
14 VU 051*	38	3.0	indeterminate	darkgreen
15 VU 054*	40	4.0	indeterminate	green
16 Shaipin #1*	40	4.5	indeterminate	green
17 Big-1*	40	4.5	indeterminate	darkgreen
18 SR00-0402	42	1.5	determinate	blue-green
19 SR00-863	38	2.0	semi-indeterminate	green
20 VU 063*	39	2.0	indeterminate	green
21 KU 20*	40	2.0	indeterminate	purple-red
22 Pranomsarakram*	41	4.5	indeterminate	green
23 VU 176	39	2.0	determinate	lightgreen
24 VU 174	40	1.0	semi-indeterminate	lightgreen

Table 3 (Cont'd) Morphological characters of 37 yardlong bean and cowpea accessions

Accession	Date to 50% flowering	Consumed 1/ qualities	Growth habit	Pod color
25 VU 189*	35	4.0	determinate	lightgreen
26 SR00-379A	39	2.0	determinate	darkgreen
27 NR 007*	41	4.5	indeterminate	green
28 VU 173	39	1.0	determinate	lightgreen
29 VU 178	37	1.5	determinate	lightgreen
30 IT 82E-9	36	2.0	determinate	darkgreen
31 VU 179	39	1.5	determinate	lightgreen
32 IT 82E-16	37	2.0	determinate	darkgreen
33 Kaohinsornt*	39	4.0	determinate	green
34 SR00-1139	44	1.5	semi-indeterminate	gray-green
35 IT 84D-666	37	2.0	determinate	darkgreen
36 SR00-0274*	40	3.5	indeterminate	green
37 SR00-379	39	1.5	determinate	green

Note * yardlong bean accession

1/ 1.0-5.0 score; 5.0 = best, 1.0 = very poor

Table 4 Pod characteristics of 37 yardlong bean and cowpea accessions

Accession	Pod length (cm.)	Pod diameter (mm.)	Pod wt. (g./pod)
1 SR99-334*	53.6	6.7	15.0
2 VU 163*	55.3	6.8	17.9
3 VU 171*	48.7	6.8	17.9
4 VU 012*	52.9	6.9	16.7
5 VU 162*	58.3	7.1	17.7
6 VU 124*	55.1	6.7	13.6
7 VU 041-A*	44.1	7.5	15.8
8 Selected PSU #1*	57.1	7.0	15.5
9 VU 146*	52.2	6.9	14.4
10 VU 135*	50.4	7.1	17.8
11 VU 144*	45.7	7.1	14.2
12 Evergreen*	56.7	7.2	16.2
13 VU 136*	54.0	7.0	15.1
14 VU 051*	44.4	7.8	18.5
15 VU 054*	47.6	6.8	13.7
16 Shaipin #1*	50.1	6.5	13.8
17 Big-1*	42.7	6.9	17.5
18 SR00-0402	39.8	6.4	13.2
19 SR00-863	23.6	5.7	7.2

Table 4 (Cont'd) Pod characteristics of 37 yardlong bean and cowpea accessions

Accession	Pod length (cm.)	Pod diameter (mm.)	Pod wt. (g./pod)
20 VU 063*	46.3	6.7	13.7
21 KU 20*	44.1	6.6	12.7
22 Pranomsarakram*	50.3	6.8	14.2
23 VU 176	18.5	4.7	4.0
24 VU 174	20.8	5.1	4.6
25 VU 189*	34.9	6.6	10.7
26 SR00-379A	17.8	5.4	5.5
27 NR 007*	44.5	7.4	17.7
28 VU 173	19.4	4.6	4.6
29 VU 178	17.2	4.5	3.6
30 IT 82E-9	15.5	6.3	4.9
31 VU 179	19.0	5.1	4.0
32 IT 82E-16	15.9	6.5	5.0
33 Kaohinsornt*	34.0	6.9	11.4
34 SR00-1139	37.7	7.0	12.1
35 IT 84D-666	16.3	6.1	4.9
36 SR00-0274*	45.2	6.7	11.0
37 SR00-379	14.9	5.2	4.8
F-test	**	**	**
LSD. 01	3.26	2.72	1.33
C.V. (%)	3.16	3.69	4.13

Note ** significant difference at 0.01 level

Table 5 Yield, relative yield and aphid resistance of 37 yardlong bean and cowpea accessions.

	Accession	No. of pod per plant	Pod yield (g/plant)	Relative yield (%)	Aphid 1/ resistant
1	SR99-334*	24.1	360.6	155.0	0.5
2	VU 163*	19.3	346.5	148.9	3.0
3	VU 171*	17.1	306.9	131.9	3.0
4	VU 012*	16.7	278.3	119.6	3.0
5	VU 162*	14.7	260.5	112.0	3.5
6	VU 124*	18.7	254.5	109.4	3.5
7	VU 041-A*	15.0	238.2	102.4	2.5
8	Selected PSU # 1*	15.0	232.6	100.0	2.5
9	VU 146*	15.6	225.3	96.9	2.0
10	VU 135*	12.6	223.7	96.2	3.0
11	VU 144*	15.6	221.3	95.1	3.0
12	Evergreen*	13.5	218.9	94.1	2.0
13	VU 136*	14.0	211.1	90.7	3.0
14	VU 051*	11.2	207.2	89.1	3.0
15	VU 054*	14.6	199.8	85.9	3.0
16	Shaipin # 1*	14.4	199.4	85.7	3.0
17	Big-1*	11.2	196.0	84.2	2.5
18	SR00-0402	14.8	195.3	83.9	0.5
19	SR00-863	24.5	176.9	76.0	0.5
20	VU 063*	12.0	164.2	70.6	3.5
21	KU 20*	12.2	154.8	66.5	4.0
22	Pranomsarakram*	10.7	152.5	65.5	3.0
23	VU 176	38.3	152.4	65.5	2.5
24	VU 174	33.1	150.8	64.8	3.0
25	VU 189*	13.6	145.8	62.7	3.5
26	SR00-379A	25.3	139.5	60.0	0.5
27	NR 007*	7.7	136.3	58.6	3.5
28	VU 173	26.9	123.6	53.1	0.5
29	VU 178	31.2	112.4	48.3	0.5
30	IT 82E-9	21.1	104.0	44.7	4.0
31	VU 179	25.5	101.2	43.5	1.0
32	IT 82E-16	19.7	98.5	42.3	4.0
33	Kaohinsornt*	8.3	94.5	40.6	2.5
34	SR00-1139	6.3	76.5	32.9	0.5
35	IT 84D-666	13.5	66.3	28.5	3.0
36	SR00-0274*	5.5	60.3	25.9	0.5
37	SR00-379	6.1	29.3	12.6	0.5
F-test		**	**	-	-
LSD. 01		3.67	50.98	-	-
C.V. (%)		8.27	10.77	-	-

Note ** significant difference at 0.01 level

1/ 0.0-4.0 score; 0.0 = very susceptible, 4.0 = high resistance

1.2 RAPD marker evaluation

One hundred and twenty 10-base oligonucleotide primers were screened among 36 yardlong bean and cowpea accessions. Only five primers (OPC-06, OPR-12, OPZ-03, OPZ-08 and OPZ-13) were chosen to use for genetic diversity analysis. The total number of clear visible and polymorphic bands across 36 accessions varied among primers (Table 6). A total of 38 visible bands, 23 polymorphic bands was generated from five primers with the mean of 7.6 and 4.6 bands/primer, respectively (Figures 2-6). OPZ-03 gave the highest number of fragments (11 fragments) and 7 from these fragments were polymorphisms (Figure 4). The size of the amplified fragment ranged from approximately 225 bp to 1650 bp (Table 6).

1.3 Genetic relatedness of collected germplasm

A dendrogram constructed from 23 polymorphic bands revealed fairly good separation of genetic groups between yardlong bean and cowpea (Figure 7). However, VU189 and Kaohinsornt, two improved yardlong bean accessions derived from a cross between yardlong bean and cowpea which exhibited most characters resembling cowpea and were classified in the cowpea group. The result revealed some good relationship between growth habit and genetic relatedness. Genetic diversity among yardlong bean was relatively higher than that of cowpea. Similarity coefficient among yardlong bean and cowpea accessions were 0.515 to 1.000 and 0.548 to 1.000, respectively. Relatedness among accessions was not influenced by geographical location, except all accessions from Bangladesh (VU173, 174, 176, 178 and 179) which originated from the same cross. They were grouped in the same cluster with similarity coefficient higher than 0.7. Among 36 accessions, VU176 (cowpea) and SR99-334 (yardlong bean) exhibited the lowest genetic relatedness with similarity coefficient of 0.484. There were three pair of very closely related accessions based on RAPD markers showing identical DNA patterns, there were IT82E-9/IT82E-16, Pranomsarakram/NR007 and VU063/VU136.

In this study, we considered the desirable male parents not only based on field experiment but genetic relatedness information derived from molecular markers analysis were also used. Varieties that had less genetic relatedness to female parent (VU162) were chosen. Among these four interesting listed based on morphological characters and yield (VU163, VU171, VU135, VU189), VU163 and VU135 were highly closely related to VU162 with similarity coefficient of 0.935 and 0.903, respectively, while VU171 and VU189 showed relative

low genetic relationship to VU162 with similarity coefficient of 0.742 and 0.813, respectively. Thus, VU171 and VU189 were chosen and used as male parents to make crossed with VU162 to produce two single cross: cross 4501 (VU162 × VU189) and cross 4502 (VU162 × VU171) which used as original populations for further study.

Table 6 Total fragments, polymorphic and size of RAPD fragments produced by yardlong bean and cowpea accessions.

Primer ID	Primer sequences	Total fragments	Polymorphic fragments	Range of fragment size (bp)
OPC-06	GAACGGACTC	8	4	275-1,350
OPR-12	ACAGGTGCGT	5	3	675-1,200
OPZ-03	CAGCACCGCA	11	7	225-1,175
OPZ-08	GGGTGGGTAA	7	5	350-1,500
OPZ-13	GACTAAGCCC	7	4	250-1,650
Total	-	38	23	225-1,650

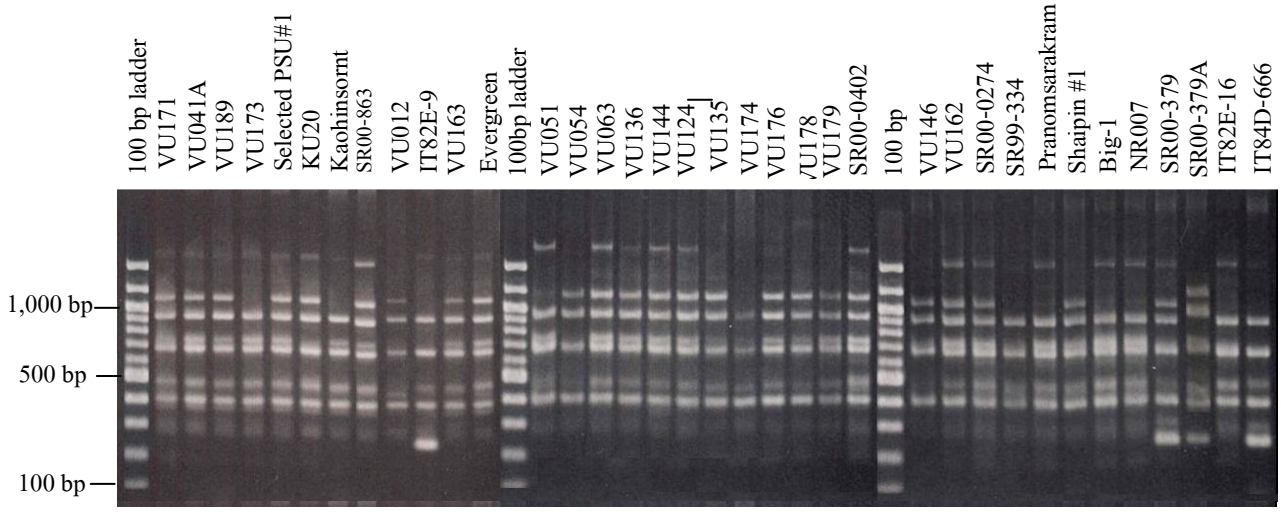


Figure 2 RAPD amplification products of 36 yardlong bean and cowpea accessions generated from primer OPZ-13.

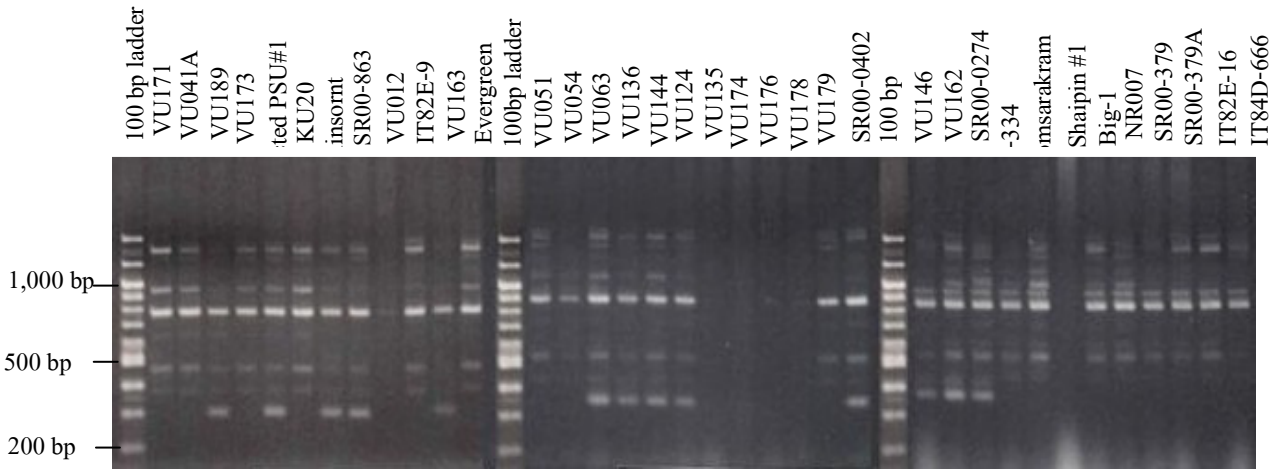


Figure 3 RAPD amplification products of 36 yardlong bean and cowpea accessions generated from primer OPC-06

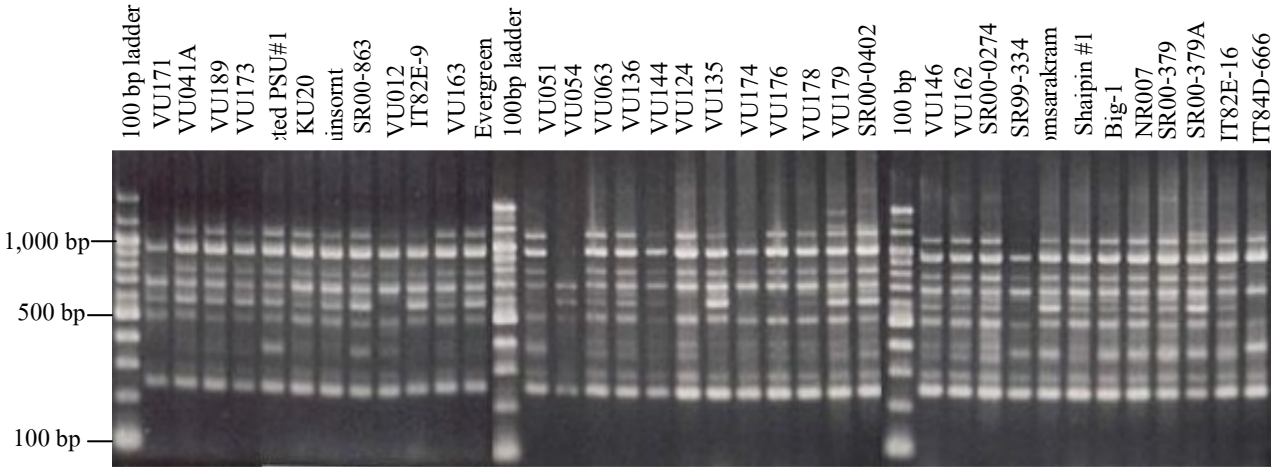


Figure 4 RAPD amplification products of 36 yardlong bean and cowpea accessions generated from primer OPZ-03

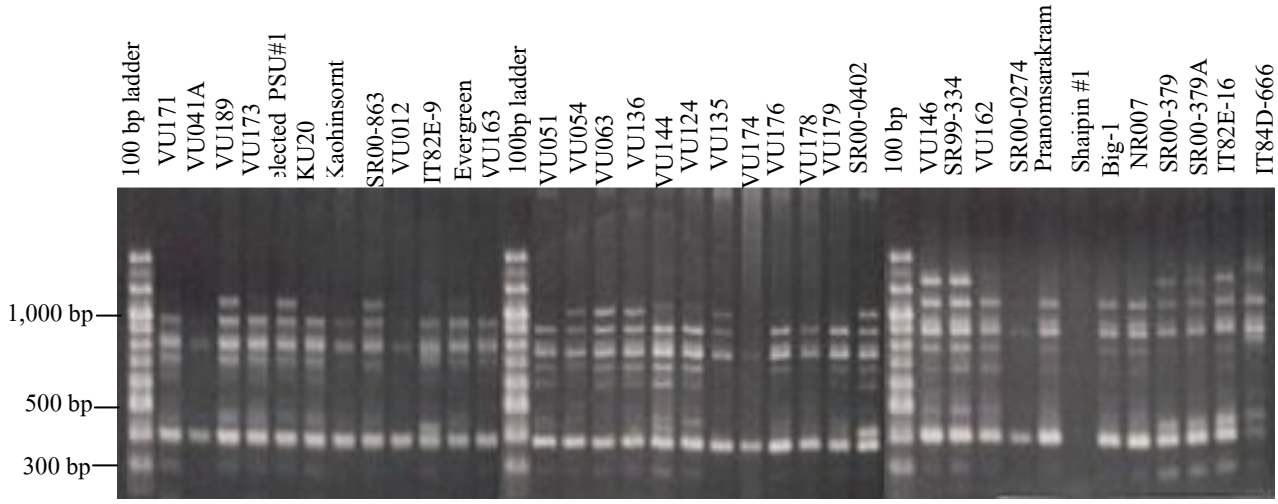


Figure 5 RAPD amplification products of 36 yardlong bean and cowpea accessions generated from primer OPZ-08

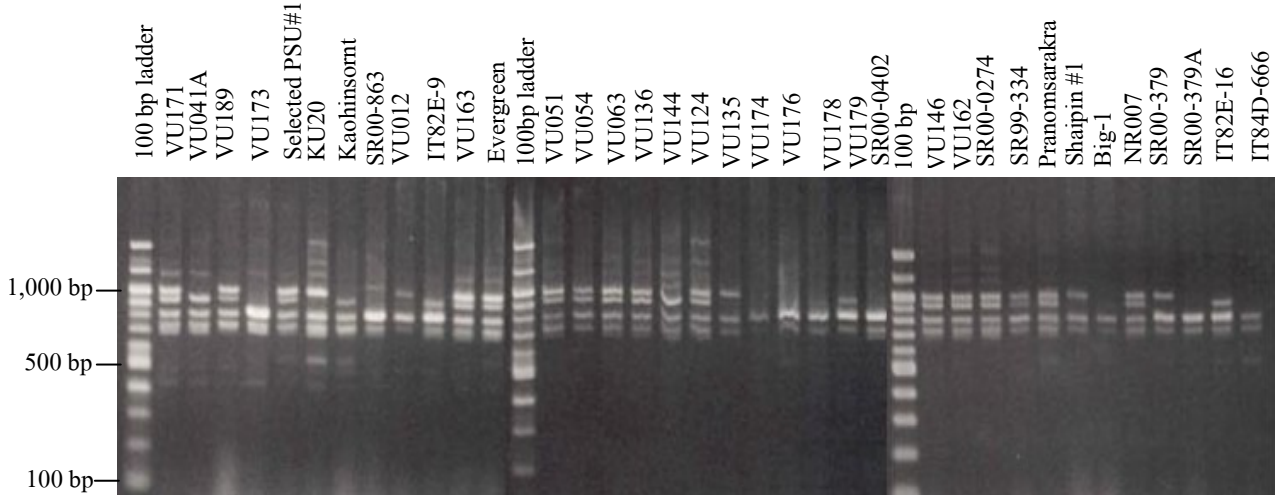


Figure 6 RAPD amplification products of 36 yardlong bean and cowpea accessions generated from primer OPR-12

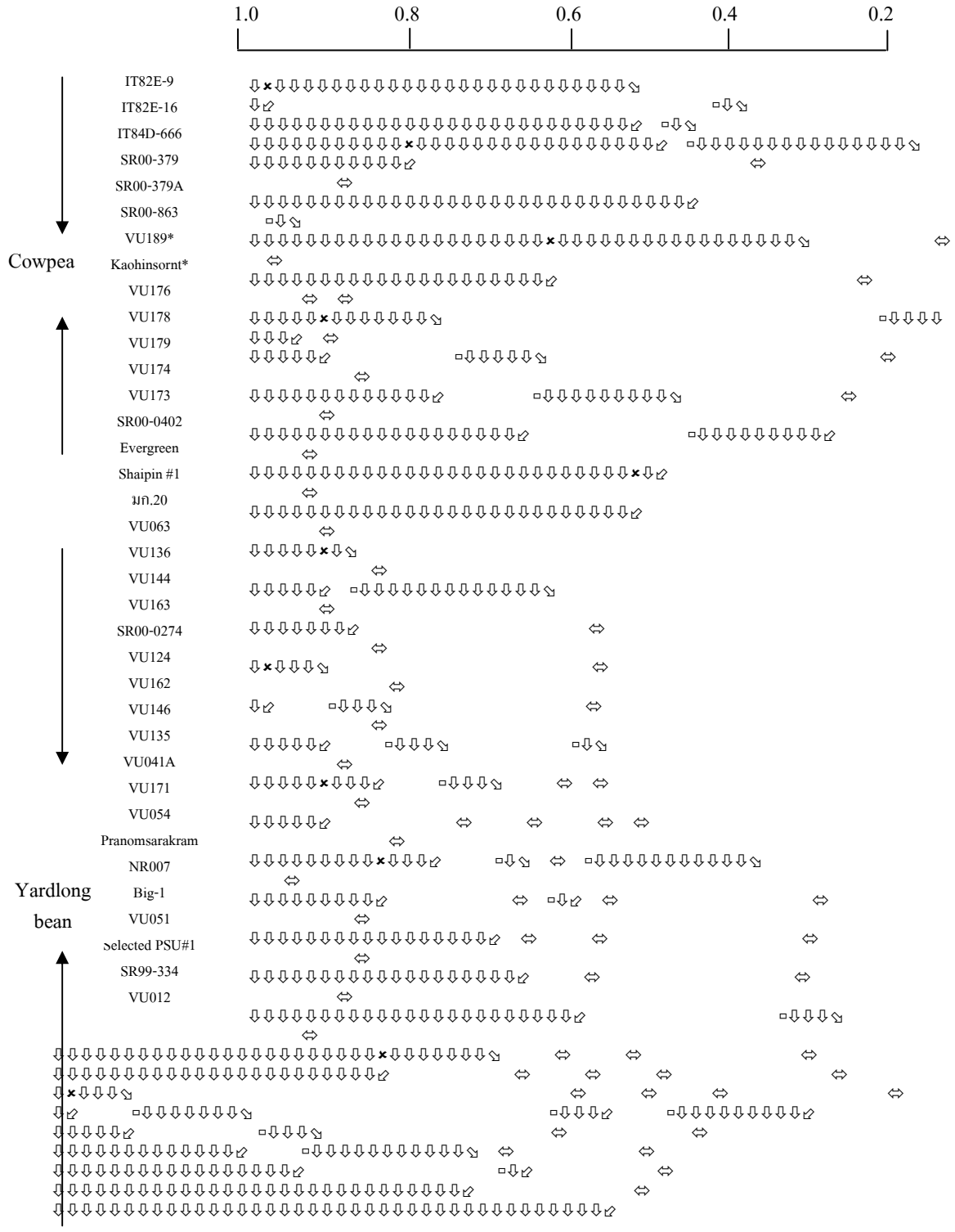


Figure 7 Dendrogram of genetic relationships between 36 yardlong bean/ cowpea accessions using unweighted pair-group arithmetic average (UPGMA) based on the Jaccard similarity matrix. (* accession that was classified as yardlong bean)

Table 7 Similarity coefficient among 36 yardlong bean and cowpea accessions

	ITK2E-9	ITK2E-16	IT84D-666	SR 00-379	SR 00-379A	SR 00-863	Kaoshinsan1	VU 176	VU 178	VU179	VU 174	VU 173	SR 00-0402	Evergreen	Shaipin	KU 20	VU 063	VU 136	VU 144	VU 163	SR 00-0274	VU 124	VU 162	VU 146	VU 135	VU041A	VU 171	[illegible]	Pranomsarakram	NR 007	Big-1	VU 051	PSU#1	SR 99-334	VU 012
IT82E-9	1.000																																	
IT82E-16	1.000*	1.000																																
IT84D-666	.821	.821	1.000																															
SR00-379	.800	.800	.815	1.000																														
SR00-379A	.800	.800	.815	.926	1.000																													
SR00-863	.800	.800	.750	.793	.793	1.000																												
VU189	.618	.618	.563	.710	.656	.710	1.000																											
Kaoshisont	.606	.606	.548	.594	.594	.645	.857	1.000																										
VU176	.625	.625	.679	.724	.724	.667	.759	.690	1.000																									
VU178	.656	.656	.655	.759	.759	.700	.793	.724	.960	1.000																								
VU179	.667	.667	.613	.710	.767	.656	.800	.733	.889	.926	1.000																							
VU174	.667	.667	.731	.714	.714	.655	.690	.679	.917	.880	.815	1.000																						
VU173	.806	.806	.7000	.688	.688	.742	.719	.710	.793	.828	.774	.786	1.000																					
SR00-0402	.706	.706	.656	.750	.806	.750	.781	.719	.800	.833	.839	.733	.813	1.000																				
Evergreen	.697	.697	.545	.636	.588	.688	.774	.656	.733	.767	.774	.667	.806	.706	1.000																			
Shaipin	.667	.667	.563	.656	.606	.710	742	.625	.759	.793	.742	.690	.833	.727	.964	1.000																		
ส.น.20	.688	.688	.581	.677	.625	.733	.710	.594	.724	.759	.710	.714	.800	.697	.929	.963	1.000																	
VU063	.676	.676	.529	.618	.571	.719	.867	.742	.656	.688	.697	.594	.781	.735	.900	.867	.833	1.000																
VU136	.616	.676	.529	.618	.571	.719	.867	.742	.656	.688	.697	.594	.781	.735	.900	.867	.833	1.000	1.000															
VU144	.706	.706	.559	.647	.600	.697	.839	.719	.688	.719	.727	.625	.813	.765	.871	.839	.806	.967	.967	1.000														
VU163	.676	.676	.529	.618	.571	.719	.806	.688	.710	.742	.750	.645	.781	.735	.966	.931	.897	.933	.933	.903	1.000													
SR00-0274	.647	.647	.500	.588	.543	.688	.833	.710	.677	.710	.719	.613	.750	.706	.931	.897	.862	.966	.966	.933	.966	1.000												
VU124	.629	.629	.529	.571	.528	.667	.806	.742	.656	.636	.647	.594	.727	.686	.839	.806	.774	.933	.933	.903	.871	.900	1.000											
VU162	.686	.686	.543	.629	.586	.727	.813	.750	.667	.697	.706	.606	.788	.743	.903	.871	.839	.935	.935	.906	.935	.903	.935	1.000										
VU146	.697	.697	.545	.636	.588	.742	.774	.656	.625	.656	.667	.613	.750	.706	.867	.833	.862	.900	.900	.871	.900	.867	.839	.903	1.000									
VU135	.600	.600	.500	.588	.543	.688	.774	.710	.625	.656	.618	.563	.750	.706	.806	.833	.800	.900	.900	.871	.839	.867	.900	.903	.806	1.000								
VU041A	.656	.656	.500	.545	.545	.645	.733	.667	.690	.724	.733	.621	.767	.667	.893	.857	.821	.862	.862	.833	.871	.893	.800	.806	.706	.828	1.000							
VU171	.645	.645	.533	.581	.531	.633	.663	.548	.567	.600	.613	.500	.645	.559	.821	.786	.750	.793	.793	.767	.793	.821	.733	.742	.700	.700	.778	1.000						
VU054	.700	.700	.533	.633	.581	.690	.667	.548	.516	.548	.563	.500	.645	.559	.759	.724	.750	.793	.793	.767	.733	.759	.733	.742	.821	.700	.714	.840	1.000					
Pranomsarakram	.800	.800	.633	.677	.625	.733	.656	.594	.613	.645	.656	.600	.742	.647	.862	.828	.793	.774	.774	.750	.833	.800	.710	.781	.742	.688	.759	.815	.750	1.000				
NR007	.800	.800	.613	.677	.625	.733	.656	.594	.613	.645	.656	.600	.742	.647	.862	.828	.793	.774	.774	.750	.931	.800	.710	.781	.742	.688	.759	.815	.750	1.000*	1.000			
Big-1	.774	.774	.633	.656	.606	.710	.636	.625	.594	.625	.636	.581	.719	.629	.833	.800	.767	.750	.750	.727	.806	.774	.750	.813	.719	.719	.733	.786	.724	.963	.963	1.000		
VU051	.742	.742	.613	.677	.625	.733	.656	.545	.667	.645	.656	.600	.688	.647	.862	.828	.793	.774	.774	.750	.833	800	.774	.781	.742	.688	.759	.815	.750	.926	.926	.839	1.000	
PSU#1	.781	.781	.625	.719	.667	.833	.750	.636	.606	.636	.674	.545	.727	.735	.893	.806	.774	.871	.871	.844	.871	.839	.813	.875	.839	.781	.742	.793	.793	.897	.897	.867	.897	1.000
SR00-334	.724	.724	.552	.600	.548	.655	.581	.516	.484*	.516	.531	.517	.613	.529	.724	.690	.714	.700	.700	.677	.700	.724	.645	.656	.667	.667	.741	.800	.800	.846	.846	.815	.778	.759
VU012	.645	.645	.586	.633	.581	.750	.813	.500	.567	.548	.515	.552	.594	.606	.700	.724	.750	.733	.733	.710	.733	.759	.733	.688	.700	.759	.714	.704	.704	.750	.750	.724	.815	.793

2. Comparison of pedigree and single seed descent methods

2.1 Pod yield and yield components between generations

Pod yield and five yield components between F_2, F_3 and F_4 generations were non-significant difference in both 4501 and 4502 population (Tables 8 and 9). Mean pod yield of F_4, F_3 and F_2, of cross 4501 were 313.5, 337.5 and 327.0 g/plant, respectively. They produced non-significant difference with mean parent (262.3 g/plant) and mean check (288.6 g/plant) (Table 8). The same result was obtained in the 4502 population. F_4, F_3 and F_2 produced pod yield of 367.3, 330.0, 320.9 g/plant, respectively that non-significant difference with mean parental (260.0 g/plant) and mean check cultivars (288.6 g/plant) (Table 9). In the 4502 population, pod yield was slightly increased during succeeded generations.

2.2 Pod yield and yield components of F_4 progenies derived from SSD and PS methods

The results showed that the F_4 progenies from SSD and PS of both populations produced relatively higher pod yield than the mean parental and check cultivars. However, mean pod yield of F_4 progenies from both SSD and PS was non-significantly different in both populations (Table 10). Five yield components of SSD and PS in both populations, except number of pod/plant in the 4501 population were also non-significant difference (Table 10). In the 4501 population, SSD progenies produced higher number of pod/plant than PS progenies.

Yield and five yield components from functional analysis for the best and the three top lines derived from SSD and PS were non-significant difference in both populations (Table 11). In the 4501 population, the best progeny from SSD produced non-significant higher pod/plant (27.93 pods) and mean pod yield (470.30 g/plant) than the best of PS (23.97 pods and 354.20 g/plant, respectively) (Table 12). In the 4502 population, the best progeny from PS produced significant more pod/plant than the best of SSD (28.10 vs 22.63 pods, respectively) (Table 13). The best progeny of PS in the 4502 population produced non-significant pod yield from the best of SSD (503.53 vs 445.03 g/plant) (Table 13). Only in the 4502 population, the best and the three tops from two selection methods produced significant higher pod yield and pod number per plant than mean parental and two check cultivars (Table 13). For other yield

components, such as number of inflorescence/plant, pod diameter and pod length, non significantly different were found among F_4 progenies, parental and check cultivars in both populations (Tables 12 and 13).

Among F_4 progenies**,** pod yield, number of pod/plant and pod weight of the 4501 population were significant difference, while number of inflorescence/plant, pod diameter and pod length were non-significant differences (Tables 14 and 15). In the 4502 population, significant differences among F_4 progenies were found in pod yield and number of pod/plant while number of inflorescence/plant, pod diameter, pod length and pod weight were non-significant difference (Tables 16 and 17). Large variation of pod number/plant and pod yield from SSD and PS methods in both populations was observed (Figures 8 and 9). SSD progenies showed larger variation of number of pod/plant and pod yield than of PS in the 4501 population (Figures 8 and 9) while in the 4502 population, pod yield of PS revealed larger variation than of SSD (Figure 9). Almost the same variation was observed in pod number/plant of SSD and PS methods in the 4502 population (Figure 8). Pod yield, number of pod/plant and pod weight of individual of SSD and PS lines showed resemble small variation in both populations (Figures 10, 11 and 12).

3. Heritability

Narrow-sense heritability (h^2) estimates for yield and yield components are presented in Table 18. Heritability was estimated by the regression of F_4 progenies on the F_3 parents. Low estimated and non-significant heritability were obtained for pod yield and five yield components in both populations. Narrow-sense heritability values for pod yield in the 4501 and 4502 populations were 2.64 ±0.36 and 1.69±0.28 %, respectively (Table 18). Number of pod/plant had a heritability of 10.53±0.19 and 7.39±0.47% in the 4501 and 4502 populations, respectively. Pod diameter and pod length had high heritability in the 4501 population but very low in the 4502 population because of the limitation variability on pod diameter and pod length in the 4502 population. VU162 and VU171, the parentages of cross 4502 have almost the same pod size. In the 4501 population, heritability for pod diameter (21.83±0.30%) and pod length (22.36±0.26%) were higher than that of other traits.

4. Correlation coefficient

Correlation coefficients among pod yield and five yield components of each population were calculated by SAS program (SAS, 1985). Pod number/plant was highly correlated with pod yield in both populations, with correlation coefficients (r) of 0.7540** in the 4501 and 0.9229** in the 4502, respectively (Table 19). In the 4501 population, pod weight was significant correlated positively with pod diameter (r = 0.5211*) while number of inflorescence/ plant was non-significant correlated with pod yield (r = 0.4438). Pod weight was non-significant correlated negatively with pod number/plant (r = 0.4839).

In the 4502 population, pod diameter and pod length were non-significant correlated positively with pod yield with correlation coefficients of 0.3626, 0.3488, respectively. Pod weigh and number of pod/plant were non-significant correlated negatively with pod yield in both populations with correlation coefficient of -0.4839 and -0.2563 in the 4501 and 4502 populations, respectively.

Table 8 Mean pod yield and five yield components among generations of cross 4501

Generations/Varieties	Pod yield (g/plant)	No. of pod/ plant	Pod wt. (g/pod)	No. of inflores-cence/plant	Pod diameter (cm)	Pod length (cm)
1 F_4 progenies (PS)	298.0	17.6	17.1 ab	17.8	0.75 bc	46.3
2 F_4 progenies (SSD)	329.0	20.7	16.1 bc	14.9	0.75 bc	44.4
3 F_3 lines (PS)	367.2	22.7	15.8 bc	16.2	0.75 bc	49.2
4 F_3 population (SSD)	307.8	19.0	16.4 bc	15.3	0.74 bc	47.9
5 F_2 generation	327.0	18.2	17.9 ab	16.0	0.73 cd	44.8
6 Mean parent	262.3	17.7	13.7 c	12.2	0.71 d	43.7
7 National check	278.2	14.6	18.8 ab	14.8	0.79 a	50.0
8 Local check	299.0	15.3	19.5 a	12.2	0.77 ab	50.9
F-test	NS	NS	*	NS	**	NS
$LSD_{.05/.01}$	-	-	3.1	-	0.03	-
C.V. (%)	22.2	19.3	10.5	11.5	2.7	7.4

Note means within the same column followed by the same letter are not significant difference by LSD

NS non-significant difference

* significant difference at 0.05 level

** significant difference at 0.01 level

Table 9 Mean pod yield and five yield components among generations of cross 4502

Generations/Varieties	Pod yield (g/plant)	No. of pod/ plant	Pod wt. (g/pod)	No. of inflores-cence/plant	Pod diameter (cm)	Pod length (cm)
1 F_4 progenies (PS)	368.3	19.3	19.1	15.1	0.76	49.0
2 F_4 progenies (SSD)	366.3	18.1	20.2	15.1	0.77	48.8
3 F_3 lines (PS)	305.5	15.8	19.3	13.8	0.77	48.5
4 F_3 population (SSD)	354.4	16.3	21.9	15.8	0.78	48.7
5 F_2 generation	320.9	14.6	22.5	14.8	0.74	46.0
6 Mean parent	260.0	12.5	20.9	13.6	0.74	47.0
7 National check	278.2	14.6	18.8	14.8	0.79	49.4
8 Local check	299.0	15.3	19.5	12.2	0.77	50.9
F-test	NS	NS	NS	NS	NS	NS
$LSD_{.05}$	-	-	-	-	-	-
C.V. (%)	16.2	17.2	8.6	10.7	2.7	4.1

Note NS non-significant difference

Table 10 Mean pod yield and yield components of fifteen F_4 progenies derived by SSD and PS from two yardlong bean crosses

Cross	Selection method	Pod yield (g/plant)	No. of pod/ plant	Pod wt. (g/pod)	No.of inflores-cence/plant	Pod diameter (cm)	Pod length (cm)
4501	Mean (SSD)	328.8	20.7 a	16.1 ab	15.7 a	0.75 bc	44.4 c
	Mean (PS)	298.0	17.4 b	17.1 ab	14.8 a	0.75 bc	46.3 bc
	Mean parent	262.3	17.7 b	13.7 b	12.2 b	0.72 d	43.7 c
	National check	278.2	14.6 c	18.8 a	14.8 a	0.79 a	50.0 ab
	Local check	299.0	15.3 c	19.5 a	12.2 b	0.77 ab	50.9 a
	F-test	NS	**	*	*	**	*
	C.V. (%)	12.6	6.1	11.2	9.1	1.6	4.6
4502	Mean (SSD)	366.3	18.1 ab	20.1	15.1	0.77	48.8
	Mean (PS)	368.3	18.9 a	19.1	15.1	0.76	48.9
	Mean parent	260.0	12.5 c	20.9	13.6	0.74	47.0
	National check	278.2	14.6 bc	18.8	14.8	0.79	49.4
	Local check	299.0	15.3 abc	19.5	12.2	0.77	50.9
	F-test	NS	*	NS	NS	NS	NS
	C.V. (%)	15.4	13.1	10.0	10.0	2.4	3.6

Note means within the same column followed by the same letter are not significant difference by LSD

NS non-significant difference

* significant difference at 0.05 level

** significant difference at 0.01 level

Table 11 Mean squares from the analysis of variance for pod yield and four yield components of 30 F_4 lines derived by two selection methods from two crosses of yardlong bean

Cross/Source of variance	d.f.	Pod yield (g/plant)	No. pod/ plant	No.inflores-cence/plant	Pod diameter (cm)	Pod length (cm)
Cross 4501 (VU162 x VU189)						
- All treatments	32	8,570.1*	28.9	4.6	0.0007	5.9
- best (SSD) vs best (PS)	1	13,651.7	44.8	8.9	0.00001	32.2
- 3 top (SSD) vs 3top (PS)	1	10,887.9	35.6	2.1	0.0018	4.1
- all 15 (SSD) vs all 15 (PS)	1	27,699.6	140.1*	7.6	0.00001	37.7
Cross 4502 (VU162 x VU171)						
- All treatments	32	7,383.7	23.4	7.1*	0.0008	6.8
- best (SSD) vs best (PS)	1	856.8	0.5	5.0	0.0001	21.3
- 3 top (SSD) vs 3top (PS)	1	1,193.9	9.7	0.9	0.0007	0.5
- all 15 (SSD) vs all 15 (PS)	1	17,469.6	40.9	12.7	0.0012	0.2

note * significant difference at 0.05 level

Table 12 Mean pod yield and five yield components of F_4 yardlong bean progenies derived by PS and SSD from cross 4501

Progenies/Selection method	Pod yield (g/plant)	No. of pod/ plant	Pod wt. (g/pod)	No. of inflores-cence/plant	Pod diameter (cm)	Pod length (cm)
1 Best (SSD)	470.30 a	27.93 a	17.2 ab	17.40	0.77	47.60
2 Best (PS)	354.20 ab	23.97 ab	17.8 ab	17.50	0.77	49.87
3 3top (SSD)	430.47 ab	26.20 a	17.5 ab	17.10	0.77	47.37
4 3top (PS)	341.77 bc	20.82 abc	18.3 ab	16.40	0.76	49.57
5 All 15 F_4 (SSD)	328.77 bc	20.67 abc	16.1 b	15.70	0.75	44.37
6 All 15 F_4 (PS)	297.93 c	17.43 bc	17.1 ab	14.83	0.75	46.27
7 Mean parents	262.27 c	17.73 bc	13.7 b	12.23	0.72	43.67
8 National check	278.20 c	14.60 c	18.8 ab	14.77	0.79	49.43
9 Local check	299.03 c	15.30 c	19.5 a	12.20	0.77	50.86
F-test	*	**	*	NS	NS	NS
$LSD_{.05}$	116.17	8.12	2.8	-	-	-
C.V. (%)	14.6	21.3	9.4	9.3	1.6	4.9

Note means within the same column followed by the same letter are not significant difference by LSD

NS non-significant difference

* significant difference at 0.05 level

** significant difference at 0.01 level

Table 13 Mean pod yield and five yield components of F_4 yardlong bean progenies derived by PS and SSD from cross 4502

Progenies/Selection method	Pod yield (g/plant)	No. of pod/ plant	Pod wt. (g/pod)	No.of inflores-cence/plant	Pod diameter (cm)	Pod length (cm)
1 Best (SSD)	445.03 ab	22.63 bc	19.7	16.80	0.80	52.50
2 Best (PS)	503.53 a	28.10 a	18.0	16.53	0.78	52.70
3 3top (SSD)	442.10 ab	21.50 bc	20.5	16.43	0.78	52.20
4 3top (PS)	447.33 ab	23.73 b	19.4	16.43	0.77	52.10
5 All 15 F_4(SSD)	366.30 bc	18.13 d	20.1	15.10	0.77	48.76
6 All 15 F_4(PS)	368.33 bc	18.87 cd	19.1	15.07	0.76	48.90
7 Mean parents	259.95 d	12.47 e	20.9	13.60	0.74	47.03
8 National check	278.20 cd	14.60 de	18.8	14.77	0.79	49.43
9 Local check	299.03 cd	15.30 de	19.5	12.20	0.77	50.86
F-test	*	*	NS	NS	NS	NS
$LSD_{.05}$	95.64	4.25	-	-	-	-
C.V. (%)	15.5	13.4	7.0	13.5	2.6	6.2

Note means within the same column followed by the same letter are not significant difference by LSD

NS non-significant difference

* significant difference at 0.05 level

Table 14 Mean pod yield and yield components of F_4 progenies of cross 4501

	F_4 progenies, Parent and Check	Pod yield (g/plant)	No. of inflorescence/plant	No. of pod/plant
1	$4501F_3$(SSD)-08	470.30	15.3	27.9
2	$4501F_3$(SSD)-07	420.33	16.4	25.4
3	$4501F_3$(SSD)-09	400.67	17.4	21.3
4	$4501F_3$(SSD)-12	382.77	16.5	23.6
5	4501-027-02	354.20	15.1	19.8
6	$4501F_3$(SSD)-03	350.77	15.5	25.4
7	4501-015-01	343.93	15.4	20.8
8	$4501F_3$(SSD)-06	333.07	16.8	17.7
9	$4501F_3$(SSD)-13	330.33	14.6	21.2
10	4501-032-01	327.17	15.1	16.2
11	4501-032-02	326.73	17.5	19.9
12	$4501F_3$(SSD)-11	323.93	16.9	21.5
13	$4501F_3$(SSD)-10	319.80	15.8	19.0
14	4501-037-01	318.30	16.1	18.0
15	4501-034-01	316.83	15.5	24.0
16	4501-025-01	313.47	15.3	18.0
17	4501-024-08	311.90	15.2	18.0
18	$4501F_3$(SSD)-14	311.60	15.7	21.0
19	4501-027-01	309.00	13.4	18.8
20	$4501F_3$(SSD)-15	306.57	16.5	19.8
21	4501-024-01	306.03	14.4	15.7
22	$4501F_3$(SSD)-04	279.27	16.9	18.9
23	4501-039-01	277.77	13.6	16.6
24	4501-015-02	258.57	13.1	16.6
25	$4501F_3$(SSD)-02	253.47	14.3	17.9
26	4501-023-01	248.70	15.7	14.5
27	$4501F_3$(SSD)-05	246.07	15.0	14.9
28	4501-002-01	235.03	14.3	12.7
29	4501-002-02	221.80	14.4	14.2
30	$4501F_3$(SSD)-01	206.03	12.3	14.5
Mean parent		262.23	12.5	17.7
Mean check		298.61	15.5	15.0
F-test		*	NS	**
LSD.05		116.17	-	8.12
C.V.(%)		22.6	16.1	26.4

Note NS non-significant difference

* significant difference at 0.05 level

** significant difference at 0.01 level

Table 15 Pod characteristics of F_4 progenies of cross 4501

	F_4 progenies, Parent and Check	Pod diameter (cm.)	Pod length (cm.)	Pod wt. (g/pod)
1	4501F_3(SSD)-08	0.76	47.2	17.0
2	4501F_3(SSD)-07	0.77	47.6	16.5
3	4501F_3(SSD)-09	0.76	46.0	19.5
4	4501F_3(SSD)-12	0.75	47.3	17.7
5	4501-027-02	0.76	48.7	17.8
6	4501F_3(SSD)-03	0.75	44.8	13.8
7	4501-015-01	0.77	43.8	16.8
8	4501F_3(SSD)-06	0.77	46.3	18.8
9	4501F_3(SSD)-13	0.71	41.5	14.5
10	4501-032-01	0.75	44.3	20.2
11	4501-032-02	0.75	44.9	16.3
12	4501F_3(SSD)-11	0.74	44.2	17.0
13	4501F_3(SSD)-10	0.71	44.6	16.8
14	4501-037-01	0.76	49.9	17.6
15	4501-034-01	0.69	44.4	13.5
16	4501-025-01	0.75	43.7	17.6
17	4501-024-08	0.77	49.8	17.2
18	4501F_3(SSD)-14	0.74	42.1	14.7
19	4501-027-01	0.75	46.0	16.4
20	4501F_3(SSD)-15	0.77	43.2	16.9
21	4501-024-01	0.76	49.2	19.6
22	4501F_3(SSD)-04	0.77	44.3	17.7
23	4501-039-01	0.76	45.3	16.6
24	4501-015-02	0.75	44.9	15.5
25	4501F_3(SSD)-02	0.72	42.8	14.2
26	4501-023-01	0.72	44.3	17.1
27	4501F_3(SSD)-05	0.77	43.5	16.9
28	4501-002-01	0.74	48.9	18.2
29	4501-002-02	0.75	45.7	15.6
30	4501F_3(SSD)-01	0.73	40.8	14.0
Mean parent		0.72	43.7	13.7
Mean check		0.78	50.2	19.1
F-test		NS	NS	**
LSD.05		-	-	2.6
C.V.(%)		4.7	8.9	9.6

Note NS non-significant difference

** significant difference at 0.01 level

Table 16 Mean pod yield and yield components of F_4 progenies of cross 4502

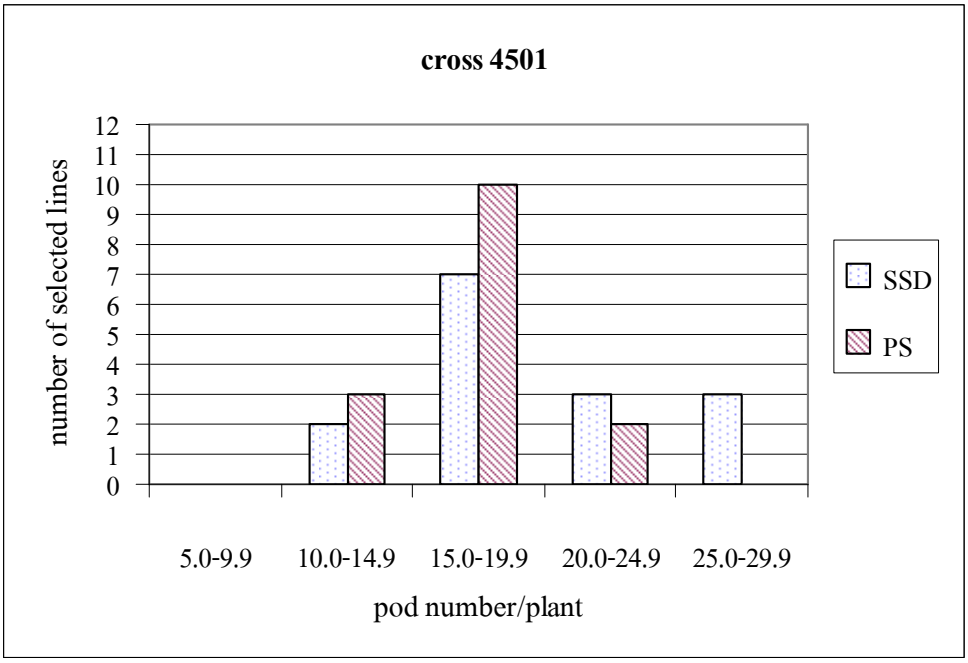
	F_4 progenies, Parent and Check	Pod yield (g/plant)	No. of inflorescence/plant	No. of Pod/plant
1	4502-005-02	503.53	15.2	28.1
2	4502 F_3(SSD)–07	445.03	16.5	22.6
3	4502-014-02	442.20	15.5	20.5
4	4502 F_3(SSD)-05	440.70	14.3	21.0
5	4502 F_3(SSD)-04	440.57	14.4	21.0
6	4502 F_3(SSD)-02	420.53	16.0	20.5
7	4502-005-01	396.27	13.8	21.1
8	4502 F_3(SSD)-06	394.87	14.6	19.5
9	4502 F_3(SSD)–14	391.50	15.1	18.9
10	4502-009-01	389.73	15.2	19.7
11	4502-022-01	387.10	16.5	22.0
12	4502-022-01	384.23	14.4	19.8
13	4502 F_3(SSD) – 03	378.13	15.2	19.3
14	4502-018-02	377.47	16.4	19.8
15	4502-014-01	368.07	15.1	18.4
16	4502-029-01	364.40	14.6	19.5
17	4502 F_3(SSD)-11	356.90	16.8	17.6
18	4502 F_3(SSD)-15	351.83	13.8	17.5
19	4502 F_3(SSD)-01	351.53	15.3	17.6
20	4502-017-01	343.87	14.7	16.7
21	4502-018-01	329.57	14.7	18.4
22	4502 F_3(SSD)-08	329.37	14.7	16.0
23	4502-029-02	326.00	16.3	17.5
24	4502 F_3(SSD)-12	315.33	15.1	16.1
25	4502-030-01	314.10	15.2	15.9
26	4502-022-02	313.30	14.7	16.8
27	4502 F_3(SSD)-10	301.83	14.5	15.6
28	4502 F_3(SSD)-13	294.80	14.2	15.2
29	4502-022-03	284.80	14.0	15.7
30	4502 F_3(SSD)-09	281.47	16.1	13.7
Mean parent		259.93	13.6	12.4
Mean check		298.61	13.5	15.0
F-test		*	NS	*
LSD.05		95.64	-	4.2
C.V.(%)		25.7	13.9	24.1

Note * significant difference at 0.05 level
NS non-significant difference

Table 17 Pod characteristics of F_4 progenies of cross 4502

	F_4 progenies, Parent and Check	Pod diameter (cm)	Pod length (cm)	Pod wt. (g/pod)
1	4502-005-02	0.77	49.3	18.0
2	4502 F_3 (SSD)–07	0.79	52.5	19.7
3	4502-014-02	0.77	48.6	21.4
4	4502 F_3 (SSD)-05	0.77	51.3	20.9
5	4502 F_3 (SSD)-04	0.77	52.4	21.0
6	4502 F_3 (SSD)-02	0.78	50.4	20.4
7	4502-005-01	0.76	49.9	18.6
8	4502 F_3 (SSD)-06	0.76	49.2	20.3
9	4502 F_3 (SSD)–14	0.75	45.3	20.6
10	4502-009-01	0.78	51.1	20.2
11	4502-022-01	0.78	48.5	18.0
12	4502-022-01	0.80	52.7	19.4
13	4502 F_3 (SSD) – 03	0.75	45.0	19.6
14	4502-018-02	0.76	49.1	19.1
15	4502-014-01	0.76	46.4	19.8
16	4502-029-01	0.75	52.6	18.4
17	4502 F_3 (SSD)-11	0.77	45.8	20.3
18	4502 F_3 (SSD)-15	0.77	48.8	19.9
19	4502 F_3 (SSD)-01	0.78	51.8	19.8
20	4502-017-01	0.76	50.5	20.7
21	4502-018-01	0.76	49.2	18.0
22	4502 F_3 (SSD)-08	0.77	46.4	20.6
23	4502-029-02	0.78	44.9	18.6
24	4502 F_3 (SSD)-12	0.73	47.4	19.6
25	4502-030-01	0.76	48.7	19.7
26	4502-022-02	0.75	45.7	18.5
27	4502 F_3 (SSD)-10	0.78	50.9	19.4
28	4502 F_3 (SSD)-13	0.75	46.5	19.4
29	4502-022-03	0.75	47.0	18.4
30	4502 F_3 (SSD)-09	0.80	48.7	20.6
Mean parent		0.73	47.1	20.0
Mean check		0.78	50.2	19.1
F-test		NS	NS	NS
LSD.05		-	-	-
C.V.(%)		2.8	8.1	7.8

Note NS non-significant difference



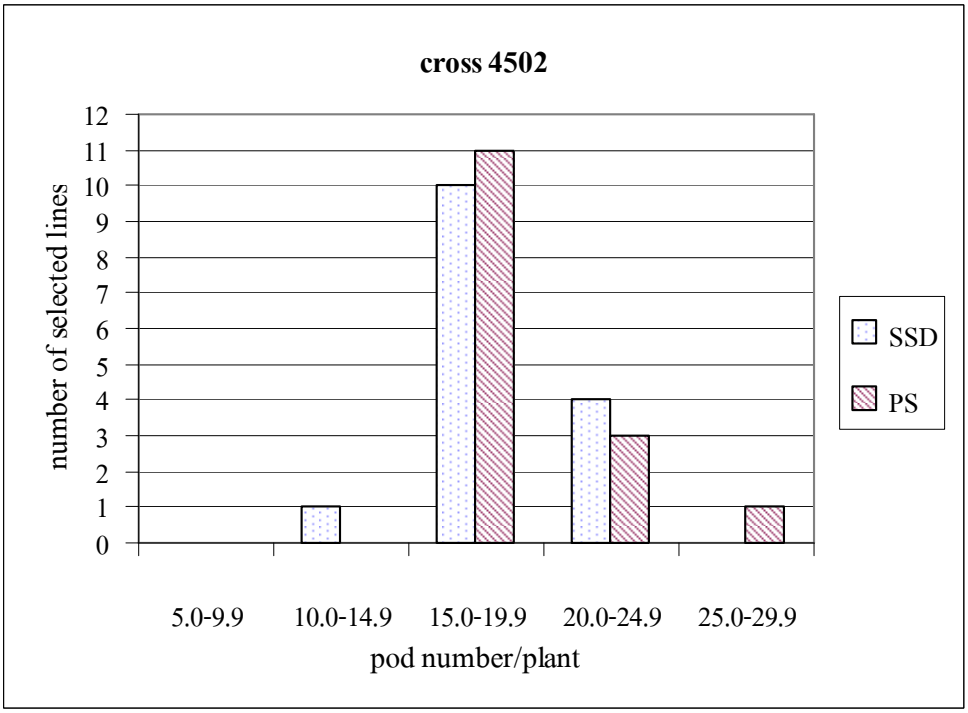
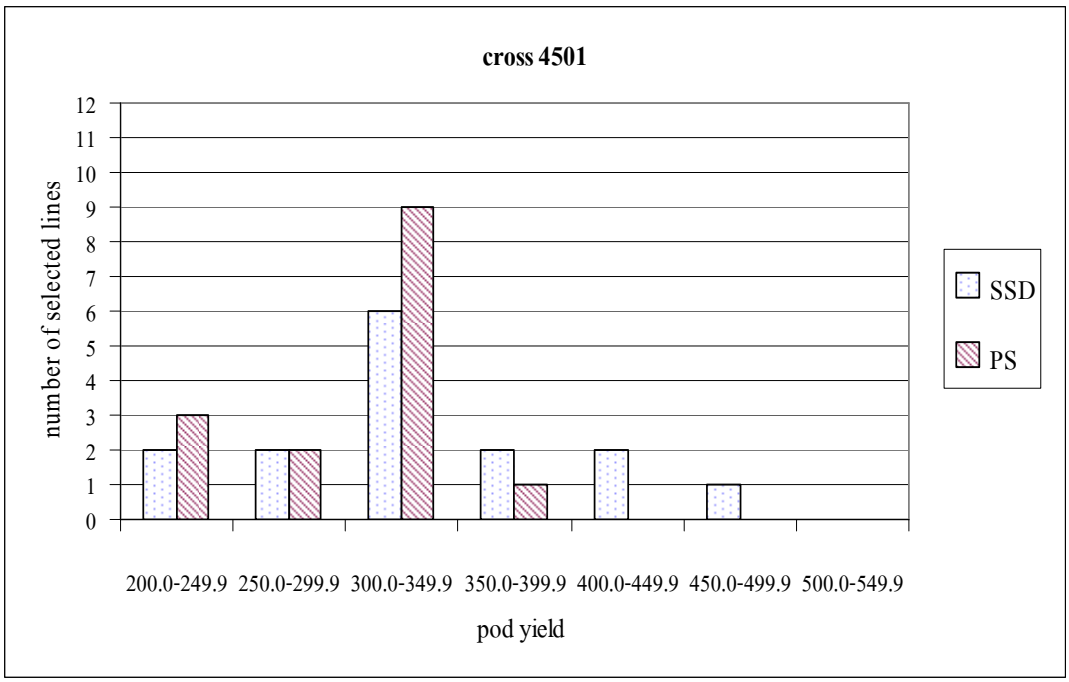


Figure 8 Pod number per plant of F_4 lines derived by single seed descent (SSD) and pedigree selection (PS) methods from two yardlong bean crosses



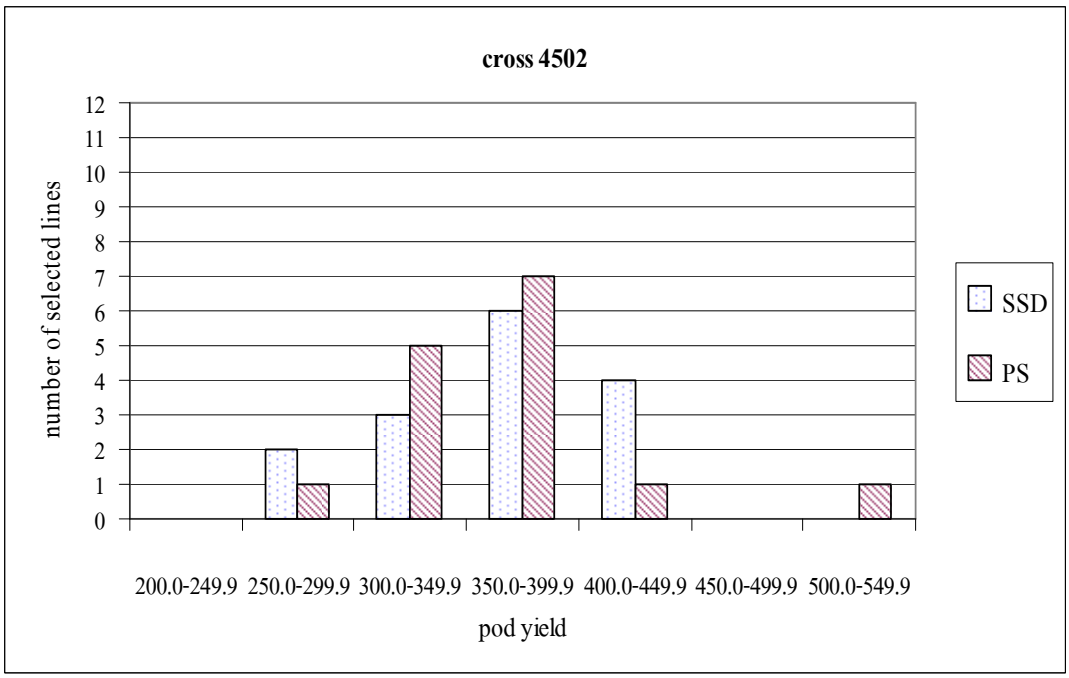


Figure 9 Pod yield (g/plant) of F_4 lines derived by single seed descent (SSD) and pedigree selection (PS) methods from two yardlong bean crosses

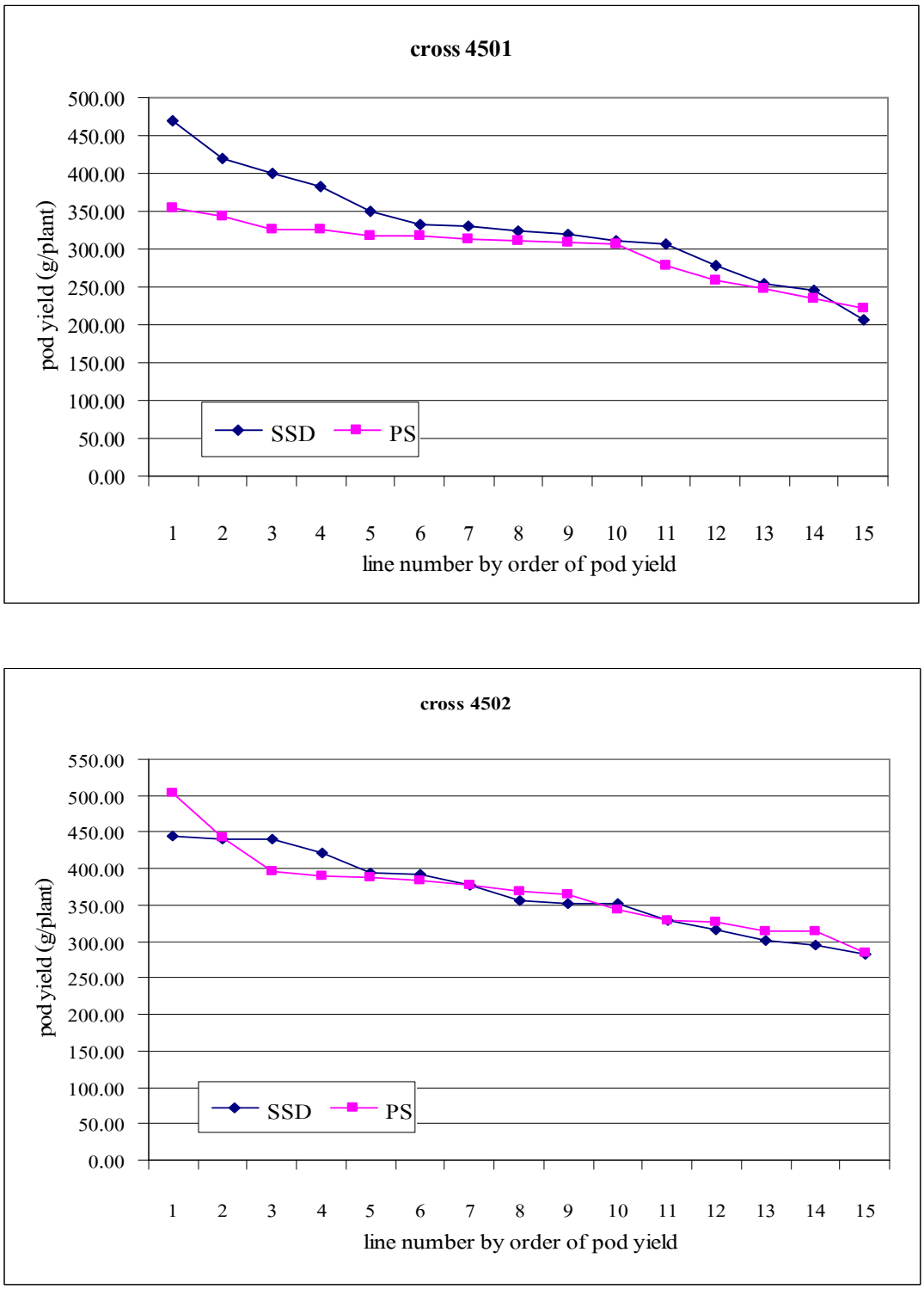


Figure 10 Pod yield (g/plant) of fifteen F_4 lines derived by single seed descent (SSD) and pedigree selection (PS) methods from two yardlong bean crosses

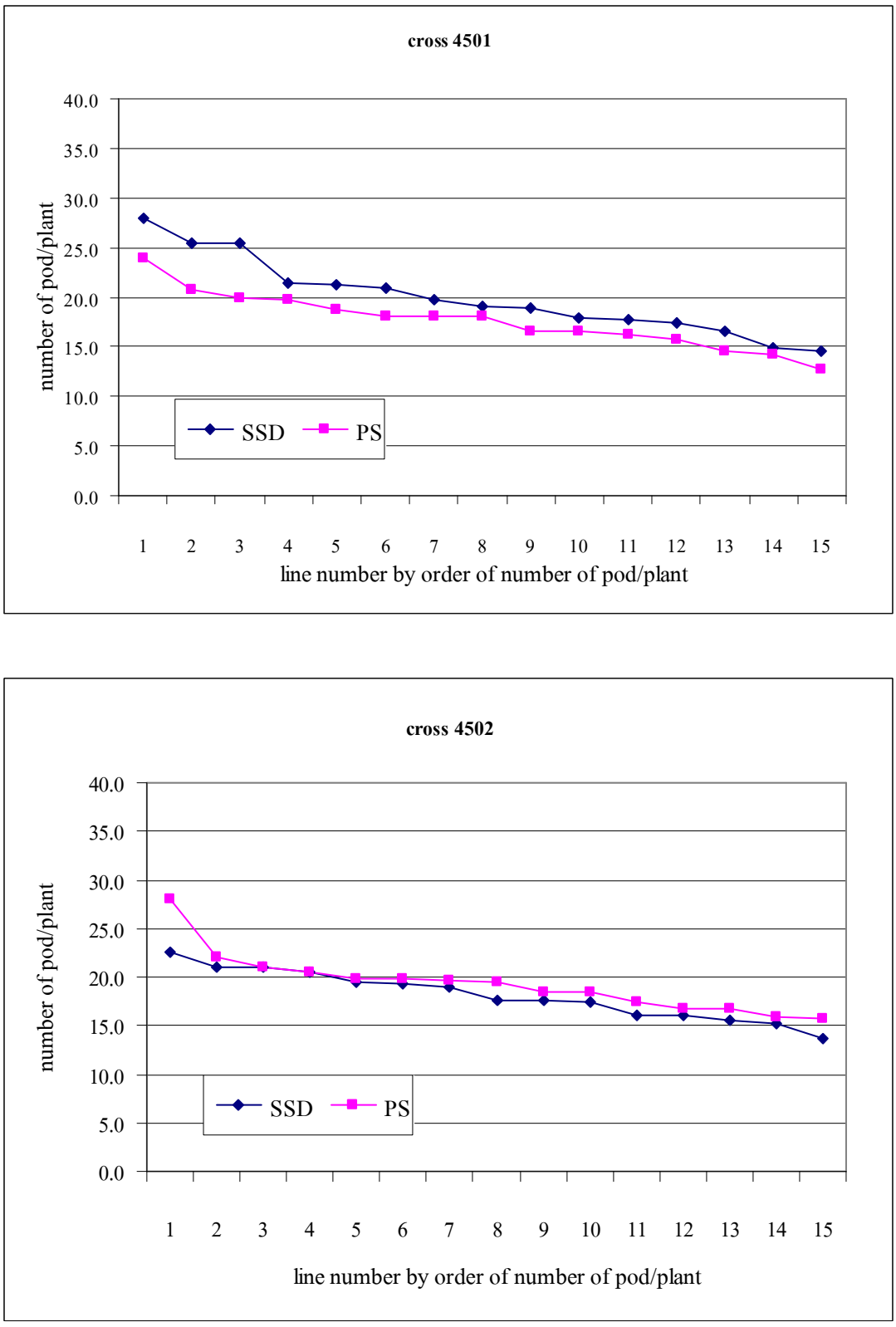
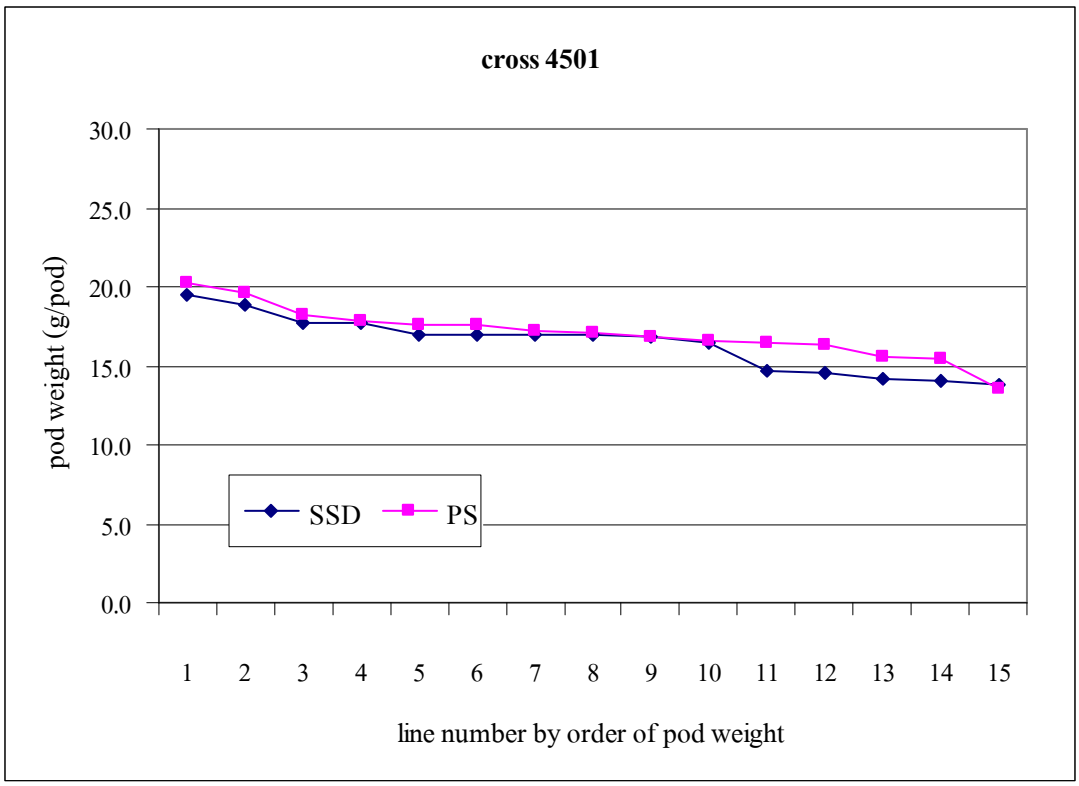


Figure 11 Number of pod per plant of fifteen F_4 lines derived by single seed descent (SSD) and pedigree selection (PS) methods from two yardlong bean crosses



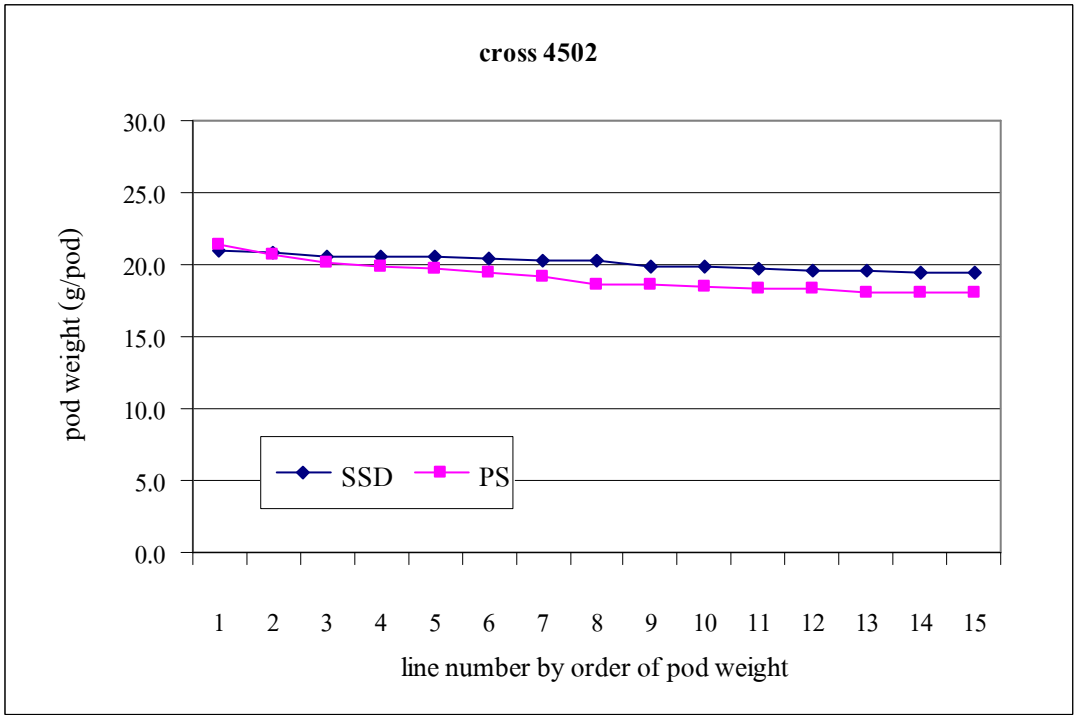


Figure 12 Pod weight (g/pod) of fifteen F_4 lines derived by single seed descent (SSD) and pedigree selection (PS) methods from two yardlong bean crosses

Table 18 Heritability (h^2) in narrow sense for pod yield and five yield components in two yardlong bean crosses

Traits	Heritability (%)	
	cross 4501	cross 4502
Regression of F_4 progenies on F_3 parents		
Pod yield	2.64 (±0.36)	1.69 (±0.28)
Pod weight	10.45 (±0.18)	4.50 (±0.21)
No of pod/Plant	10.53 (±0.19)	7.39 (±0.47)
No. of inflorescence/plant	0.07 (±0.04)	0.84 (±0.32)
Pod diameter	21.83 (±0.30)	0.03 (±0.18)
Pod length	22.36 (±0.26)	0.01 (±0.07)

Table 19 Correlation coefficients between pod yield and its components among F_4 progenies of cross 4501 and 4502

Cross	Traits	Pod weight. (g./pod)	No.of inflores-cence/plant	No. of pod/plant	Pod diameter	Pod length	Pod yield
4501	Pod weight.	1.0000	0.0389	-0.4839	0.5211*	0.3546	0.1913
	No. inflorescence/plant		1.0000	0.3727	-0.0453	-0.0306	0.4438
	No. pod/plant			1.0000	-0.3645	-0.02334	0.7540**
	Pod diameter				1.0000	0.2681	0.0961
	Pod length					1.0000	0.0301
	Pod yield						1.0000
4502	Pod weight.	1.0000	0.0368	-0.2563	0.3529	0.1628	0.1247
	No. inflorescence/plant		1.0000	0.1959	0.0353	-0.3118	0.1914
	No. pod/plant			1.0000	0.2114	0.2874	0.9229**
	Pod diameter				1.0000	0.3405	0.3626
	Pod length					1.0000	0.3488
	Pod yield						1.0000

Note * significant 0.05 level

** significant 0.01 level

DISCUSSION

A yardlong bean breeding program comprises of four important procedures as other self-pollinated plants. First, breeder must collect and evaluate germplasm materials to select as parental lines. Morphological characters obtained from field experiment are important information in breeding program. However, genetic characterization and relatedness among collected germplasm at DNA level also provide useful information for breeder to select a suitable parental lines. Second step, making crosses between parental lines to produce genetic variability population as source materials for selection. Third step, selection desirable recombinant genetic lines. Breeder must consider which effective selection procedure should be used in their breeding program. Evaluation of selected lines finding for best lines use as new elite varieties was the fourth or the last step in any breeding program.

1. Evaluation of germplasm for parental varieties

In this study a 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 were evaluated. Data from field experiment showed various morphological characters. Most yardlong bean, 22 of 24 accessions were indeterminate growth habit, while 10 of 13 cowpea were determinate and other 3 accessions had semi-determinate growth habit. Almost yardlong bean had higher pod yield than cowpea. This study showed that the top seventeen highest pod yield were found in yardlong bean accessions. Mean pod yield of 24 yardlong bean was 212.1 g/plant while that of 13 cowpea was 117.4 g/plant, or only 45.4% compared to yardlong bean. Yardlong bean produced higher pod yield than cowpea because of its indeterminate in growth habit. Indeterminate growth habit allowed yardlong bean had long reproductive time periods. During harvesting, it is still developed new inflorescences and produced consecutive new pods yield from newly inflorescences. The top five highest pod yield found in yardlong bean were SR99-334, VU163, VU171, VU012 and VU162. VU162 was considered as the best among southern domestic germplasms. It was expected to be the most suitable female parent. SR99-334 which produced the highest pod yield was susceptible to aphid,

one of an important insect pest in yardlong bean production. VU012, the fourth top yield rank had relative low consuming qualities with score of 3.0 while VU135, the ten top yield showed the best consuming qualities (score of 5.0). Another interested variety was VU189. It showed vigorous peduncle and produced consecutive multiple well developed pods. It was the earliest variety with short harvested time period. VU163, VU171, VU135 and VU189 were the first round four list of interest superior varieties for used as male parents.

2. RAPD marker and genetic relatedness

Conventionally, the genetic diversity has been estimated on the basis of morphological characters. However, morphological studies alone do not provide sufficient information to understand genetic diversity within the species, as well as relatedness to related species. A number of studies about relationship within the Asian *Vigna* using DNA markers have been carried out, including RFLP (Fatokun *et al*., 1993), RAPD (Kaga *et al*., 1995; Pooprompan *et al*.,1996), AFLP (Yee *et al*., 1999: Yoon *et al*., 2000) and microsatellite (Li *et al*., 2001). The choice of appropriate marker depends not only on the particular objectives of the study, but also on the cost and time investments. In this study, RAPD was used to evaluate genetic variation as well as revealed genetic relatedness among yardlong bean and cowpea. RAPD is an effective and relatively inexpensive, not requiring any prior sequence information and therefore can be applied to a wide range of plant and animal taxa. (Karp and Edwards, 1997). This technique also allow the analysis of a large number of samples in a short time (Williams *et al*., 1990). RAPD analysis has been used for diversity studies in several legume species for example, food and feed legumes such as common lima and adzuki beans, broadbeans, soybean, chickpeas alfalfa and lupin etc. (Weder, 2002).

With 5 primers used in this study (OPC-06, OPR-12, OPZ-03, OPZ-08 and OPZ-13), the size of the amplified fragment ranged from approximately 225 bp to 1650 bp. Pooprompan *et al*. (1996) identified various varieties of yardlong bean and cowpea accessions across Thailand and some Asian countries by RAPD and reported size of fragment varied from 500 to 2200 bp, while 940 to 1100 bp fragments were reported by Phansak *et al.* (2001). However, primers used by each research groups were different from primers used in our

experiment. Based on a dendrogram constructed from 23 polymorphic bands revealed fairly good separation of genetic groups between yardlong bean and cowpea indicating some good relationship between growth habit and genetic relatedness. However, VU 189 and Kaohinsornt, two improved yardlong bean accessions derived from crossing between yardlong bean and cowpea which exhibited determinate growth were classified in cowpea group with long pod (34.9 and 34 cm., respectively). The relationship between growth habit and genetic relatedness was also realized among 38 Malawian cowpea (Nkongolo, 2003) and 7 cultivated Senegalese cowpea (Laity *et. al*., 2003). No specific fragment was found to be linked to growth habit from 5 primers used in the present study. More RAPD primers have to be screened or other appropriate technique will be used for further studies. Similarity coefficients among yardlong bean and cowpea in our study ranged from 0.484-1.00. The highest distant pairs are SR99-334 (yardlong bean) and VU176 (cowpea). Three pairs of accessions appeared to be identical, which were IT 82E-16 and IT82E-9, VU 136 and VU 063, Pranomsarakram and NR007. These results can be explained by 1) they originated from the same parental lines (particular for the first and the second pairs) resulting in very closed relation between them or 2) in case of Pranomsarakram and NR007, accessions brought from the local market, they may be the same but in different name. Phansak *et al*. (2005) used Sequence Tagged Microsatellite Site (STMS) to evaluate genetic diversity among *Vigna* and reported VU 173 and VU 174 was identical. Based on RAPD analysis from this present study, these 2 accessions were different with similarity coefficient 0.786. Genetic diversity among yardlong bean was relatively higher than that of cowpea. Vaillancourt *et al*. (1993) reported that cultivated cowpea had little diversity while wild cowpea was very diverse. This finding was supported by Li *et al*. (2001) who studied genetic diversity in cultivated cowpea accessions by microsatellite. They concluded that the cultivated cowpea is relatively low in genetic diversity compared with other crops.

Results from field experiment and molecular markers, could provide useful tools for germplasm characterization, conservation and utilization as well as genetic and breeding studies in *Vigna unguiculata*. From the first lists of interested superior accessions chosen (VU 163, VU171, VU 135, and VU189), VU 163 and VU135 had high related to VU 162 with similarity coefficient of 0.935 and 0.903, respectively while VU 171 and VU 189 showed relative low genetic relationship to VU 162 with similarity coefficient 0.742 and 0.813, respectively.

Genetic relationship analysis could be useful to select parent to be crossed for genetic appropriate population intended for genome mapping and breeding purposes. The most distantly two sexually compatible individually are related taxonomically, the higher of frequency of polymorphism detected between them. From this reason, VU 171 and VU 189 were then chosen and used as male parents to make cross with VU 162 to produce two single cross: cross 4501 (VU 162 × VU 189) and 4502 (VU 162 × VU 171) which used as original population materials for yardlong bean improvement focus on yield and yield components. VU 162 and VU 171 had good consumed quality, pod length at 58.3 and 48.7 cm., respectively and produced higher yield than VU 189. VU 162 and VU 171 have indeterminate growth habit while VU 189 is determinate with shorter pod length and flower earlier (35 days after planting).

3. Comparison of pedigree and single seed descent methods

Effective selection procedure during succeeding generations is the most important role of any breeding program to achieve a successful ultimate goal. Breeders must carefully make decision which appropriate selection procedure should be used in their project. There are many selection procedures but no perfect method for general used in all crop plants. Effective of selection depends on different items including the trait will be improved, genetic inheritance of the trait, environment-genotype interaction and final variety type products of the breeding program (Ranalli and Cubero, 1997). There are three most commonly used selection methods in self-pollinated crops: pedigree (PS) single seed descent (SSD) and bulk method (BM). Genetic variability and gene frequency of population succeeded by bulk method was highly associated to environmental conditions. We considered that BM was non-flavourable for yardlong bean improvement because yardlong bean was very susceptible to environmental conditions.

3.1 Pod yield and yield components between generations

Pod yield and yield components among F_3 and F_4 derived by two selection methods and F_2 showed similarly result in both 4501 and 4502 populations. They produced non-significant difference in pod yield and five yield components among generations, indicating that

selection for pod yield and five yield components in early (F_2 and F_3) generations were ineffective in yardlong bean. Tee and Qualset (1975) compared yield and agronomic traits among generations of two wheat populations. They reported a very similar results to our studied. Generation means increased from F_3 to F_6 only for plant height in both populations where recessive genes for dwarfness were segregating. Their result indicated that selection for certain highly heritable characters such as tallness/shortness was effective. On the contrary, Ntare *et al.* (1984) reported that yielding ability of F_3 cowpea lines persisted over later generations indicating that selection for pod yield was effective in cowpea. Their results was confirm by the high significant correlations between F_3 yields and those of later generations which ranged from r = 0.51* to 0.85*. They also found significant linear correlation between visual rating of F_3 and F_6 yields with actual yields revealed that it is possible to identify promising lines of cowpea visually.

Salas and Friedt (1995) compared the efficiency between SSD and PS in four linseed populations. They found that early selection for seed yield, a character with low heritability was not successful. They suggested that selection for seed yield in linseed should be postponed to later inbred generations. Their results showed minimum differences between PS and SSD lines for grain yield, while in only one cross, the SSD lines were significantly superior to the pedigree lines.

3.2 Pod yield and yield components of F_4 progenies from PS and SSD methods

Mean pod yield of F_4 progenies derived by SSD and PS was non-significantly different in both populations (Table 10). In the 4501 population, the SSD progenies produced non-significant higher pod yield than the PS progenies (328.8 and 298.0 g/plant, respectively), while the SSD and PS progenies from the 4502 population produced almost the same yield (366.3 and 368.3 g/plant, respectively). The results from the present study found that selecting lines from early generations for pod yield was ineffective in yardlong bean, as has been previously reported in wheat (De Pauw and Shebeski, 1973; Inagaki *et al*., 1998), barley (Hanson *et al*., 1979), mungbean (Gill *et al*., 1995), linseed (Salas and Friedt, 1995), rice (Nagai, 1962) and blackgram (Arshad, 2004).

For yield components, significant differences were found in almost all characters in the 4501 population, notably number of pods/plant, pod weight, pod length, pod diameter and number of inflorescens/plant. F_4 progenies of SSD method had a higher number of pods/plant than PS, while only the number of pods/plant was significantly different from the mean parents (Table 10). The best SSD progeny also produced non-significantly higher pods/plant than the best PS (27.93 vs. 23.97 pods/plant, respectively) (Table 12), while the best PS progeny in the 4502 population produced significantly ($p< .05$) higher pods/plant than the best SSD (28.10 vs. 22.63 pods/plant, respectively) (Table 13). The mean number of inflorescences per plant, pod diameter and pod length of F_4 progenies of both methods, mean parents and check cultivars were non-significantly different in both populations (Tables 12, 13). Since non-significant differences were found between the two methods of selection, SSD may be suitable for yield improvement in yardlong bean because it required less selection effort than the pedigree and early generation yield testing procedures. Example of cultivars released, Inca and Inca-LD are two yardlong bean cultivars created through SSD (Ponce and Casanova, 1999). Gill *et al*. (1995) studied in mungbean and reported the SSD method was preferable to the PS and bulk methods because of the shorter time required and the better cost effectiveness in handling segregating generations. The same recommendation and reasons were given for wheat by Van Oeveren (1992). In contrast, pedigree selection was found to be superior to SSD for seed size of greengram (*Vigna radiata* L.) (Dahiya and Singh, 1986). Several cultivars of cowpea have previously been derived from the PS method, such as Mouride, Melakk, Ein El Gazal, etc (Singh *et al*., 2002). The success of pedigree selection is based on the number of segregation plants to be selected and the traits of interest should be highly heritable and predominantly controlled by additive gene.

Padi and Ehlers (2008) made selection for grain yield in cowpea and reported F_4 lines derived from the highest 10% performing F_3 individuals were no higher yielding than F_4 lines derived from the remaining F_3 individuals, indicating that early generation selection for yield was ineffective. Single-seed descent (SSD) or bulk breeding methods will be more efficient than pedigree breeding for developing cowpea varieties with high yield potential.

4. Heritability

For the improvement of any crops, knowledge of the relation among various characters with yield is essential in order to find appropriate selection criteria. Also type of selection to be done and progress from selection for a particular character depends in part on the magnitude of heritability estimate. This is because the expected response under selection is a function of heritability, variation and selection density. The response can be predicted if the correlation and the heritability of the characters are known.

In two populations of yardlong bean: 4501 and 4502, as in most crops, yield has a low heritability (2.64 and 1.69%, respectively) because of environmental variable. The same findings was reported by Santhadphanich (1987), she found that heritability in broad senses for pod yield were varied from 4.03-25.30%. In contrast, Pornsuriya (1994) estimated heritability from 3 yardlong bean population and reported the highest narrow- sense heritability for pod yield of 66.08%. This heritability is quite high because he estimated by using variance components so the environmental and its interaction variance were confound with genetic variance. Tee and Qualset (1975) estimated heritability in F_4-F_6 of two wheat crosses and they reported a high varying heritability for wheat yield from 3.2 to 72.4%. Low estimated heritability for pod yield from the present study confirmed that lines selection in early generation was ineffective for yield improvement in yardlong bean. It has been reported that pedigree was the most effective selection method when the heritability was high (75%) and moderate (50%). With heritability around 10%, SSD without prior selection would be the preferred method (Tigchelaar and Casali, 1976). In the case of early generation testing, Cooper (1990) reported that it is effective in identifying superior pure lines, but requires extra yield testing.

5. Correlation coefficient

Based on low heritability in yield, a number of breeders have been used other traits that are related to yield and express high heritability for indirect selection. Results from the present study showed that pod number/plant showed highly significant positive correlated with pod yield in both populations with 0.754** and 0.923**, respectively. However pod number/

plant has low heritability (18.48% in the 4501 population and 12.93% in the 4502 population). Higher estimation of heritability was found in other characters such as pod length and pod diameter. Arshad (2004) reported a similar results to present study, he reported that number of pod per plant was correlated positively to grain yield in all eleven crosses of blackgram. Santhadphanich (1987) reported that pod weight was negative correlation to number of pod/plant with correlation coefficient range of -0.031 to -0.394. Mehta and Zaveri (1998) studied the association between traits of *Vigna unguiculata* and they found that seed yield/plant was strongly and positively associated with branches/plant and clusters/plant. In grain legume such as black gram seed yield/plant showed significant positive correlation with petiole length, total dry matter, height and primary leaf area (Arshad, 2004). In faba bean, Sinhu *et al*. (1986) reported that the efficiency of selection has been improved up to 30% by some combinations of characters. In order to get the good choice of characters for indirect selection, correlation and path coefficient must be analyzed (Ranalli and Cubero, 1997).

CONCLUSION

A total of 37 yardlong bean and cowpea accessions were grown for morphological evaluation. Genetic analysis and relatedness among all accessions were also studied. A dendrogram based on 23 RAPD polymorphic fragments obtained from 5 primers (OPC-06, OPR-12, OPZ-03, OPZ-08 and OPZ-13) revealed fairly good separation of groups between yardlong bean and cowpea. Based on morphological characters and genetic relatedness, VU162 was chosen as a female parent while VU171 and VU189 were used as male parents. Crossing were made between VU162 × VU189 (cross no. 4501) and VU162 × VU171 (cross no. 4502) to produce two F_1 hybrids. Each F_1 hybrid was self and two segregated F_2 populations were used as sources for yardlong bean improvement. The effectiveness between 2 selection methods, pedigree and single seed descent were studied. Results indicated that no significant difference was found between PS and SSD for pod yield and yield components in two crosses of yardlong bean. However, the best and the three top F_4 progenies derived by PS and SSD of both populations produced higher pod yield than the mean parent and check cultivars. The number of pods per plant had the highest positive correlation to pod yield in both populations with correlation coefficients (r) of 0.7540** and 0.9229**, respectively. Narrow-sense heritability for pod yield in the 4501 and 4502 populations were low estimation of 4.62 and 2.96 %, respectively. The results obtained from this study indicated that genetic advances in yield and yield components of F_4-based yardlong bean progenies from PS and SSD method were not effective. F_4 is still very heterogenic and will be segregating in the subsequent generations, at least F_6 or more generation should be performed. Early generation testing is effective in identifying superior pure lines, but requires extra yield testing.

The present study revealed equally effective of SSD and PS methods for pod yield improvement in yardlong bean. We concluded that the SSD was preferred for yardlong bean improvement because of the shorter time required and the better cost effectiveness in handling segregating generations.

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