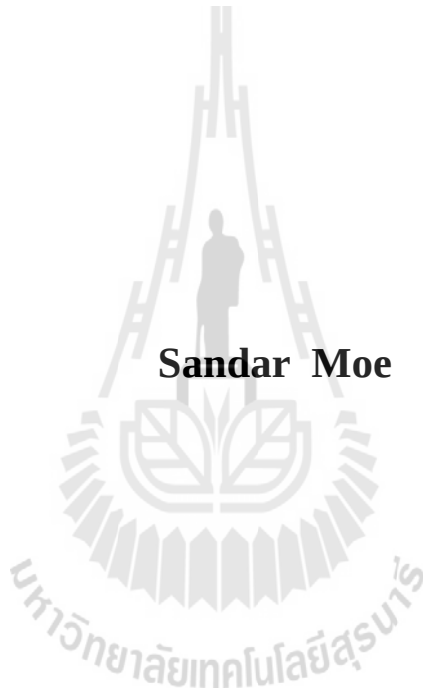
RELATIONSHIPS OF SOYBEAN (*Glycine max* L.) ACCESSIONS BASED ON AGRO-MORPHOLOGICAL, PHYSIOLOGICAL TRAITS AND DNA POLYMORPHISMS

Sandar Moe



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ความสัมพันธ์ของถั่วเหลือง (*Glycine max* L.) สายพันธุ์ต่าง ๆ โดยใช้ลักษณะทางสัณฐานวิทยา สรีรวิทยา และความแตกต่างของดีเอ็นเอ

นางสาวแซนดาร์ โม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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RELATIONSHIPS OF SOYBEAN (*Glycine max* L.) ACCESSIONS BASED ON AGRO-MORPHOLOGICAL, PHYSIOLOGICAL TRAITS AND DNA POLYMORPHISMS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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SANDAR MOE : RELATIONSHIPS OF SOYBEAN (*Glycine max* L.) ACCESSIONS BASED ON AGRO-MORPHOLOGICAL, PHYSIOLOGICAL TRAITS AND DNA POLYMORPHISMS

THESIS ADVISOR : TEERAYOOT GIRDTHAI, Ph.D., 150 PP.

SOYBEAN ACCESSIONS/MORPHOLOGICAL TRAIT/PHYSIOLOGICAL TRAIT/DNA POLYMORPHISMS/ISSR MARKERS

In breeding program, genetic diversity evaluation among germplasms is an importance and a prerequisite. The objectives of this research were; 1) to evaluate the diversity of soybean accessions based on agro-morphological, physiological traits and DNA polymorphism using ISSR markers and 2) to identify the correlation of the tested traits. ANOVA test for agro-morphological traits showed that the variations due to genotypes were highly significant. For agro-morphological traits, 94 accessions were grouped into 7 different clusters at similarity coefficient 0.52 by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The analysis revealed that cluster I, II, III, IV, V, VI, and VII consisted of 23, 37, 5, 4, 18, 5, and 2 soybean accessions. Positive and significant correlations were observed between yield and all other traits, except seeds per pod. Physiological traits were studied on 27 soybean accessions with maturity date of more than 100 days. The analysis of variance for physiological traits showed that the variations due to genotypes were significant. In the physiological traits, there were negative and highly significant correlations between specific leaf area (SLA) and yield (-0.54^{**}) while SLA showed no association with SPAD chlorophyll meter reading, SCMR (-0.39^{ns}). Yield and SCMR were also not correlated (0.20^{ns}). For the physiological traits, 4 major groups were divided

for 27 soybean accessions by using UPGMA method. Cluster I, II, III, and IV consisted of 11, 5, 9, and 12 soybean accessions. A large genetic diversity was detected among the samples based on estimation of DNA products amplified from seven selected ISSR primers, with the similarity coefficient varying from 0.5 to 1.0. The highest similarity with S_{ij}, 1.0 was observed between Forrest and OTOOTAN and between Ka La Dam and Prolina. Genetic patterns and correlation information obtained from this study can be helpful for parental selection in the future breeding program.

School of Crop Production Technology Student's Signature_____

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แซนดาร์ โม : ความสัมพันธ์ของถั่วเหลือง (*Glycine max* L.) สายพันธุ์ต่าง ๆ โดยใช้ลักษณะทางสัณฐานวิทยา สรีรวิทยา และความแตกต่างของดีเอ็นเอ (RELATIONSHIPS OF SOYBEAN (*Glycine max* L.) ACCESSIONS BASED ON AGRO-MORPHOLOGICAL, PHYSIOLOGICAL TRAITS AND DNA POLYMORPHISMS)
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การประเมินความหลากหลายทางพันธุกรรมเป็นสิ่งที่สำคัญ และควรทำเป็นอันดับแรกในโปรแกรมการปรับปรุงพันธุ์ งานวิจัยนี้มีวัตถุประสงค์เพื่อ 1. ประเมินความหลากหลายทางพันธุกรรมของถั่วเหลืองสายพันธุ์ต่าง ๆ โดยใช้ลักษณะทางสัณฐานวิทยา สรีรวิทยา และความแตกต่างของดีเอ็นเอโดยใช้เครื่องหมาย ISSR และ 2. เพื่อศึกษาความสัมพันธ์ระหว่างลักษณะต่าง ๆ จากการศึกษาพบว่าถั่วเหลืองสายพันธุ์ต่าง ๆ มีความแตกต่างทางสัณฐานวิทยาอย่างมีนัยสำคัญยิ่งทางสถิติ โดยถั่วเหลืองทั้ง 94 สายพันธุ์สามารถแบ่งกลุ่มได้ 7 กลุ่มที่ระดับสหสัมพันธ์ความคล้ายคลึง 0.52 โดยวิธี Unweighted Pair Group Method with Arithmetic Mean (UPGMA) ซึ่งกลุ่มที่ 1, 2, 3, 4, 5, 6 และ 7 ประกอบด้วยถั่วเหลือง 23, 37, 5, 4, 18, 5 และ 2 สายพันธุ์ตามลำดับ และพบว่าผลผลิตมีความสัมพันธ์ทางบวกกับลักษณะทางสัณฐานวิทยาที่ศึกษาอย่างมีนัยสำคัญยิ่งทางสถิติยกเว้นกับลักษณะเมล็ดต่อฝัก และจากการศึกษาลักษณะทางสรีรวิทยาโดยคัดเลือกจากสายพันธุ์ต่าง ๆ 27 สายพันธุ์ ที่มีอายุเก็บเกี่ยวมากกว่า 100 วันหลังปลูก พบว่าทั้ง 27 สายพันธุ์ มีความแตกต่างทางสรีรวิทยาอย่างมีนัยสำคัญทางสถิติ โดยพบว่าผลผลิตมีความสัมพันธ์ทางลบกับลักษณะพื้นที่ใบจำเพาะ (SLA) (-0.54**) ในขณะที่ลักษณะ SLA ไม่มีความสัมพันธ์กับลักษณะ SPAD chlorophyll meter reading (SCMR) (-0.39^{ns}) และยังพบว่าผลผลิตไม่มีความสัมพันธ์กับลักษณะ SCMR (0.20^{ns}) โดยถั่วเหลืองทั้ง 27 สายพันธุ์ สามารถแบ่งกลุ่มออกด้วยวิธี UPGMA ได้เป็น 4 กลุ่ม ซึ่งกลุ่มที่ 1, 2, 3, และ 4 ประกอบด้วยถั่วเหลือง 11, 5, 9 และ 2 สายพันธุ์ นอกจากนี้ยังพบความแตกต่างของสายพันธุ์จากการประเมินโดยใช้เครื่องหมาย ISSR 7 ชนิดที่สหสัมพันธ์ความคล้ายคลึงตั้งแต่ 0.5 ถึง 1.0 โดยที่ระดับสหสัมพันธ์ความคล้ายคลึงสูงสุด 1.0 พบความแตกต่างระหว่างสายพันธุ์ Forrest และ OTOOTAN และระหว่างสายพันธุ์ KaLa Dam และ Prolina รูปแบบของลักษณะทางพันธุกรรม และข้อมูลความสัมพันธ์ของลักษณะต่าง ๆ จากการทดลองนี้สามารถนำไปใช้ในการคัดเลือกสายพันธุ์พ่อแม่ในโปรแกรมการปรับปรุงพันธุ์พืชได้อีกด้วย

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LIST OF ABBREVIATIONS

%	=	Percentage
etc.	=	et cetera (and so on)
et al.	=	et alia (and others)
DNA	=	Deoxyribonucleic acid
RAPD	=	Random Amplified Polymorphic DNA
AFLP	=	Amplified Fragment Length Polymorphism
SSR	=	Simple Sequence Repeat
ISSR	=	Inter Simple Sequence Repeat
Mbp	=	Million base pairs
n	=	Number of chromosomes
cm	=	Centimeter
in	=	Inch
m	=	Meter
ft	=	Feet
mm	=	Millimeters
P	=	Phosphorus
P_2O_5	=	Phosphorus pentoxide
K	=	Potassium
oz	=	Ounce (equal to approximately 28 grams)
kJ	=	Kilojoules
kcal	=	Kilocalories

LIST OF ABBREVIATIONS (Continued)

g	=	Gram
µg	=	Microgram
mg	=	Milligram
°C	=	Degree celsius
°F	=	Degree fahrenheit
NA	=	Non avilable
ANOVA	=	Analysis of variance
HI	=	Harvest Index
SLA	=	Specific Leaf Area
CO_2	=	Carbon dioxide
UV	=	Ultraviolet
SCMR	=	SPAD Chlorophyll Meter Reading
r	=	Correlation coefficient
PCR	=	Polymerase Chain Reaction
UPGMA	=	Unweighted pair group method with arithmetic mean
US	=	United States
AVRDC	=	Asian Vegetable Research and Development Center
Kg	=	Kilogram
cc	=	Cubic centimetres
lit.	=	Litre
IBPGR	=	International Board for Plant Genetic Resource
hrs	=	Hours
Co. Ltd.	=	Company limited

LIST OF ABBREVIATIONS (Continued)

UBC	=	University of British Columbia
A	=	Adenine
T	=	Thymine
C	=	Cytosine
G	=	Guanine
Y	=	Thymine or cytosine
CTAB	=	Hexadecyltrimethylammonium bromide
N_2	=	Nitrogen gas
µl	=	Microlitre
mM	=	Millimolar
HCl	=	Hydrogen chloride
M	=	Molar
NaCl	=	Sodium chloride
EDTA	=	Ethylenediaminetetraacetic acid
w/v	=	Weight by volume
rpm	=	Revolutions per minute
min.	=	Minute
ml	=	Millilitre
EtOH	=	Ethanol
OD	=	Optical density
ng	=	Nanogram
dNTP	=	Deoxynucleotide triphosphate
		(dATP, dCTP, dGTP, dTTP)

LIST OF ABBREVIATIONS (Continued)

dATP	=	Deoxyadenosine triphosphate
dCTP	=	Deoxycytidine triphosphate
dGTP	=	Deoxyguanosine triphosphate
dTTP	=	Deoxythymidine triphosphate
$MgCL_2$	=	Magnesium chloride
sec.	=	Second
mA	=	Milliampere
CV%	=	Coefficient of Variation
NTSY	=	Numerical Taxonomy and Multivariate Analysis system
LSD	=	Least Significant Difference
F- test	=	Fisher test
Min.	=	Minimum
Max.	=	Maximum
SD	=	Standard Deviation
ha	=	Hectare
DF	=	Days to 50% flowering
DP	=	Days to pod formation
DM	=	Days to maturity
PH	=	Plant height
NF	=	Number of filled pods per hill
SW	=	100 seed weight
PY	=	Yield per hill

LIST OF ABBREVIATIONS (Continued)

**	=	Highly significant
*	=	Significant
ns	=	Nonsignificant
S_{ij}	=	Pair wise genetic similarities between genotypes

CHAPTER I

INTRODUCTION

1.1 Rationale and background

The soybean (*Glycine max* L.) is one of the oldest cultivated crops. It originated in China where first written records date back to 2328 B.C. (Smith and Huyser, 1987). Cultivated soybean is under family Leguminosae, subfamily Papilionoidea, Genus *Glycine*. It is widely distributed and broadly cultivated in diverse geographical locations and under different growing conditions. Among pulses, soybean is one of the commercially potential crops. Comparing to animal protein, it is the best and cheapest protein source and its demand is tremendous for food and feed supply. In nutritional point of view, on dry matter basic, Openshaw and Hadley (1981) indicated that the primary constituent of soybean seed contained about 40% protein, 21% oil, and 11% soluble carbohydrates.

Soybean is usually grown for its seed protein and oil. For protein sources, it can be used in different ways, such as soy milk, soy meat, snaps, tofu, etc. For oil source, some products have been produced from soybean oil, such as cooking oil, margarine, cosmetics, biodiesel, etc. Besides these products, it can also be used as soy ink. It is more superior to petroleum based inks because it is not toxic, renewable and easily cleans up. Although demand for soybean has been increased, the genetic improvement for soybean cultivars is extremely narrow. There are several limitations for soybean production such as low yield, susceptibility to pests and diseases and adverse environmental conditions, etc. These limitations can be overcome in different

ways. They are field selection, variety improvement, cultural practices, post harvest technology, etc. Among these, variety improvement program can be done by breeding techniques.

In plant breeding program of new cultivars, it is essential to proper characterize and evaluate in the germplasm. Genetic diversity evaluation among germplasms is an importance and a prerequisite in any hybridization program. Paterson et al. (1991) suggested that evaluation of genetic diversity would promote the efficient use of genetic variations in the breeding program. To improve an efficient crop, it is essential to obtain the information on genetic diversity and relationships among breeding materials for a plant breeder.

It is also very useful that the knowledge of genetic diversity can be effectively used in gene-bank management, breeding experiments like tagging of germplasm, identification and/or elimination of duplicates in the gene stock and establishment of core collections, etc. To improve the chances of selection for various characters, genetic diversity among the parents is a prerequisite (Dwivedi et al., 2001).

Genetic diversity among genotypes regarding to agro-morphological and phsiological characteristics are either indirect or direct representations of differences at the DNA level. Therefore, they can be expected to provide information about genetic relationships. The assessment of genetic diversity is important not only for crop improvement but also for efficient management and conservation of germplasm resources (Tahir and Karim, 2011). In the selection of diverse parental combinations, it is invaluable to estimate accurately in genetic diversity for generating segregated progenies with maximum genetic variability. Although genetic diversity can be determined by morphological and agronomic traits, they can be influenced by the environment and also laborious and mechanical error.

Therefore, these problems could be overcome by molecular characterization. In the study, to achieve the best result, it will be done to examine agro-morphological and physiological characterization conjugating with molecular characterization.

1.2 Research objectives

The objectives of this research were 1) to evaluate the diversity of the tested soybean accessions at agro-morphological, physiological traits and DNA polymorphisms, 2) to study the ISSR polymorphism among the tested soybean accessions and 3) to determine the correlations between the yield and other traits.

1.3 Need assessment

The estimations of genetic distance among genotypes are helpful in the selection of parents to be used in a breeding program (Becelaere et al., 1994). Varieties developed with wider genetic base may be helpful in enhancing the yield under various agro-climatic conditions (Asif et al., 2005). Diverse genetic base may also resist the spread of diseases in approved varieties (Zhu et al., 2000).

Genetic diversity can be accessed from pedigree analysis by using morphological traits (Pejic et al., 1998). However, diversity estimates based on pedigree analysis have generally been found inflated and unrealistic (Fufa et al., 2005). Naseem et al. (2007) suggested that morphological characters have insufficient genetic relationship in correctly identifying varieties and therefore they should be used in conjunction with more reliable methods of characterization such as molecular markers.

It is fundamentally important to know the degree of genetic similarity among different genotypes for efficient plant breeding programs. Such information is useful

for organizing a working collection, identifying heterotic groups, and selecting parents for crossing (Bonato et al., 2006).

The estimation of genetic diversity based on morphological traits suffers from the drawback that such traits are limited in number and are influenced by the environment (Maric et al., 2004). Molecular markers are useful tools to estimate genetic diversity because these are not influenced by the environment (Bohn et al., 1999).

Under molecular technique, there are several DNA markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), inter simple sequence repeat (ISSR) etc.. When ISSR markers are compared with RAPD, AFLP, and other molecular markers, the main advantages of ISSR are: no need for DNA sequence information prior to amplification, low cost, simple operation, high stability, abundance of genomic information, no requirement of radioactivity and high polymorphism.

In fact, ISSR techniques are nearly identical to RAPD techniques except that ISSR primer sequences are non random and designed from microsatellite regions, and the annealing temperatures used are higher than those used for RAPD markers. Therefore, it can overcome the limitations of RAPD. Based on the published, unpublished and in-progress studies that have been conducted using ISSR markers, it is clear that ISSR markers have great potential for studies of natural populations. ISSR markers are very useful for correct botanical identification. They can clearly distinguish intra and inter species variation. There are several studies in which these markers are used for species or cultivar identification (Aghaei, 2012).

Therefore, in this study, it will be done to identify the relationships among the tested soybean accessions based on agro-morphological, physiological traits and DNA

polymorphisms by using ISSR markers and to identify the correlations between the yield and other traits.

1.4 Hypothesis of this study

1.4.1 Morphological, physiological traits and DNA polymorphism can determine genetic diversity and relationships of the tested soybean accessions.

1.4.2 The characterization of the soybean accession can provide the genetic materials with novel variation to soybean breeders.

1.4.3 The study will enable the development of strategies for effective breeding and for the isolation of agronomically important genes in the future.

1.4.4 Correlation of soybean yield can be formed among agro-morphological and physiological traits in the tested soybean accessions.

1.5 Expected outcomes

The results obtained from this experiment will be invaluable for specific objectives in breeding program. This information can be useful for plant breeders to make informed decisions in an effort to devise breeding or crossbreeding programs for the development of the crop.

Researchers can use the information on genetic similarity to make decision regarding selection of superior genotypes for improvement or use as parents for the development of future cultivars through hybridization.

Application of direct genetic analyses in soybean cultivars, based on DNA polymorphism, morphological and physiological characters, seems to be suitable tool for reliable variety identification and an effective breeding process.

Wide diversity in the tested soybean accessions will indicate a considerable potential for improving soybean for both agronomic and quality traits. The diversity encountered in the tested soybean accessions will point out that there is a large potential for the improvement of soybean for both agronomic and quality traits (Muhammad et al., 2008).

The study will also work as indicator for soybean breeders to evolve varieties with diverse genetic background to achieve sustainability in soybean production. Knowledge of genetic diversity and relationships among the cultivars of a crop species will be an essential component in germplasm characterization and conservation.

Therefore, this result will be useful for the breeders to cross the cultivars that are the most distance for maximum heterosis achievement. We can also inform the breeders the correlations that are valuable in indirect selection for increasing yield in future breeding program.

CHAPTER II

REVIEW OF LITERATURES

2.1 Soybean classification

The soybean is one of the oldest cultivated crops. It is originated in China where first written records date back to 2328 B.C. (Smith and Huyser, 1987; Chanprasert, 1988). Cultivated soybean belongs to family Leguminosae, subfamily Papilionoidea, Genus *Glycine*. Genus *Glycine* is divided into two subgenera; *Glycine* (perennials) and *Soja* (annuals). The subgenus *Soja* includes the cultivated soybean, and the wild annual soybean, *Glycine soja*. The haploid soybean genome consists of 1,100 million base pairs (Mbp), which is relatively larger than the model plant, *Arabidopsis* (120 Mbp). Both the cultivated and wild soybean are $2n = 40$ with base chromosome number of 20 and perfectly cross compatible (Hymowitz, 2004). The subgenus *Glycine* contains 22 species including important species like *G. tabacina* and *G. tomentella*. Soybean genome evolved by polyploidization or duplication (Shoemaker et al., 1996; Schlueter et al., 2004) and 35% of the soybean genome is diploidized (Shultz et al., 2006).

The soybean has a fairly wide range of adaptation involving a wide array of climatic, soil and growth conditions though it is mostly grown on rain-fed area (Fageria et al., 1997). It is now cultivated throughout East and South East Asia for food, animal feed and medicine. It is a miraculous crop due to its extraordinary qualities, it contains about 37-42% good qualities protein, 6% ash, 29% carbohydrate and 17-24% oil comprising 85% poly-unsaturated fatty acid with three essential

fatty acids (oleic, lenoleic and linolenic acid) which are not synthesized by the human body (Antalina, 2000; Balasubramaniyan and Palaniappan, 2003).

2.2 Soybean utilization

Soybeans are high in protein and most soybeans are processed for their oil and protein. It is used in different kinds of products including soy milk, soy flour, soy protein, tofu and many retail food products. Soybeans are also used in many non-food (industrial) products. Soybean oil is used in cooking and frying foods. Margarine is a product made from soybean oil. Salad dressings and mayonnaises are made from soybean oil. Some foods are packed in soybean oil (tuna, sardines, etc.) Soybean is usually used as an ingredient for baked breads, crackers, cakes, cookies and pies.

The high-protein fiber (which remains after processing has removed the oil) is toasted and prepared into animal feed for poultry, pork, cattle, other farm animals and pets. The poultry and swine industries are major consumers of soybean meal. Over half of the soybeans processed for livestock feed are fed to poultry, about one-quarter is fed to swine, and the rest is used for beef cattle, dairy cattle and pet. Soy protein is increasingly found in fish food, both for home aquariums and for the fish grown for eating.

Biocomposites are building materials made from recycled newspaper and soybeans. They can be replaced in other products traditionally made from wood, such as furniture, flooring, and countertops. Biodiesel fuel for diesel engines can be produced from soybean oil by a simple process called transesterification**.** Soy oil produces an environmentally friendly solvent that safely and rapidly removes oil from creeks, streams and shorelines without harming people, animals and the environment. Soy is an ingredient in many industrial lubricants, solvents, cleaners and paints. Soy ink is more superior to petroleum-based inks because it is not toxic,

renewable and environmentally friendly, and cleans up easily. Soy-based lubricants are as good as petroleum-based lubricants, but can withstand higher heat. Especially, they are non-toxic, renewable and good for environment. Soy-based foams are currently being developed for use in coolers, refrigerators, automotive interiors and even footwear. Beginning in October 2007, Ford Mustangs rolled off the production line with soy flexible foam in the seats (North Carolina Soybean Producers Association, 2011). Therefore, soybean is very useful for different kinds of materials.

2.3 Morphological characters of soybean

The height of soybean plant can vary from below 20 cm (7.9 in) up to 2 metres (6.6 ft). The pod, stem, and leaf are usually covered with fine brown or gray hairs. The leaves are trifoliolate, having three to four leaflets per leaf, and the leaflets are 6-15 cm (2.4-5.9 in) long and 2-7 cm (0.79-2.8 in) broad. The leaves fall before the seeds are mature. The inconspicuous, self-fertile flowers are developed in the axil of the leaf and are white, pink or purple. The pod is a hairy one that grows in clusters of three to five; each pod is 3-8 cm long (1-3 in) and usually contains two to four (rarely more) seeds with 5-11 mm in diameter. Soybeans occur in various sizes, and in different seed coat colors, including black, brown, blue, yellow, green, and mottled (Gask, 2012).

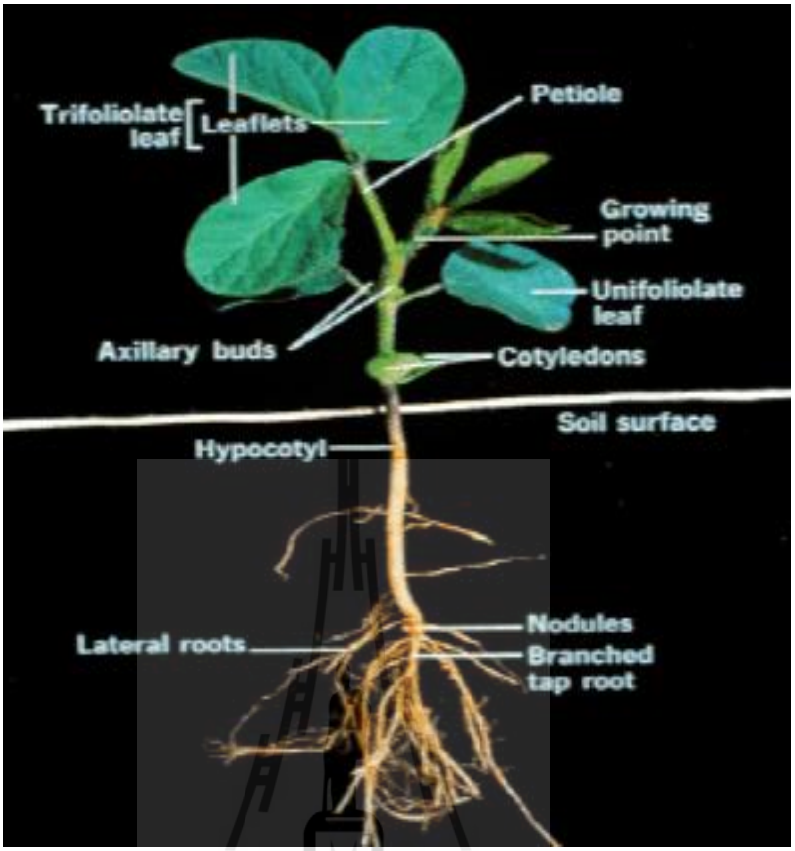

Figure 2.1 Soybean seedling morphology

2.4 Growth stages of soybean

Soybeans can be classified as indeterminate, semi-determinate or determinate in growth habit. Determinate growth of soybean ceases their vegetative growth when the main stem terminates in a cluster of flowering. Indeterminate varieties develop leaves and flowers simultaneously throughout a portion of their reproductive period, with one to three pods at the terminal apex. A fully developed leaf node for the vegetative stages has a leaf above it with unrolled or unfolded leaflets. These unfolded leaflets have their edges no longer touching. Stages are counted from the unifoliolate leaf node and upward. All other stages have true leaves that are trifoliolate and produced singularly on different nodes with these leaves alternating on the stem. The

reproductive stages are divided into 4 parts: R_1 and R_2 describe flowering; R_3 and R_4 describe pod development; R_5 and R_6 describe seed development; and R_7 and R_8 describe plant maturation (McWilliams et al., 2004).

Vegetative growth stages of soybean

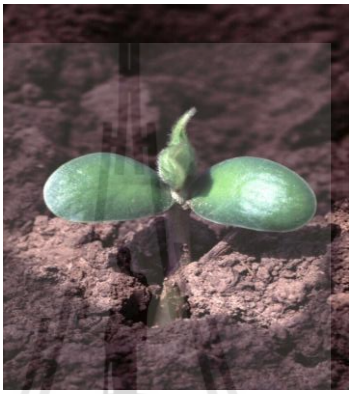

Figure 2.2 VE - emergence - 5 to 14 days after planting

Figure 2.3 VC - cotyledon - unifoliolate leaves have unrolled-leaves are opposite

Figure 2.4 V_1 - one trifoliolate - one node above the unifoliolate - trifoliolates are produced singularly and alternately

Figure 2.5 V_2 - 2 nd node - two trifoliolates -nodules have been established

Figure 2.6 V_3 - 3rd node - 3 nodes above unifoliolate - cotyledons gone

Figure 2.7 V_6 - New V stages at every 3 days

Reproductive stages and development

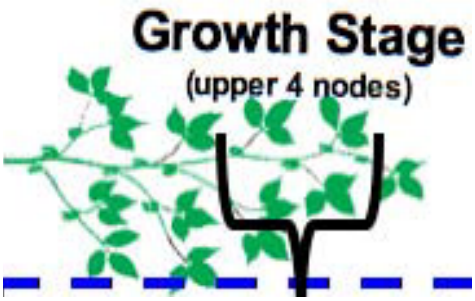

Figure 2.8 R_1 - Beginning bloom (one open flower at any node)

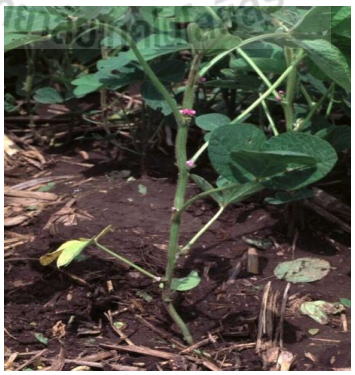

Figure 2.9 R_2 -Full bloom (one open flower at one of the two uppermost nodes)

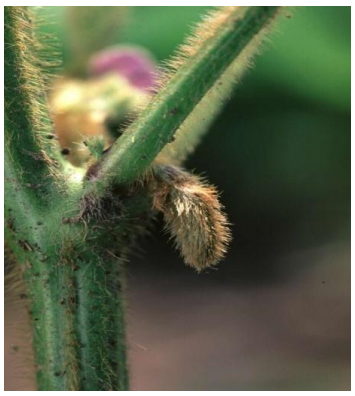

Figure 2.10 R_3 -Beginning pod (Pod is 3/16 in long at one of the four uppermost nodes)

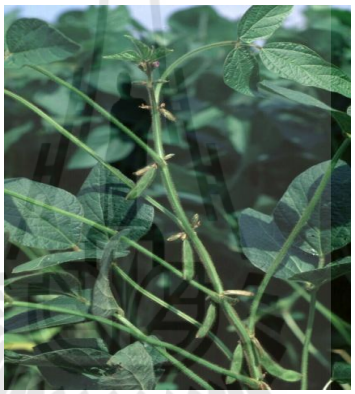

Figure 2.11 R_4-Full pod (Pod is 3/4 in long at one of the four uppermost nodes)

Figure 2.12 R_5 - Beginning seed (Seed is 1/8 in long in pod at one of the four uppermost nodes)

Figure 2.13 R_6 -Full seed (Pod containing a green seed that fills the pod cavity at one of the four uppermost nodes)

Figure 2.14 R_7 -Beginning maturity (one pod anywhere with its mature color)

Figure 2.15 R_8-Full maturity

(95% of the pods have reached mature color, harvestable 7-10 days after R_8)

(Gaska, 2012)

2.5 Nitrogen fixation of soybean

Symbiotic nitrogen fixation occurs in plants that keeps nitrogen-fixing bacteria within their tissues. The best nitrogen fixation activity between legumes and *Rhizobium* was found as a symbiotic relationship. *Rhizobium* is gram negative bacilli that live freely in the soil especially where legumes have been grown. They cannot fix atmospheric nitrogen until they have invaded the roots of the appropriate legume. *Rhizobium* and legume interaction is a well known symbiotic association occurring in nature and responsible for biological nitrogen fixation. It was determining the threshold concentration of fungicide for soybean seed dressing for effective nitrogen fixation and crop yield**.** Nitrogen fixing contributes to fertility of soil resulting in increased production of subsequent crop (Bikrol et al., 2005). Under field conditions, the first nodules form within a week after seedling emergence and become visible as they increase in size. Active fixation begins in the V_2 to V_3 stage, after which the number of nodules and the amount of fixed nitrogen continue to increase. The soybean demand for nitrogen is highest from the R_5 to R_8 stages (Kandel, 2012).

2.6 Role of nutrient elements on soybean growth and soil fertility

2.6.1. Nitrogen (N)

Leguminous crops, such as soybean, that have been inoculated properly with nitrogen-fixing bacteria rarely give an economical yield response to N fertilization. The species of symbiotic bacteria that fix atmospheric nitrogen in the nodules of soybean roots is *Bradyrhizobia japonicum*. Inoculation of the seed with these bacteria is especially recommended for fields where soybean has not been grown recently. The innoculant is not expensive because improved strains have been developed

and seed inoculation is a good practice every year for ensuring effective nodulation and adequate nitrogen availability. Barker et al. (2005) reported that N was not applied to soil during early reproductive stages as a method to increase soybean yield or grain quality.

2.6.2. Phosphorus (P)

Many soils have very high or above optimum levels of phosphorus due to high frequency of fertilizer and manure application in every planting year. P fertilizer is usually not recommended for soybean grown on such soils. The amount of P_2O_5 recommended is only enough to replace the phosphorus removed by the crop. Fertilizer sources of phosphorus for soybean may include superphosphate or triple-superphosphate (Heckman, 2009).

However, the quality of manure must be taken into consideration as the nutrient supplying power depends on the conditions under which the manure was stored (Chiezey et al., 2009). Phosphorous is an essential element for plant growth, hence it is an important soil fertility indicator. Makoi et al. (2008) suggested that soil pH and excessive sodium in the soil followed by calcium, soil organic matter and cation exchange capacity are the major soil fertility constraints to crop production in the area. Aduloju et al. (2009) concluded that application of 30 kilograms phosphorous per hectare is beneficial to soybean growth and grain yield in soils with low available phosphorous. Application of 30 kilograms phosphorous per hectare produced significantly higher number of branches (Mahamood, 2009). It may reduce the benefit of applying phosphorous to soybean according to weather, nutrient, and soil moisture interaction.

2.6.3. Potassium (K)

Soybean production has a relatively high demand for potassium. Providing an adequate supply of potassium for economic yields is a concern for

producing soybean on many New Jersey soils. Applying adequate amounts of this element to soils that test low or medium for potassium helps to ensure maximum economic soybean yield. Sandy soils have a more limited supply of potassium than loamy soils and are thus more prone to potassium deficiency. Soybean takes up potassium in large amounts. On soils that test high for potassium, it is important to apply enough potassium to maintain this optimum level of fertility. When the soil fertility level is high, applying potassium in a band generally provides no benefits (Heckman, 2009).

2.7 Nutritional composition in soybean seeds

Nutritional value per 100 g (3.5 oz)

- Energy 1,866 kJ (446 kcal)
- Carbohydrates 30.16 g
- Sugars 7.33 g
- Dietary fiber 9.3 g
- Fat 19.94 g
  - saturated 2.884 g
  - monounsaturated 4.404 g
  - polyunsaturated 11.255 g
- Protein 36.49 g
  - Tryptophan 0.591 g
  - Threonine 1.766 g
  - Isoleucine 1.971 g
  - Leucine 3.309 g
  - Lysine 2.706 g

- Methionine	0.547 g
- Cystine	0.655 g
- Phenylalanine	2.122 g
- Tyrosine	1.539 g
- Valine	2.029 g
- Arginine	3.153 g
- Histidine	1.097 g
- Alanine	1.915 g
- Aspartic acid	5.112 g
- Glutamic acid	7.874 g
- Glycine	1.880 g
- Proline	2.379 g
- Serine	2.357 g
- Water	-8.54 g
- Vitamin A	-equiv. 1 µg (0%)
- Vitamin B_6	-0.377 mg (29%)
- Vitamin B_{12}	-0 µg (0%)
- Choline	-115.9 mg (24%)
-Vitamin C	-6.0 mg (7%)
-Vitamin K	-47 µg (45%)
-Calcium	-277 mg (28%)
-Iron	-15.70 mg (121%)
-Magnesium	-280 mg (79%)
-Phosphorus	-704 mg (101%)
-Potassium	-1797 mg (38%)

-Sodium -2 mg (0%)

-Zinc -4.89 mg (51%)

Source: USDA Nutrient database (2011),

(National Soybean Research Laboratory, 2012)

2.8 Soybean nutrition

Soybeans consist of the macro-nutrients required for good nutrition: complete protein, carbohydrate and fat, as well as vitamins and minerals, including calcium, folic acid and iron. Soybeans are the only common plant food that contains complete protein. Soybean protein provides all the essential amino acids in the amounts needed for human health. The amino acid profile of soy protein is nearly equivalent in quality to meat, milk and egg protein.

Almost 40% of the calories in soybeans are derived from protein causing soybeans to be higher in protein source than other legumes and many animal products. Unlike many other good sources of protein, soybeans are low in saturated fat and are cholesterol-free. Soybeans, especially the outer hull, are an excellent source of dietary fiber.

The whole soybean foods are high in protein, fiber and unsaturated fat, and rich in vitamins and minerals. They also show many anticarcinogenic properties related to the unique benefits of soy isoflavones, phytochemicals which exert biological effects in humans and other animals. Soybean sprouts are rich in vitamins A, B and C, and D are eaten raw in salads or cooked (National Soybean Research Laboratory, 2010).

2.9 General climatic requirement and cultural practices for soybean growing

Soybean cultivation is usually successful in climates with hot summers, with optimum growing conditions in mean temperatures of 20 to 30°C (68 to 86°F); temperatures of below 20°C (68°F) and over 40°C (104°F) retard growth significantly. It can be grown in a wide range of soils, with optimum growth in moist alluvial soils with a good organic content. Soybean germination will be best at soil temperatures of 21°C to 32°C (70°F to 90°F) and poor at temperatures above 35°C (95ºF).

Soybean is a hardy plant and well adapted to a variety of soils and soil conditions. Ideal soil for optimum soybean production is a loose, well-drained loam. Growth and productivity of soybean can be decreased in many fields that have tight soil, high clay soil. When the soil dries out, a hard crust surface may cause a barrier to seed germination.

Land preparation for soybeans will provide for deep rooting and a moist seedbed for planting. Deep turning or chiseling is also acceptable if soil is not re-compacted with roto-tillers, disks and other seedbed preparation equipment. The ideal seedbed for soybeans should provide moisture and the appropriate temperature warmth for rapid germination and seedling emergence. Soil should remain friable without crusting over when dry. Germination of weed seeds should be delayed or prevented. If soybeans have not been grown in a particular location for three to five years, it is best to inoculate the seed with the proper strain of nitrogen-fixing bacteria, Rhizobium.

2.9.1. Optimum planting dates

The optimum period for planting soybeans is from May to June. Planting can be started as early as May if soils are warm > 21°C (> 70ºF). Planting before May

usually causes premature flowering, plant stunting and reduced seed quality. Very early-maturing soybean varieties tend to have a more narrow range of favorable planting dates than late-maturing varieties do. This occurs because the photoperiod response induces early varieties to flower before obtaining adequate growth necessary for optimum yields. Planting after June 10 may reduce plant growth, axillary limb branching, root nodulation/nitrogen fixation, and yield. However, the planting period can be extended as late as June 30 if adapted tall growing late maturing varieties are used. All soybean plantings should be completed before July. Growth and yield, even with the best of efforts, are generally not economical after this time.

Above planting date guidelines can be slightly modified for the Early Soybean Production System which uses early indeterminate soybeans. These varieties can be planted as early as April 20 if soil temperatures are above 21°C (70°F), but should not be planted after May 20 (Whitaker, 2012).

2.9.2. Row Spacing

Highest yields are generally obtained from soybean with row widths of 20 to 30 inches. However, most soybean varieties will reach to their potential with wider row spacing of 30 to 36 inches if planted at the optimum time. When it is planted in May and in close rows, short growing varieties will lodge less and often give higher yields than tall-growing varieties (Food and Agriculture Organization, 2012a). Rajput (1984) observed that the combination of 45 cm row spacing and 20 cm plant spacing gave best results.

2.9.3. Planting depth

Soybean seeds should be deeply planted enough to meet the moisture and temperature requirements for germination. Planting depth may be determined by variety, and some varieties can emerge from greater depths than others (usually the

larger seeded varieties). Typical planting depths are 1-1.5 inches, but if soil is low in moisture or sandy, plant 2 inches deep. In cool, moist soil, seed can be planted 1 inch deep. Seldom should soybeans be planted deeper than 2.5 inches.

2.9.4. Plant Population / Seeding Rates

The optimum plant stands for soybean is between 85,000 and 100,000 plants per acre. Final stands as low as 60,000 plants per acre can produce reasonable yields if plants are evenly distributed. Under good planting conditions, final stands will be about one soybean plant for every two planted seeds. The seeding rate should be increased by 10 to 20% if planting late, or in a dry or trashy seedbed. The seeds should be at least 80% germination. Seed can be treated with fungicide, but this is not necessary, if the soil temperatures are warm and if the germination rate is over 85% because there is little advantage in using fungicide-treated seed. Lower germination seed may have a 5 to 10% increasing in emergence if treated (Whitaker, 2012).

Planting an excessive population may result in increased lodging, but an inadequate stand may lead to higher weed population. At lower population, plants branch more and lodge less, while at high populations the opposite is true. Pods form higher on the plant with high populations. Weeds are more of a problem in low populations. Populations should be adjusted to reduce lodging and keep pods high on the plant. Populations can be increased growing determinate, semi-dwarf and non-branching varieties. Additionally, the local soil type, environment, and seed quality can influence plant density (North Carolina Soybean Producers Association, 2011).

2.10 Soybean planting and harvest seasons in the world

Soybean crops around the world have their own unique production cycles of planting and harvest timeframes. The followings are the window of opportunities for planting and harvesting soybean crops within the largest soybean production countries.

In United States (38% of world production), soybean crop is planted at the beginning of late April and last through June. It is mainly harvested in late September and is finished by the end of November.

In Brazil (25% of world production), soybean crop is planted at October through December and harvested at April through May.

In Argentina (19% of world production), soybean crop is planted at October through December and harvested April through early June.

In China (7% of world production), soybean crop is planted at late April through mid-June and harvested through early October (Kowalski, 2012).

2.11 Soybean production area in the world

In the world, the five largest producers of soybean are the United States (47%), Brazil (20%), China (11%), Argentina (11%) and India (4%). Soybean is the world's leading grain legume. World soybean production has increased at a faster rate than any other oilseed except rape. Considering the world's demand for proteins and carbohydrates for human diets, soybean is an extremely important crop. The world soybean cultivation during 2006 is estimated about 93.63 million hectares. The world average soybean yield is about 2.34 metric tons per hectare. World soybean production has increased steadily in the last decade, rising from 133 million metric tons in 1996 to 258.4 million metric tons in 2010 (USDA, 2010).

In Thailand, soybean has been cultivated as rice-soybean cropping pattern in the upper north of the country since 1930s. The planted area has expanded to the lower part of the northern area and it was later extended to the northeastern region and central plains. In Thailand, it is grown in three main seasons, early rainy season (40% of the total planted area), late rainy season (35%) and dry season (25%). In 2003, the soybean planted area of Thailand was 161,600 ha, production was 240,000 tons with yield 1512 kg/ha (Charoenrath, 2005).

In Myanmar, about 50% of total soybean cultivated area is in Shan State which is located above 1000-15000 meter above sea level. In Shan State, soybean is cultivated during rainy season. It is usually grown as sole, mixed or intercropped with maize, sorghum or sunflower. The rest of soybean is grown in the central plain region (especially Sagaing, Mandalay and Bago Division) and other hilly region (especially, Kachin State) as a winter crop, mainly after harvesting rice. In lower Myanmar, soybean is grown on alluvial soils of unbounded area as a winter crop when water is receded. In Myanmar, soybean is grown in 165, 000 ha with a total production of 258594 metric tons and the average productivity of 1567 kg/ha (FAO, 2012).

2.12 The importance of genetic diversity in breeding program

Knowledge of diversity patterns will allow breeders to better understand the evolutionary relationships among accessions, to sample germplasm in a more systematic fashion and to develop strategies in incorporating useful diversity in their breeding programs (Bretting and Widrlechner, 1995). Among the different kinds of usefulness, hybridization is the most widely and commonly used technique in most of the crop species including soybean. For creating desirable variability, parents should

be carefully selected and some biometrical tools can be used. Of these findings, diversity among genotypes is very useful (Bhatt, 1973).

Breeding strategies need to exploit existing variation within germplasm to broaden the genetic base of currently used cultivars (Muhammad et al., 2006). Aravind (2006) indicated that the estimations of genetic distance might help in identifying suitable germplasm for introgression into breeding stocks.

Knowledge of genetic diversity in a crop species is fundamental in its improvement. A variety of molecular, biochemical and morphological descriptors are used to characterize the genetic diversity among and within crop species (Ozkaya et al., 2006).

The study of genetic diversity is also important for varietal identification, proper purity maintenance and the implementation of plant variety protection (Ahmed, 2010). The estimations of genetic similarity/distance among genotypes are helpful in the selection of parents to be used in a breeding program (Becelaere et al., 2005).

Varieties developed with wider genetic base may be helpful in enhancing the yield under various agro-climatic conditions (Asif et al., 2005). Diverse genetic base may also resist the spread of diseases in approved varieties (Zhu et al., 2000). Researchers can use the information on genetic similarity to make decisions regarding in selection of superior genotypes for improvement or for use as parents for the development of future cultivars through hybridization.

The use of plant introductions for the development of soybean cultivars will be an important approach to create diversity in soybean breeding in the future (Sneller et al., 1997). The estimates of genetic relationship can be helpful for organizing germplasm for conservation of genetic resources for the identification of cultivars for selection of parents for hybridization, for predicting favorable heterotic combinations (Ozkaya et al., 2006).

In the breeding program, the evaluation of the genetic diversity would promote the efficient use of genetic variation (Paterson et al., 1991). Identification of new and different sources of diversity may help breeders to decide which genotypes should be used to cross for making new genetic combinations and to determine which genetic resources should be retained in a collection in order to conserve maximum genetic diversity in the gene bank (Saghir and Salam, 2011).

For the effective use of cultivated crops, it is critical to understand the extent and distribution of genetic diversity within species (Padulosi et al., 1999). Variety identification and varietal purity assessment are very important for varieties, hybrids and their parents. Therefore, to produce new cultivars, proper characterization and evaluation of soybean is essential for initiated successful breeding program. Genetic diversity is an important factor and also a prerequisite in any hybridization program. Evaluation of genetic variations in the breeding program (Paterson et al., 1991), the accurate combination is to generate segregation progenies with maximum genetic variability. It is also required to be a substantial need for research on many aspects of the extent and distribution of genetic diversity. Genetic diversity data on the extent, structure and distribution of genetic diversity is necessary for several purposes. Most of them have significant direct or indirect consequences on the conservation and use of genetic diversity (Rao and Hodgkin, 2002).

2.13 The importance of exotic germplasm

Exotic germplasm refers to crop varieties that well adapted to a breeder's target environment, and is also an important resource for crop improvement. Because genetic diversity within elite cultivars of a crop is limited compared to the variability within the species and its relatives worldwide, genes from exotic germplasm can

protect the crop against new biotic and abiotic stresses, and may represent unique alleles for productivity that are absent from elite crop gene pools. Improvements in crop productivity may be achieved by incorporating exotic germplasm into elite gene pools**.** Germplasm is the source of the genetic potential of living organisms. Among other things, diversified germplasm allows organisms to adapt to changing environmental conditions. No single individual of any species, however, contains all the genetic diversity of that species. This means that the total genetic potential is represented only in populations made up of many individuals (Wilkes, 1992).

Genetic similarity causes vulnerability of crops to epidemics and environmental disasters. The availability of plant genetic resources and genetic diversity allows the plant to adapt to changing environments such as new pests, diseases and climatic conditions. Therefore, protection of genetic diversity is very important. Measuring genetic variation is very useful for selective breeding, rapid domestication and/or conservation in populations or species.

2.14 Soybean germplasm

There are more than 1,750 individual genebanks worldwide, about 130 of which hold more than 10,000 accessions each. Total world soybean accessions are about 229,944. China holds the largest collection of soybean germplasm (14% of the world's accessions), (Food and Agriculture Organization, 2012b). World-wide, there are over 170,000 soybean accessions held in more than 70 countries. There is certainly much duplication among these collections but perhaps as many as 30% of the accessions could be unique. The USDA Soybean Germplasm Collection have collected more than 20,000 lines and send out 25,000-30,000 seed samples per year to private and public researchers (Bennett, 2009). In Thailand, soybean germplasm

consists of approximately 200 indigenous varieties (introduced from various unknown sources), 16 certified varieties and various exotic soybean introductions in different breeding programs (Chotiyarnwong et al., 2007).

2.15 Morphological characterization

The value of the germplasm collection depends upon the availability of information relative to the accessions. Morphological and agronomic traits that are known to be in the individual accessions increase the importance of the germplasm. Moreover, systematic description leads to a more efficient use of germplasm in the collection.

The basic requirement for an accession to be included in the characterization planting is the absence of information about this collection, thus newly acquired samples always make it on to the list of materials for characterization. Another basis for selection of materials for characterization is the completeness of information about the accession. Materials from previous characterization plantings with incomplete morphological and agronomic data are retrieved and are included in succeeding characterization plantings. Morphological and agronomic characters of plants are best scored at different growth stages of the crop, thus characterization is done at three different stages, vegetative, reproductive, and at post-harvest stages. (International Maize and Wheat Improvement Center, 2012).

The extent of genetic diversity in germplasm can be assessed through morphological characterization and genetic markers. Then, the characterized material helps the plant breeders to select the accessions to be utilized in hybridization program (Ghafoor et al., 2002). For the management of collections and determining genetic diversity, phenotypic evaluation of soybean germplasm is a fundamentally important step. The knowledge of the genetic variation within accessions from germplasm

collections is essential to the choice of strategy to incorporate useful diversity into the program, to facilitate the introgression of genes of interest into commercial cultivars, to understand the evolutionary relations among accessions, to better sample germplasm diversity and to increase conservation efficiency (Fu, 2003).

Malik et al. (2011) assessed the relationship of some morphological traits in 92 genotypes of soybean that are potential new sources of genetic variation for soybean breeding programs in Pakistan. They reported that phenotypic selection could be made on the basis of some morphological traits (leaf area, pods per plant, branches per plant, 100-seed weight and grain yield per plant) that have high level of diversity.

Dayaman (2007) investigated 45 soybean accessions from different geographical areas were screened for genetic diversity using 22 morphological traits. The investigation revealed out the accessions into 6 clusters by the analysis of morphological traits.

Genetic diversity of a primary core collection of 91 soybean landraces from Shaanxi Province, China, was analyzed by using agronomic traits. ANOVA analysis showed that a significant proportion of variance (94.28%) was due to variation within populations (Liu et al., 2011).

Edwardsjeromedies (1943) classified 26 soybean varieties on the basis of varietal characters of days to maturity, flower colour, pubescence colour and pod colour.

Edgar et al. (1970) studied soybean morphological characters for growth habit, nature of stem hairiness, flower colour and pubescence colour. Szabo et al. (1983) characterized 40 soybean cultivars. Observations were recorded and grouped on the basis of flower colour, pubescence colour and pod colour at maturity. Koszykowski and Burgoon (1983) tabulated 64 soybean cultivars for plant characters and classified based on the leaflet shape, days taken for flowering, flower colour, plant height,

growth habit and maturity days. Agrawal (1984) classified soybean varieties based on spreading type, presence of pubescence on stem, leaf shape and size, flower color, pod color at maturity and days taken for maturity. All these characters were exhibited considerable differences between the varieties. Rasaily et al. (1986) characterized twenty soybean genotype characters based on plant height, number of branches, pods per plant and seed yield per plant. Diazcarrasco et al. (1986) grouped seventeen soybean varieties based on days to maturity, plant height and seed yield per plant.

Reddy et al. (1989) recorded five soybean cultivar variations for days to maturity, period from flower initiation to maturity, plant height, seeds per plant and yield per plant. Muhammad et al. (2006) thirty-three soybean genotypes were evaluated for days to flowering, days to maturity, pod length, number of branches, number of unfilled, filled pods and total pods, 100 seed weight and seed yield (kg/ha). Manjaya and Bapat (2008) grouped 55 soybean genotypes by using quantitative characters viz, days to 50% flowering, days to maturity, plant height, number of branches per plant, number of pods per plant, number of seeds per plant, 100 seed weight, yield per plant.

2.16 Harvest index

Harvest index (HI) is a measurement of crop yield expressed as the weight of a harvested product as a portion of the total plant weight of a crop. HI is used to denote the fraction of economically useful products of a plant in relation to its total productivity. HI reflects the division of photosynthates between the seeds and the vegetative part and improvements in HI emphasize the importance of carbon allocation in seed production. Dry matter weight is an important plant component for determining grain yield and crop improvement has been primarily based on the

concept of maximizing seed yield per unit of dry matter produced. The economic yield can be raised by biological enhancing without changing the harvest index, or by partitioning more of the dry matter weight into the economic yield thereby increasing HI (Maobe et al., 2010). The formula for calculating HI is the following:

$$HI(\%) = \frac{\text{Seed dry weight (g)}}{\text{Total dry weight (g)}} \times 100$$

Amauliah and Muhammad (2011) evaluated harvest index on 33 germplasm of common bean. Harvest index varied from 14.3 to 66.2% among different germplasm.

Cui and Yu (2005) estimated the relative contribution of increased biomass, harvest index and yield components to seed yield gain of soybean. The results indicated that harvest index was a larger contributor to the progress of soybean yield improvements than biomass in China.

Harvest index was studied to determine the variability in 139 soybean genotypes. Results of analysis of variance showed significant differences among genotypes and the existence of genetic variation (Iqbal et al., 2010).

2.17 Specific leaf area

Specific leaf area (SLA) is the ratio of leaf area to leaf dry weight. SLA is the ratio of one sided leaf area to the dry matter of the leaf (cm^2/g) and is used to calculate the rate of change in leaf area based on the rate of change in leaf dry matter. SLA can determine the thickness of leaves. In nature, it is very difficult and time consuming to measure accurately leaf thickness. Leaf thickness plays an important role in leaf and plant functioning and is related to species' strategies of resource acquisition and use. The amount of light absorbed by a leaf, and the diffusion pathway of CO_2 through its

tissues depend, at least partially, on its thickness (Givnish, 1979; Agusti et al., 1994; Syvertsen et al., 1995). Enriquez et al., 1996 and Garnier et al., 1999 indicated that negative relationships between leaf thickness and photosynthetic and growth. Therefore, leaf thickness has often been used as a tool to screen species and/or cultivars for productivity (Dornhoff and Shibles, 1976; White and Montes-R, 2005) or ecological performance (Witkowski et al., 1992; Dı´az et al., 2004).

SLA is an important ecological variable because of its links with plant ecophysiology and leaf biochemistry. Variations in SLA are associated with variations in leaf optical properties, and these changes in leaf optical properties have been found to result in changes in canopy. Jongrungklang et al. (2008) studied SLA to identify drought resistant peanut genotypes from a collection of peanut germplasm. Vile et al. (2005) examined specific leaf area and dry matter content estimate thickness in laminar leaves. They reported that this is an easy and rapid way to estimate leaf thickness from other, widely measured leaf traits, which are also easier to measure.

$$\text{Specific Leaf Area} = \frac{\text{total leaf area } (cm^2)}{\text{total leaf dry weight } (g)}$$

2.18 The determination of chlorophyll by using SPAD chlorophyll reading

SPAD chlorophyll meter reading (SCMR) is a quick, easy measurement of the chlorophyll content of plant leaves without damaging leaf. It measures green color intensity in leaves in *vivo*, and is an ideal instrument for collecting large amount of data on chlorophyll in the field within a short time without any destructive sampling. Close associations between SCMR value and chlorophyll density have been reported

for maize and soybean (Markwell et al., 1995), cotton (Wu et al., 1998), rice (Jinwen et al., 2009), potato (Bindi et al., 2002), wheat (Ommen et al., 1999), lauraceae, lindera, pondberry (Hawkins et al., 2009) and peanut (Arunyanark et al., 2009). The use of SCMR to assess relative chlorophyll density as alternative to the standard method is very attractive because it is easy to operate, low cost, non-destructive and can be applied in the field conventionally. SCMR could help to increase the effectiveness of breeding if it can be used to identify genotypes with high chlorophyll density and high biomass productivity.

Anything that can alter the color of plants (e.g., diseases, nutrient deficiencies, variety differences) can influence chlorophyll meter readings. Therefore, we should not take readings on leaves with lesions or with bronzing around the margins. Lesions on leaves are usually caused by blast, sheath blight, or brown spot diseases. Plants with bronze leaf margins may be deficient in potassium (Spectrum Technologies, 2013).

Bindu et al. (2003) reported that there was a direct close relationship of transpiration efficiency with SCMR in groundnut. Yadava (1986) also indicated that SCMR is a direct linear relationship through extracted leaf chlorophyll and it is related leaf nitrogen concentration (Bullock and Anderson 1998). The advantages such as easy and rapid measurement, nondestructive method and light weight make SPAD meters the best choice for use in the trait-based groundnut breeding program to improve the drought tolerance of groundnut at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Serraj et al., 2004). Kashiwagi et al., 2006 indicated that the top and second leaf had significantly lower SCMR than the other leaves and on the other hand there was no significant difference in SCMR among the leaves below the third leaf. Therefore, they suggested that the third leaf can

be considered as representative of the plant canopy for SCMR measurement. Thus, the third leaf can be used for further SCMR measurement.

Chlorophyll meters are extensively used in agriculture for estimation of foliar chlorophyll and nitrogen in numerous crop species. Coste et al. (2010) assessed foliar chlorophyll contents with SPAD-502 chlorophyll meter in thirteen tree species of tropical rainforest in French Guiana. They indicated that the SPAD-502 meter provides a simple, non-destructive method for estimating foliar chlorophyll that quickly reports a large number of readings, thus paving the road for immediate assessment of physiological variables (Hawkins et al., 2009). They also conclude that it should be possible to use the SPAD-502 as a tool for a variety of research and management applications, including the assessment of physiological changes over time, the assessment of relative health status or to delineate the effects of management and logging practices on the photosynthetic performance.

The report of Arunyanark (2009) about SCMR is that chlorophyll density and SCMR can vary depending on water regimes, time of sampling and genotypes but water regime x genotype interactions are not significant for chlorophyll density and SCMR. They indicated that the correlation coefficients between chlorophyll density and SCMR were positive and significant across irrigation treatments ($r = 0.76^{**}$, 0.94^{**} and 0.96^{**}) and each water regime, plant age and leaf position ($r = 0.31$ to 0.99^{**}). Their results suggest that evaluation of chlorophyll density by SCMR can be carried out at any water regime conditions in the second or third-fully expanded leaves after 40 days of crop growth. Peng et al. (1993) reported that leaf N status can be estimated by a chlorophyll meter (SPAD-502). Nitrogen is an important nutrient for plant growth to obtain high yield or high quality (Li and He, 2008).

Karademir et al. (2009) studied the correlations between leaf chlorophyll content, yield and yield components in cotton. They mentioned that there were significant correlations between leaf chlorophyll content and seed cotton yield ($r = 0.231^{*}$), however positive but non-significant correlations were observed among leaf chlorophyll content and other investigated characteristics except for plant height and 100 seed weight.

2.19 Molecular markers

In the past, genetic diversity studies in crop plants mostly relied on the evaluation of morphological and agronomic traits (Upadhyaya et al., 2002). Currently, there are many molecular marker systems routinely used to evaluate genetic diversity in plants. These include randomly amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs) and ISSRs (inter simple sequence repeats) (Patzak, 2001).

DNA markers are the most widely used types of marker due to their abundance. DNA markers may be broadly divided into three classes based on the method of their detection; 1) hybridization based, 2) polymerase chain reaction (PCR based), and 3) DNA sequence based. DNA markers are particularly useful if they reveal difference between individuals of the same or different species. Molecular markers are useful tools for estimating genetic diversity as these are not influenced by the environment, are abundant and do not require previous pedigree information (Bohn et al., 1999).

Genetic variation is a pre-requisite for any crop improvement program to be successful. DNA based molecular markers acted as versatile tools to study variability

and diversity in different plant species. Although a range of plant characters are currently available for distinguishing between closely related individuals, their sensitivity to environment and less genome coverage hinder their usage. DNA based molecular markers clearly allow the comparison of genetic material of two individual plants avoiding any environmental influence on gene expression. Presently, many kinds of DNA based molecular markers such as RFLP, RAPD, ISSR and AFLP etc. are available which detect polymorphism at the DNA level (Aravind, 2006). The present study will employ ISSR technique to assess genetic polymorphism.

The introduction of molecular markers in plant breeding has presented a valuable tool for the characterization of genetic materials (Tahir, 2011).This is a useful tool for assessing genetic variations and resolving cultivar identities. The application of DNA technology in agricultural research is progressed rapidly over the last two decades, especially in the area of variety identification. More recently, molecular marker systems based on the Polymerase Chain reaction (PCR) technique have become increasingly popular for fingerprinting and variety identification (Hussein et al., 2008).

DNA based markers are effective and reliable tools for measuring genetic diversity in crop germplasm and studying evolutionary relationship. Molecular genetic techniques using DNA polymorphism is increasingly used to characterize and identify a novel germplasm for uses in the crop breeding process.

Molecular markers, less influenced by the environment, is important to provide additional information about the characterization, degree of diversity and genetic constitution of the existing cultivars. The molecular markers are proven to be a powerful tool that can yield significant information that enhances the scope of using germplasm in the crop improvement programs. The major advantage of molecular methods for characterization is their direct investigation of the genetic situation which

allows them to detect variation at the DNA level, thereby excluding all environmental influences (Hammer, 2012).

2.20 The usefulness of ISSR (inter-simple sequence repeats) markers

PCR-based molecular markers are playing an increasingly important role in the analysis of genetic diversity of horticulture and field-crop species (Torres et al., 1993; Wolf et al., 1995; Debener etal., 1996; Swoboda and Bhalla, 1997). The similarity values based on ISSR data were reported to be higher than those based on RAPD. In this regard, parallel study using RAPD and inter-simple sequence repeats (ISSR) techniques showed that RAPD required the testing of six times more primers than ISSR (Korbin et al., 2002). It is reported that ISSR profiling is a powerful method for the identification and molecular classification of *Leucadendron* varieties (Pharmawati et al., 2005) and proved to be a potentially useful tool for the identification of strawberry varieties, because it is simple, fast-cost, highly discriminant and reliable (Arnau et al., 2003).

ISSR is PCR based marker. It is a fast, inexpensive genotyping technique based on variation in the regions between microsatellites. ISSR usually detects of polymorphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotides simple sequence repeats. ISSR markers have been extensively used for DNA finger-printing (Moreno et al., 1998), genetic diversity studies (S'anchez et al., 1996), population genetic studies (Wolfe et al., 1998) and phylogenetic studies (Hess et al., 2000).

ISSR marker system shows polymorphism in inter-microsatellite DNA regions without any prior sequence knowledge (Zietkiewicz et al., 1994). Primers are based on a repeat sequence and amplify the sequence between two microsatellites. Large numbers of amplification products per primer are produced, providing high reproducibility and low cost. Although morphological and biochemical markers can be

influenced by environmental conditions, molecular markers (RAPD, ISSR, SSR, etc.) cannot be influenced by environment. Therefore, they can accurately characterize the plants portraying the extent of genetic diversity among taxa (Thimmappaiah et al., 2009). Of the different molecular markers, RAPD and ISSR has been widely used in the last two decades in cultivar identification program (Ebrahimi et al., 2009) and assessing genetic variations among different taxa at DNA level because of their cost effectiveness and simple operation without requiring prior knowledge of species DNA sequences (Williams et al., 1990) and can provide vital information for development of genetic sampling, conservation and improvement strategies.

In particular, ISSR markers can be highly variable within a species and have the advantage over RAPDs in utilizing longer primers that allow more stringent annealing temperatures and reveal more polymorphic fragments (Fang and Roose, 1997). ISSR technique is very simple, fast, cost effective, highly discriminative, reliable, require small quantity of sample DNA, do not need any prior primer sequence information and non-radioactive (Lakshmanan et al., 2007). The use of ISSR primers for assessment of genetic identification is well documented (Leroy et al., 2001; Lakshmanan et al., 2007).

The ISSR marker can produce much larger numbers of fragments per primer, with the advantages of high reproducibility and relatively low cost (Fang et al., 1997; Nagaoka and Ogihara, 1997). Since the last two decades, it has been used in the application of DNA technology for agricultural research, especially in the area of variety identification. ISSR is a type of molecular marker proposed by Zietkiewicz et al. (1994) for fingerprinting. Nowadays, molecular marker systems based on the polymerase chain reaction (PCR) technique become increasingly popular for fingerprinting and variety identification. One of DNA markers is ISSR marker that

uses simple sequence repeats anchored at the 5’ or 3’ end by a short arbitrary sequence as PCR primers (Zietkiewicz et al., 1994).

ISSRs are ideal markers for genetic mapping and population studies due to their abundance and the high degree of polymorphism between individuals with a population of closely related genotypes (Jarret and Bowen, 1994; Lanham and Brennan, 1998).

Therefore, this method has a wide range of uses, including the characterization of genetic relatedness among populations, genetic fingerprinting, gene tagging, detection of clonal variation, cultivar identification, phylogenetic analysis, detection of genomic instability, and assessment of hybridization.

ISSR is a general term for a genome region between microsatellite loci. The complementary sequences to two neighboring microsatellites are used as PCR primers; the variable region between them gets amplified. Sequences amplified by ISSR-PCR can be used for DNA fingerprinting. There are some literature reviews that among PCR-based methods, inter-simple sequence repeats (ISSRs) has been found to be an efficient and reliable technique established by Zietkiewicz et al. (1994) for the identification of species or varieties, population authentication and population genetic structure, etc. (Shen et al., 2006; Liu et al., 2009).

When ISSR markers are compared with random amplified polymorphic DNA (RAPD) method, amplified fragment length polymorphism (AFLP), and other molecular markers, the main advantages of ISSR are: no need for DNA sequence information prior to amplification, low cost, simple operation, high stability, abundance of genomic information, and no requirement of radioactivity and high polymorphism.

In fact, ISSR techniques are nearly identical to RAPD techniques except that ISSR primer sequences are non random designed from microsatellite regions and the

annealing temperatures used are higher than those used for RAPD markers, so it can overcome limitations of RAPD. Based on the published, unpublished and in-progress studies that have been conducted using ISSR markers it is clear that, ISSR markers have great potential for studies of natural populations (Kurane et al., 2009).

ISSR markers are very useful for correct botanical identification. They can clearly distinguish intra and inter species variation. There are several studies in which these markers are used for species or cultivar identification.

Fang et al. (1998) studied ISSR markers in phylogenetic relationships among selected *Citrus* germplasm accessions revealed by inter-simple sequence repeat (ISSR) markers. They reported that ISSR markers can generate phylogenetic relationships among *Citrus* accessions and could be useful for expanding the germplasm base of breeding programs.

Baloch et al. (2009) examined ISSR markers in 21 soybean and 32 peanut accessions for assaying of diversity. In their study, they observed that the ISSR analysis, which was performed with 46 primers in soybean and 47 primers in peanut, yielded 31 and 26 polymorphic band, respectively.

Yan et al. (2003) reported that a relatively large genetic diversity was detected among a wild soybean population based on estimation of DNA products amplified from 15 selected ISSR primers, with the similarity coefficient varying from 0.17 to 0.89.

ISSR markers were also studied on 25 soybean varieties to detect the genetic diversities (Yan et al., 2011). They reported that 9 polymorphic ISSR primers among 28 tested primers could reveal out 65 bands and contained 58 polymorphic bands showing their polymorphism percentage (89.23%).

Monte-Corvo et al. (2001) analyzed ISSR markers on 24 pear cultivars. They observed that 8 ISSR primers generated 337 markers, 79.5% of which were polymorphic.

Hussein et al. (2008) conducted for molecular identification and genetic relationships of six strawberry varieties using ISSR markers. They reported that nine ISSR primers generated 102 total amplified fragments of which 86 (84.3%) polymorphic fragments discriminated the varieties under the investigation. They also concluded that the ISSR techniques were useful tools for the varietal identification.

Liu et al. (2012) discriminated tea germplasm at the inter-specific level. Eighteen primers generated 99.4% polymorphic bands. Therefore, they concluded that ISSR markers provide a powerful tool to discriminate tea germplasm at the inter-specific level.

Qiang et al. (2008) assessed sixty-two main parents of sweetpotato by using inter-simple sequence repeat (ISSR) markers to understand their genetic differences. In the study, seventeen ISSR primers generated 490 polymorphic bands with an average of 28.8 polymorphic bands per primer, indicating that ISSR marker was efficient to analyze the genetic diversity of sweetpotato.

2.21 Correlation analysis

Association analysis measures the natural relationships between various plant characters and determines the components on which selection can be based for improvement. The association of characters may be due to either genetic linkage or pleiotrophy (Harland, 1939). Knowledge of correlation that exists among important characters may facilitate proper interpretation of results and provide basis for planning more efficient breeding programs (Aravind, 2006). Correlation analysis can be used to evaluate the strength of the relations between variables statistics.

Correlation is a term that refers to the strength of a relationship between two variables. A strong or high correlation means that two or more variables have a strong

relationship with each other while a weak or low correlation means that the variables are weakly related. Correlation coefficients can range from -1.0 to +1.0. The value of -1.0 represents a perfect negative correlation while a value of +1.0 represents a perfect positive correlation. A value of 0 means that there is no relationship between the variables being tested (Ashley Crossman, 2013).

Correlation has been applied in many agricultural analyses. Aravind (2006) analyzed the correlations of yield components with seed yield. Correlations of ten quantitative traits were studied in ninety-two genotypes of soybean.

Grain yield was positively and highly significantly correlated with leaf area, plant height, pods per plant, branches per plant and 100 seed weight suggesting thereby that phenotypic selection could be made on the basis of these characters (Malik et al., 2011).

The correlations of phenotype and genotype were examined between characters of soybean line and varieties. Significantly positive correlation was observed between seed yield and days to flowering. Genotypic correlation was observed that seed yield was positively correlated with all characters except 100 seed weight (Machikowa, 2011).

Truong et al. (2006) studied the correlations of flowering and maturing times with plant yield and yield components in soybean. Udensi (2012) examined the correlations of yield and yield related traits in *Cajanus cajan* (L.) Millsp. They found that correlation results revealed that there was significant positive correlations between plant height and number of leaves per plant (0.93^{**}), leaf area per plant (0.57^{*}) and number of seeds per plant (0.62^{*}). It also showed that the number of leaves per plant (0.68^{*}). Leaf area per plant had a positive significant association with the number of seeds per plant (0.58^{*}). Additionally, pod length per plant positively correlated with the number of seed per plant (0.85^{**}) while number of nodules per plant positively correlated with 100 seed weight (0.80^{**}).

Rad et al. (2012) studied the correlations of physiological traits under drought stress in wheat (*Triticum aestivum*). In the study, there was a positive relationship between chlorophyll content and stomatal conductance.

Table 2.1 Summary of review of literature on correlation of component traits with seed yield in soybean

No.	Component characters	Correlation	References
1.	Days to flowering	Positive	Rajasekaran et al. (1980), Amaranath et al. (1990), Harer and Deshmukh (1992), Mahajan et al. (1994), Thorat et al. (1999), Ramana et al. (2000),Bangar et al. (2003), Mukhekar et al. (2004)
2.	Plant height	Positive	Weber and Murthy (1952), Johnson et al. (1955), Kwon and Torrie (1964), Raut et al. (1982), Perraju et al. (1982), He (1987), Sharma and Abraham (1988), Amarnath et al. (1990), Harer and Deshmukh (1992), Mahajan et al. (1994), Vimala Devi (1993), Maharaddi (1996), Taware et al. (1997), Mehetre et al. (1997), Rajput et al. (1998), Ramana et al. (2000), Basavaraja (2002), Mukhekar et al. (2004).
		Negative	Rajasekaran et al. (1980), Kalaimagal (1991)
3.	Number of branches per plant	Positive	Rajasekaran et al. (1980), Dixit and Patil (1982), Perraju et al. (1982), Amarnath et al. (1990), Kalaimagal (1991), Harer and Deshmukh (1992), Lakhani (1993), Mahajan et al. (1994),Jadhav et al. (1995), Maharaddi (1996), Taware et al. (1997), Mehetre et al. (1997), Singh and Singh (1999), Basavaraja (2002), Bangar et al. (2003), Hina Kausar (2005), Mukhekar et al. (2004), Parameshwar (2006)
		Negative	Kalaimagal (1991), Shinde et al. (1996), Sunilkumar et al. (1997).

Table 2.1 Summary of review of literature on correlation of component traits with seed yield in soybean (continued)

No.	Component characters	Correlation	References
4.	Days to maturity	Positive	Amaranath et al. (1990), Harer and Deshmukh (1992), Lakhani (1993), Ramana et al. (2000), Bangar et al. (2003) , Mukhekar et al. (2004).
		Negative	Kalaimagal (1991), Parameshwar (2006).
5.	Number of pods Per plant	Positive	Dixit and Patil (1982), Perraju et al. (1982), Sharma et al. (1988), Amaranath et al. (1990), Harer and Deshmukh (1992), Lakhani (1993), Mahajan et al. (1994), Vimala Devi (1993), Maharaddi (1996), Ramgiry and Raha (1997), Thorat et al. (1999), Singh and Singh (1999), Rajput et al. (1998), Bangar et al. (2003), Hina Kausar (2005), Mukhekar et al. (2004), Parameshwar (2006)
		Negative	Kalaimagal (1991)
6.	Number of seeds per plant	Positive	Kalaimagal (1991), Vimala Devi (1993), Jagtap and Choudhary (1993), Ramgiry and Raha (1997), Pooranchand (1999), Nehru et al. (1999), Mukhekar et al. (2004)
7.	Number of seeds per pod	Positive	Perraju et al. (1982), Song et al. (1987), Sahu and Mishra (1988), Surlan and Nikolic (1990), Amaranath et al. (1990), Kalaimagal (1991), Jadhav et al. (1995), Mishra et al. (1987), Taware et al. (1997), Ramgiry and Raha (1997), Nehru et al. (1999), Basavaraja (2002), Hina Kausar (2005).
		Negative	Thorat et al. (1999)
8.	Harvest index	Positive	Bhardwaj et al. (1990), Yao et al. (1987), Ramgiry and Raha (1997), Mehetre et al. (1997).

Review of inter-correlation among yield components in soybean

Table 2.2 Days to 50% flowering with

No.	Component characters	Correlation	References
1.	Plant height	Positive	Harer and Deshmukh (1992), Ramgiry and Raha (1997), Mehetre et al. (1997), Bhandarkar (1999), Nehru et al.(1999), Rajanna et al. (2000), Mukhekar et al. (2004), Dev Vart (2005)
2.	Days to maturity	Positive	Harer and Dehmukh (1992), Mehetre et al. (1997), Ramgiry and Raha (1997), Bhandarkar (1999), Nehru et al.(1999), Ramana et al. (2000), Mukhekar et al. (2004), Dev Vart (2005)
3.	No. of pods per plant	Positive	Harer and Deshmukh (1992), Mehetre (1997), Ramgiry and Raha (1997), Bhandarkar (1999), Nehru et al. (1999), Mukhekar et al. (2004) and Dev Vart (2005)
4.	No. of seeds per pod	Negative	Harer and Deshmukh (1992), Bhandarkar (1999), Nehru et al. (1999), Dev Vart (2005)
		Positive	Ramgiry and Raha (1997) and Ramana et al. (2000)
5.	Harvest index	Negative	Ramgiry and Raha (1997) and Dev Vart (2005)

Table 2.3 Plant height with

No.	Component characters	Correlation	References
1.	Days to maturity	Positive	Harer and Deshmukh (1992), Mehetre et al. (1997), Ramgiry and Raha (1997), Nehru et al. (1999), Bhandarkar (1999), Ramana (2000), Mukhekar (2004), Dev Vart (2005)
		Negative	Ramgiry and Raha (1997) and Dev Vart (2005)
2.	No. of pods per plant	Positive	Harer and Deshmukh (1992), Mehetre et al. (1997), Ramgiry and Raha (1997), Nehru et al. (1999), Bhandarkar (1999), Ramana (2000), Mukhekar (2004), Dev Vart (2005)
3.	No. of seeds per pod	Positive	Harer and Deshmukh (1992), Ramgiry and Raha (1997), Bhandarkar (1999) and Ramana et al. (2000)
		Negative	Nehru et al. (1999) and Dev Vart (2005)
4.	Harvest index	Positive	Mehetre et al. (1997)
		Negative	Ramgiry and Raha (1997) and Dev Vart (2005)

Table 2.4 Days to maturity with

No.	Component characters	Correlation	References
1.	No. of pods per plant	Positive	Harer and Deshmukh (1992), Mehetre et al. (1997), Ramgiry and Raha (1997), Nehru et al. (1999), Ramana et al. (2000), Mukhekar et al. (2004) and Dev Vart (2005)

Table 2.4 Days to maturity with (contd.)

No.	Component characters	Correlation	References
1.	No. of pods per plant	Positive	Harer and Deshmukh (1992), Mehetre et al. (1997), Ramgiry and Raha (1997), Nehru et al. (1999), Ramana et al. (2000), Mukhekar et al. (2004) and Dev Vart (2005)
2.	No. of seeds per pod	Positive	Ramgiry and Raha (1997), Nehru et al. (1999) and Ramana (2000)
		Negative	Harer and Deshmukh (1992), Bhandarkar (1999) and Dev Vart (2005)
3.	Harvest index	Negative	Mehetre et al. (1997), Ramgiri and Raha (1997) and Dev Vart (2005)

Table 2.5 No. of pods per plant with

No.	Component characters	Correlation	References
1.	No. of seeds per pod	Positive	Ramgiry and Raha (1997), Nehru et al. (1999) and Dev Vart (2005)
		Negative	Harer and Deshmukh (1992), Ramana et al.(2000) and Mukherkar (2004)
2.	Harvest index	Positive	Mehetre et al. (1997) and Dev Vart (2005)

Table 2.6 No. of seeds per pod with

No.	Component characters	Correlation	References
1.	Harvest index	Positive	Ramgiry and Raha (1997) and Dev Vart (2005)

2.22 Cluster analysis

Cluster analysis is the task of assigning a set of objects into groups (called clusters) so that the objects in the same cluster are more similar to each other than to those in other clusters.

Cluster analysis is an unsupervised learning technique that seeks to divide cases into clusters, sharing similar qualities. Cluster analysis divides data into groups (clusters) that are meaningful, useful or both. If meaningful groups are the goal, then the clusters should capture the natural structure of the data.

Cluster analysis groups data objects based only on information found in the data that describes the objects and their relationships. The goal is that the objects within a group be similar (or related) to one another and different from (or unrelated) to the objects in other groups. The greater the similarity (or homogeneity) within a group does and the greater the difference between groups, the better or more distinct the clustering also does. UPGMA (Unweighted Pair Group Method with Arithmetic mean) is one of the clustering types.

Cluster analysis has been used in different analysis. Malik et al. (2011) used cluster analysis in morphological traits of soybean. In their study, by using cluster analysis, the physical distinctness between Pakistani and US/AVRDC accessions reflects that the introgression of US and AVRDC accessions to Pakistani breeding program could broaden Pakistani soybean germplasm diversity.

Iqbal et al. (2008) clustered 139 soybean accessions based on ten quantitative traits. Based on these traits, 139 genotypes were clustered into five clusters. Cluster I showed maximum number of filled pods per plant, 100 seed weight, grain yield per plant, biological yield and harvest index. Cluster II consisted of least oil content, grain

yield per plant and harvest index. Cluster III showed maximum plant height and number of branches per plant. Cluster IV comprised of accessions having high oil content and least number of unfilled pods per plant while early maturity was observed in cluster V.

Dayaman (2007) used cluster analysis to construct a dendrogram of soybean accessions by using 22 morphological traits and SSR marker data, resulting in 14 clusters. Under drought stress, wheat genotypes were clustered by using some physiological traits, (Rad et al., 2012). Li (2001) also constructed a dendrogram among soybean populations from regions of China, Japan and S. Korea by using RAPD markers.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental materials

The materials used in this study comprised of ninety-five soybean accessions which included thirteen released varieties and eighteen local varieties of Thailand. These accessions were obtained from Khon Kaen University and Chiang Mai Field Crop Research Centre, Thailand. The list of the experimental materials is given in table 3.1.

Table 3.1 List of soybean accessions and their origin

No.	Accession name	Origin
1.	Chiang Mai 60*	Thailand
2.	Chiang Mai 5*	Thailand
3.	Chiang Mai 6*	Thailand
4.	Chiang Mai 1*	Thailand
5.	SJ 2*	Thailand
6.	SJ 4*	Thailand
7.	SJ 5*	Thailand
8.	Sukhothai 2*	Thailand
9.	Sukhothai 3*	Thailand
10.	Srisamrong 1*	Thailand
11.	Nakhon Sawan 1*	Thailand
12.	KKU-35*	Thailand
13.	Rat Mongkhon*	Thailand
14.	G 2261	NA
15.	Xanh tien tai	Vietnam
16.	Lee	United States
17.	Bethel	United States

Table 3.1 List of soybean accessions and their origin (continued)

No.	Accession name	Origin
18.	White Lion	United States
19.	LV.Su 7	Thailand
20.	Austin	United States
21.	Cha Sengoku 81	Japan
22.	Ogden USA	United States
23.	Alankar	India
24.	San Khiao**	Thailand
25.	Thua Nao**	Thailand
26.	Rahu Chiangfai**	Thailand
27.	Clark 63	Egypit
28.	TG 71	Thailand
29.	Tainung 3	Taiwan
30.	Bossier	United States
31.	Pha Bong 8**	Thailand
32.	TG 56	Thailand
33.	Davis	United States
34.	IAC 13	Brazil
35.	TG 61	Thailand
36.	Acadian	United States
37.	TG 65	Thailand
38.	Doi Kham 1**	Thailand
39.	Don Chiang**	Thailand
40.	Sansai	United States
41.	Pak chong	Japan
42.	TG 150	Thailand
43.	Klang Dong**	Thailand
44.	Jupiter	Africa
45.	TG 165	Thailand
46.	Thua Chao Khao**	Thailand
47.	GC 89001-B-33-B	NA
48.	CLOUD 190	United States
49.	Kuro (dull)	Japan
50.	Dam Tia 6**	Thailand
51.	SSR 8502-2-9	NA

Table 3.1 List of soybean accessions and their origin (continued)

No.	Accession name	Origin
52.	Chosen Kuro daizu	Japan
53.	Ka La Dam**	Thailand
54.	Yot Son (165)**	Thailand
55.	Leich hardt 5	NA
56.	Black Malla	NA
57.	Khai Mon**	Thailand
58.	Forrest	United States
59.	OTOOTAN	Taiwan
60.	Fort Lamy	Thailand
61.	KKU 252	Thailand
62.	Black seed	Korea
63.	Korea 5 (Bokwang kong)**	Korea
64.	Choe Chian (green)**	Thailand
65.	NC 103	United States
66.	Prolina	USA
67.	Kao Song**	Thailand
68.	Siam Riop**	Thailand
69.	LV. Southern Shan 1**	Myanmar
70.	Tadaeng**	Thailand
71.	Takhao Chiang Daot**	Thailand
72.	Korea 2007 no.6	Korea
73.	Korea 2007 no.47	Korea
74.	Korea 2007 no.70	Korea
75.	Korea 2007 no. 71	Korea
76.	Yezin	Myanmar
77.	DS 1099-04-01	NA
78.	China 1 Henyshoi-Heibei	China
79.	Dain 86-4	China
80.	Zhongpin 661	China
81.	44 Lb-4E	Thailand
82.	KKU 213	Thailand
83.	KKU 74	Thailand
84.	44 Ly-8	Thailand

Table 3.1 List of soybean accessions and their origin (continued)

No.	Accession name	Origin
85.	KKU 223	Thailand
86.	44 Ly-14 E	Thailand
87.	KKU-5E	Thailand
88.	KKU 282	Thailand
89.	40 Ly-15	Thailand
90.	74-T4	Thailand
91.	44 Ly-75	Thailand
92.	Nakhon Sawan 1(set 2)	Thailand
93.	Chiang Mai 60 (set 2)	Thailand
94.	SJ 5 (set 2)	Thailand
95.	KKU 35 (set 2)	Thailand

* = Released variety, ** = Local variety, NA = Non available, set 2 = the accession from Khon Kaen University

3.2 Experimental site

The field experiment was grown on June 27, 2012 at the Suranaree University of Technology Farm, Nakhon Ratchasima, Thailand. The duration of the study was about 5 months from June to October of 2012.

3.3 Field plot technique

The experiment consisting of 95 soybean accessions was laid out in a randomized complete block design with three replications. The entries were sown in one row each of 2.5 m in length, with a spacing of 50 cm between rows and 20 cm

between the plants. Each hill consisted of 2 plants. The soybean variety (SJ 5) was grown as a border around the experimental area. Before the seeds were grown, they were treated with Metalaxyl fungicide (7g per 1 kg of seed). After sowing, pre-emergence herbicide (Alachlor, 500 cc per 20 litres of water) was immediately applied. Fertilizer (12-24-12, 20 kg per 1,600 m^2) was separately applied during vegetative growth and pod formation period. Protection and cultural practices (insecticide spraying and weeding) were also carried out throughout the growth duration.

3.4 Recording of observations

3.4.1 Morphological observation

Morphological observations were recorded on five randomly selected plants for different characters in each accession and replication, IBPGR (1980). The collected morphological data consisted of leaf shape, flower color, pubescence color, mature pod color, seed coat color, hilum color, days to 50% flowering, days to pod formation, days to maturity, plant height at maturity (cm), number of filled pods per plant, number of seeds per pod, 100 seed weight (g), and seed yield (g).

Leaf shape was recorded from random three leaves of each accession and scored (1 = lanceolate, 3 = triangular, 5 = pointed ovate, and 7 = rounded ovate) at R_1 (beginning bloom, first flower).

Flower color was recorded and scored (1 = white, 2 = light purple, 3 = purple and 4 = dark purple) at R_2 (one open flower at one of the two uppermost nodes).

Pubescence color was recorded and scored (1 = grey, 2 = light brown, and 3 = brown) at R_6 (full size seed in top 4 nodes).

Mature pod color was recorded and scored (3 = tan, 5 = brown, and 7 = black) at R_7 (beginning maturity, one mature pod).

Growth habit was scored (1 = Determinate, 2 = Semideterminate, and 3 = Indeterminate) at R7 (beginning maturity, one mature pod).

Seed coat color was recorded and scored (1 = yellowish white, 2 = yellow, 3 = green, 4 = buff, 5 = reddish brown, 6 = grey, 7 = imperfect black, and 8 = black) after harvesting.

Hilum color was recorded and scored (1 = yellow, 2 = buff, 3 = brown, 4 = green, 5 = grey, 6 = imperfect black, 7 = black, and 8 = other) after harvesting.

Days to 50% flowering were recorded at R_2 (full bloom, flower in top 2 nodes). Number of days taken from the date of sowing to the day on which 50% of the plants in each genotype initiate first flower were recorded as days to flowering.

Days to pod formation were recorded from sowing to first pod development within each row at R_3 (beginning pod, 3/16 inch pod in top 4 nodes).

Days to maturity were recorded at R_8 (full maturity, 95% of pods on the plant turn color). Numbers of days taken from date of sowing to physiological maturity of the plant were recorded as days to maturity.

Plant height (cm) was recorded after harvesting. Height of the main stem from the ground level to the top of the main stem was measured.

Numbers of filled pods per plant were recorded after harvesting. These were recorded by counting the number of filled pods present on main stem and branches in each of the five selected plants.

Numbers of seeds per pod were recorded after harvesting. Numbers of seeds per pod were counted from fifty randomly selected filled pods in each of the five selected plants.

100 seed weight (g) was recorded after harvesting. 100 seed weight was computed by weighing 100 seeds which were randomly chosen filled seeds from a complete sample made by mixing the seeds of all the five plants in each replication.

Seed yield (g) was recorded after harvesting. Seeds obtained from each selected five hills were weighed and averaged.

For harvest index (HI) (%), seed dry mass (g) and total plant dry mass (g) were measured to calculate HI.

$$\text{Harvest index (\%)} = \frac{\text{Seed dry weight (g)}}{\text{Total dry weight (g)}} \times 100$$

3.4.2 Physiological observation

Physiological traits were studied only on 27 late matured soybean accessions with maturity date of more than 100 days after sowing. At 90 days after sowing, five leaves of each accession were randomly selected to measure physiological traits. Each leaf was selected from the third portion from the top (Kashiwagi et al., 2006).

For SLA, the leaf area and leaf dry weight were measured. The leaf area was measured by using LI 3100 Area meter. After that, the leaf was dried in an oven (80°C, 48 hrs) to measure dry weight. SCMR was measured to determine the chlorophyll content.

3.4.3 Molecular observation (ISSR analysis)

3.4.3.1 Plant materials

To study diversity among tested thirty-six soybean accessions [randomly selected from different groups clustered by 9 agro-morphological traits (flower color, leaf shape, plant type, pod color, pubescence color, seed coat color and helium color, plant height and days to maturity)], ISSR technology was applied. For

DNA extraction, disease free younger tender leaves were harvested from 5 plants of each variety at 40 days after sowing and stored at -20°C. The seven ISSR primers were used to screen the soybean accessions following the method of BALOCH et al. (2009). These seven ISSR primers were obtained from BioDesign Co., Ltd., Thailand. The sequence details of the primers are presented in table 3.2.

Table 3.2 ISSR primers that were used to screen 36 soybean accessions Degenerate base, Y = C/T

No.	Primer name	Primer sequence(3′-5′)
1	UBC-834	$(AG)_8YT$
2	UBC-836	$(AG)_8YA$
3	UBC-850	$(GT)_8YC$
4	UBC-822	$(TC)_8A$
5	UBC-828	$(TG)_8A$
6	UBC-857	$(AC)_8YG$
7	UBC-868	$(GAA)_6$

3.4.3.2 ISSR analysis

DNA extraction was conducted by the hexadecyltrimethyl-aammonium bromide (CTAB) method of Doyle and Doyle (1990). The detail procedures are as the following:

Step 1: About 100-150 mg of soybean young leaves were ground in liquid N_2 with a mortar and pestle.

Step 2: 600 µl of DNA extraction buffer (100 mM Tris-HCl (pH 8.0), 1.5 M NaCl, 50 mM EDTA (pH 8.0), 2% w/v CTAB) were added.

Step 3: The mixer was incubated in water bath at 65°C for 30 min and vortexed for 3-4 times before the incubation.

Step 4: 600 µl of 24:1 chloroform:isoamyl alcohol was added and gently shaken the tube by the hand until homogenize. Then the mixer was spun in centrifuge at 13,000 rpm about 20 min. at room temperature.

Step 5: Supernatant was transferred into a new tube (about 400 µl).

Step 6: Step 4 was repeated again.

Step 7: 0.5 volume of 5 M NaCl was added and the tube was gently shaken.

Step 8: 1 volume of 100% Isopropanol (cold) was added and gently shaken.

Step 9: It was incubated at -20°C (all night).

Step 10: It was then centrifuged at 13,000 rpm about 15 min. at room temperature and the solution was discarded.

Step 11: The pellet was washed with 500 ml of 70% EtOH (ethanol).

Step 12: Then it was centrifuged at 13,000 rpm about 5 min. at room temperature and the solution was discarded.

Step 13: After that, 500 ml of 100% EtOH (cold) was added and centrifuged at 13,000 rpm about 5 min. at room temperature and the solution was discarded. The pellet was air-dried at room temperature.

Step 14: The pellet was re-suspended in 30-100 µl of distilled water and was mixed as a solution.

Step 15: 10-30 µl RNase (1 mg/ml) was added and incubated at 37°C about 30 min.

Step 16: It was stored in -20°C and concentration and purity of DNA were checked by NANO drop Spectrophotometer.

Step 17: Concentration of DNA (25 ng/µl) was adjusted and prepared for use in PCR.

Step 18: The amplification was carried out in a 20 µl (0.2 mM dNTP, 1x PCR buffer, 2.5 mM $MgCl_2$, 1 unit of Taq DNA polymerase, 50 ng DNA, and 4 µM primer).

Step 19: For optimum annealing temperature for each primer, random two samples among 36 samples were analyzed regarding of annealing temperature (48 to 56°C). In PCR reaction, denaturation was done for 3 min. at 94°C; followed by 49 cycles of 1 min. at 94°C, 1 min. at 48-56°C (depend on primer) and 2 min. at 72°C; with a final 7 min. extension at 72°C. Then PCR products were stored at 4°C. The amplification products were size-separated by standard horizontal electrophoresis in 2% agarose gels and stained with ethidium bromide.

Step 20: 1xTBE was prepared and agarose for 2% was weighted. Then it was heated in microwave about (2 min. 34 sec).

Step 21: The agarose solution was transferred into the mold and the comb was inserted. After waiting until the gel strength, it was put in a horizontal machine containing 1 x TBE buffer.

Step 22: Then loading dye 2µl was dropped on plastic (parafilm), DNA (5 µl) was also dropped and mixed with loading dye. The total solution (dye+DNA) was dropped in well of agarose gel.

Step 23: The machine was set at electricity at 100 Volt, 400 mA about 25 min. Then the gel was soaked in ethydium bromide solution about 5-20 min. Finally, DNA bands were checked by UV light.

Amplifications for screened primers and DNA samples were conducted independently for two times with the same procedure to verify the reproducibility and consistency of the ISSR markers.

Molecular sizes of the amplified fragments were roughly estimated using a 100 bp ladder (Invitrogen Brazil). ISSR markers were scored as DNA fragments present or absent. Cluster analysis was performed by un-weighted

pair group method based on arithmetic mean (UPGMA). Percent of polymorphism was calculated by using the following formula.

$$\text{Percent polymorphism} = \frac{\text{number of polymorphic bands}}{\text{total number of bands}} \text{ X } 100$$

3.4.4 Statistical analysis

Analysis of variance was performed for all traits in order to test the significance of variation among genotypes. The data was analyzed for mean, coefficient of variation (CV%). All agro-morphological traits, physiological traits and ISSR polymorphisms were used for cluster analysis to study the relationship among the accessions. Moreover, the correlations between the yield and other traits were computed. The ISSR bands were manually scored as present (1) or absent (0). Only clear and strong bands were recorded and used for analysis. Genetic similarities were calculated according to the method developed by Jaccard (1908). A jaccard similarity matrix was used to build an unweighted pair-group method with arithmetic means (UPGMA) tree. These computations were done by using statistic 8, CropStat 7.2 and NTSYSpc version 2.2 softwares. NTSYpc version 2.2 software was used for genetic similarity computing dendrogram construction.

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was carried out at Suranaree University of Technology on June to October of 2012 to assess the nature and extent of genetic diversity among 95 soybean accessions. Agro-morphological traits were studied on tested 95 soybean accessions. Physiological traits were studied on 27 soybean accessions with maturity of more than 100 days after sowing. Agro-morphological and physiological traits recorded were used to estimate the variability parameters, character association, and diversity in the materials. Molecular analysis was conducted on 36 soybean accessions (randomly selected from different groups based on 9 morphological traits: flower color, leaf shape, plant type, pod color, pubescence color, seed coat color, hilum color, days to maturity, and plant height) using ISSR analysis. The experimental results are presented as the followings:

4.1 Experiment I. Agro-morphological identification

Although 95 soybean accessions were grown, accession Thva Chao Khao flowered only about 85 days after sowing. Although pod formation took place about 110 days after sowing, it cannot set seed. Therefore, agro-morphological traits were analyzed on 94 soybean accessions. The variations of the tested traits, days to emergence, days to 50% flowering, days to pod formation, days to maturity, plant height (cm), number of filled pods per hill, number of seeds per pod, 100 seed weight (g), yield per hill (g) and harvest index % due to genotypes were highly significant

among 94 soybean accessions. Significant mean squares of genotypes for yield and yield components indicated the existence of genetic variability in these characters. This result is in agreement with finding of Aravind (2006) in which the variation due to genotypes was significant for all the tested characters under the study both at 5 and 1 % probability levels. A clear understanding of the extent of variability prevalent for each of the character in the germplasm would imply the scope for improving the character studied through selection. The success of any crop improvement programs mainly depends on the amount of diversity available in the crop. Improvement of yield in soybean is attributed to use genetically diverse parents in breeding program. Hence, it is important to study the knowledge of genetic divergence in the available cultivars for the selection of the parents to be used in hybridization program for obtaining desirable genetic recombination.

The tested character mean values of 94 soybean accessions were as the followings in table 4.1.

Table 4.1 Analysis of agronomic performance of the tested soybean accessions

No.	Accession name	Days to 50% flowering	No. of filled pods/ hill	Seed/ pod	Harvest index (%)	100 seed weight (g)	Yield per hill (g)
1	Chiang Mai 60	40	71	2	51.6	17.7	14.8
2	Chiang Mai 5	38	69	3	90.8	17.6	28.9
3	Chiang Mai 6	37	65	2	81.0	14.8	26.3
4	Chiang Mai 1	38	29	2	83.5	30.8	26.9
5	SJ 2	39	88	2	47.1	16.9	21.3
6	SJ 4	38	67	2	74.7	19.3	23.8
7	SJ 5	42	79	2	67.4	18.8	23.0
8	Su khothai 2	35	34	2	42.9	15.1	10.5
9	Sukhothai 3	34	64	2	79.8	13.5	21.9
10	Srisamrong 1	33	43	3	69.0	18.3	15.1
11	Nakhon Sawan 1	32	27	3	41.7	22.8	8.0
12	KKU 35	42	71	3	73.9	20.3	36.0
13	Rat Mongkhon	38	65	2	75.8	15.9	20.3
14	G 2261	34	44	2	45.8	16.1	13.4
15	Xanh tien tai	38	99	2	79.8	10.9	20.5
16	Lee	40	76	2	56.8	12.1	31.0
17	Bethel	36	18	2	46.2	17.1	7.0
18	White lion	25	16	2	26.8	31.5	5.7
19	LV.Su 7	42	65	2	78.7	15.4	21.5
20	Austin	34	27	2	57.1	19.6	11.6
21	Cha Sengoku 81	33	47	3	40.9	10.6	17.6
22	Ogden USA	32	19	2	21.9	19.7	4.7
23	Alankar	32	33	2	55.3	15.0	10.9
24	San Khiao	40	32	2	59.4	12.8	16.0

Table 4.1 Analysis of agronomic performance of the tested soybean accessions (continued)

No.	Accession name	Days to 50% flowering	No. of filled pods/ hill	Seed/ pod	Harvest index (%)	100 seed weight (g)	Yield per hill (g)
25	Thua Nao	47	45	3	53.9	8.1	29.0
26	Rahu Chiangfai	36	40	2	72.6	13.2	20.2
27	Clark 63	29	30	2	75.2	16.1	11.2
28	TG 71	34	56	2	58.0	21.8	26.3
29	Tainung 3	34	41	2	74.4	17.1	20.7
30	Bossier	39	92	3	87.0	16.5	37.0
31	Pha Bong 8	42	133	2	77.0	14.2	51.7
32	TG 56	42	65	3	48.1	16.3	26.0
33	Davis	37	38	3	71.3	16.4	19.1
34	IAC 13	38	37	2	47.2	14.3	13.2
35	TG 61	44	124	2	81.2	17.6	50.0
36	Acadian	42	68	3	56.8	12.3	14.7
37	TG 65	38	45	3	64.7	14.5	21.0
38	Doi Kham 1	39	45	2	47.4	17.8	15.6
39	Don Chiang	40	35	2	42.2	18.4	11.2
40	Sansai	45	111	2	64.8	12.8	42.3
41	Pak chong	50	173	2	69.3	10.6	53.6
42	TG 150	46	120	2	86.0	11.7	47.2
43	Klang Dong	53	182	2	46.3	11.0	51.1
44	Jupiter	50	89	2	62.0	19.5	46.4
45	TG 165	52	145	2	78.5	9.8	54.7
46	GC 89001-B-33-B	83	48	2	81.9	26.7	20.2
47	CLOUD 190	32	10	2	33.4	13.8	7.1

Table 4.1 Analysis of agronomic performance of the tested soybean accessions (continued)

No.	Accession name	Days to 50% flowering	No. of filled pods/ hill	Seed/ pod	Harvest index (%)	100 seed weight (g)	Yield per hill (g)
48	Kuro (dull)	27	1	2	6.8	18.9	1.2
49	Dam Tia	31	119	2	81.7	12.5	30.0
50	SSR 8502-2-9	39	82	2	58.1	14.4	26.5
51	Chosen Kuro daizu	35	54	4	53.7	12.6	22.7
52	Ka La Dam	37	73	3	65.8	13.6	16.5
53	Yot Son (165)	38	72	2	77.3	12.4	23.9
54	Leich hardt 5	38	96	2	83.1	14.3	48.8
55	Black Malla	42	84	3	86.8	14.6	23.9
56	Khai Mon	34	72	2	39.5	14.1	17.8
57	Forrest	40	151	2	83.0	10.5	40.8
58	OTOOTAN	45	127	2	68.3	12.2	49.3
59	Fort Lamy	43	139	2	70.7	11.2	50.0
60	KKU 252	43	80	3	64.4	21.3	49.4
61	Black seed	38	8	2	24.8	30.2	4.0
62	Korea 5 (Bokwang kong)	31	15	2	63.5	27.5	14.2
63	Choe Chian (green)	26	25	2	57.0	21.0	15.4
64	NC 103	30	15	2	32.4	18.6	7.6
65	Prolina	27	24	2	58.9	17.9	16.0
66	Kao Song	36	15	2	21.1	27.7	3.8
67	Siam Riop	25	89	2	87.7	17.0	46.9
68	LV. Southern Shan 1	45	57	2	69.0	22.6	28.8
69	Tadaeng	40	72	2	63.9	20.2	37.0

Table 4.1 Analysis of agronomic performance of the tested soybean accessions (continued)

No.	Accession name	Days to 50% flowering	No. of filled pods/ hill	Seed/ pod	Harvest index (%)	100 seed weight (g)	Yield per hill (g)
70	Takhao Chiang Daot	38	89	2	76.9	12.9	26.8
71	Korea 2007 no.6	40	16	2	43.5	25.0	9.6
72	Korea 2007 no.47	32	19	3	35.3	18.9	6.1
73	Korea 2007 no.70	25	12	2	4.8	12.1	0.9
74	Korea 2007 no. 71	31	26	2	30.2	11.5	4.4
75	Yezin	33	78	3	78.9	13.4	31.3
76	DS 1099-04-01	36	18	3	52.3	24.7	12.6
77	China 1 Henyshoi-Heibei	33	50	2	55.7	19.2	15.7
78	Dain 86-4	32	24	3	43.2	20.7	9.6
79	Zhongpin 661	30	25	3	43.1	17.7	7.5
80	44 Lb-4E	25	88	2	65.9	19.7	30.7
81	KKU 213	38	58	2	62.0	20.5	22.7
82	KKU 74	39	63	3	62.9	23.8	25.5
83	44 Ly-8	37	85	3	66.0	17.6	32.9
84	KKU 223	34	76	3	77.5	22.3	33.1
85	44 Ly-14 E	39	50	2	77.0	20.3	21.8
86	KKU-5E	39	37	2	58.0	21.0	16.1
87	KKU 282	40	35	2	54.3	22.5	14.5
88	40 Ly-15	32	47	3	75.7	17.4	22.7
89	74-T4	37	54	2	70.1	22.7	24.2
90	44 Ly-75	40	86	3	53.7	21.4	29.1

Table 4.1 Analysis of agronomic performance of the tested soybean accessions (continued)

No.	Accession name	Days to 50% flowering	No. of filled pods/ hill	Seed/ pod	Harvest index (%)	100 seed weight (g)	Yield per hill (g)
91	Nakhon Sawan 1 (Set 2)	32	31	2	50.9	20.7	11.3
92	Chiang Mai 60 (Set 2)	22	64	2	69.9	14.8	17.8
93	SJ 5 (Set 2)	40	74	2	43.3	17.1	21.4
94	KKU 35 (Set2)	41	45	3	21.5	21.9	22.9
	Mean	37	61	2	59.8	17.4	23.1
	CV%	5.7	39.4	13.0	32.2	5.7	45.6
	$LSD_{(0.05)}$	3.5	38.8	0.5	30.9	1.6	16.9
	F-test	**	**	**	**	**	**

** = highly significant, set 2 = the accession from Khon Kaen University

The germplasm exhibited high variability for the tested 10 traits. Range of variation for 10 characters studied in the soybean accessions is present in table 4.2. High range of variations were recorded in number of filled pods per hill (1 to 182), yield per hill (0.9 to 54.7 g) and harvest index (4.8 to 90.8%), respectively revealing a high level of diversity among the accessions for these traits. Therefore, selection on the basis of these traits can be useful. About this phenomenon, Malik et al. (2011) observed that high range of variations were also recorded in number of pods per hill (26 to 130), 100-seed weight (4.2 to 21.5 g) and grain yield per hill (4.0 to 28.2 g), respectively indicating a high level of diversity among the accessions for these traits. Ojwang (2003) also indicated that the characters, including plant height, days to maturity, number of seeds per pod, days to 50% flowering, and 100-seed weight were the major sources of diversity among the soybean genotypes.

Table 4.2 Range of variation in the tested 10 traits of 94 soybean accessions

Characters	Min.	Max.	Range	Mean±SD	CV%
Days to emergence	4	9	5	6 ± 1	24.9
Days to 50% flowering	22	83	61	37 ± 8	5.7
Days to pod formation	30	115	85	48 ± 12	6.0
Days to maturity	85	119	34	100 ± 9	1.6
Plant height (cm)	13	104	91	47 ± 19.4	6.0
No. of filled pods/hill	1	182	181	61 ± 38	39.4
Seeds/pod	2	4	2	2 ± 0.4	13.0
100 seed weight	8.1	31.5	23.5	17.4 ± 4.9	5.7
Harvest index (%)	4.8	90.8	86.0	60.1 ± 18.5	32.2
Yield/hill (g)	0.9	54.7	53.8	23.1 ± 13.6	45.6

*SD = standard deviation

4.1.1 Growth habit

Based on growth habit at maturity, the accessions were classified into determinate, semideterminate and indeterminate in table 4.3. Thirty-four of soybean accessions were determinate, fifty-four were semideterminate and seven were indeterminate plant type. The plant height also varied among the genotypes. The plant height of determinate plant type ranged from 13 to 62 cm, the semideterminate plant type ranged from 27 to 97 cm and the indeterminate plant type ranged from 48 to 119 cm. Variation in growth habit might be due to the genetic characters of varieties. Similar results were reported by Boerma et al. (1990) in soybean and Muthiah (2006) in green gram.

Table 4.3 showed that plant height varied significantly from 13 cm, White Lion to 119 cm, Thva Chao Khao in different germplasm. This variation in plant height of different germplasm might be due to the difference in the genetic make-up of different germplasm (Amanuliah and Muhammad, 2011). Similar results were reported by Bahrenfus and Fehr (1984), Diazcarrasco et al. (1986), Rasaily et al.

(1986), Reddy et al. (1989), Tarasatyavathi et al. (2004), Manjaya and Bapat (2008) in soybean; Parameshwarappa et al. (2009) in sesame. Wide variation in plant height was due to genetical characters of the varieties and also might be influenced by agronomical and environmental conditions (Govindarao, 2010).

Table 4.3 Grouping of soybean accessions based on only growth habit showing plant height

No.	Accession name	Growth habit	Plant height (cm)
1	Chiang Mai 60	Determinate	62
2	Chiang Mai 1	Determinate	46
3	Srisamrong 1	Determinate	28
4	Nakhon Sawan 1	Determinate	30
5	G 2261	Determinate	30
6	Xanh tien tai	Determinate	34
7	White Lion	Determinate	13
8	LV.Su 7	Determinate	34
9	Austin	Determinate	41
10	Ogden USA	Determinate	38
11	Alankar	Determinate	19
12	Clark 63	Determinate	30
13	Davis	Determinate	25
14	GC 89001-B-33-B	Determinate	37
15	CLOUD 190	Determinate	24
16	Kuro (dull)	Determinate	18
17	Dam Tia	Determinate	37
18	Leich hardt 5	Determinate	47
19	Khai Mon	Determinate	46
20	Black seed	Determinate	19
21	Korea 5 (Bokwang kong)	Determinate	23
22	Choe Chian (green)	Determinate	26
23	NC 103	Determinate	14
24	Prolina	Determinate	23

Table 4.3 Grouping of soybean accessions based on only growth habit showing plant height (continued)

No.	Accession name	Growth habit	Plant height (cm)
25	Kao Song	Determinate	18
26	LV. Southern Shan 1	Determinate	15
27	Korea 2007 no.6	Determinate	25
28	Korea 2007 no.47	Determinate	23
29	Korea 2007 no.70	Determinate	15
30	Korea 2007 no. 71	Determinate	15
31	Yezin	Determinate	27
32	DS 1099-04-01	Determinate	34
33	China 1 Henyshoi-Heibei	Determinate	36
34	Dain 86-4	Determinate	25
35	Chiang Mai 5	Semideterminate	57
36	Chiang Mai 6	Semideterminate	57
37	SJ 2	Semideterminate	60
38	SJ 4	Semideterminate	60
39	SJ 5	Semideterminate	64
40	Sukhothai 2	Semideterminate	41
41	Sukhothai 3	Semideterminate	44
42	KKU 35	Semideterminate	75
43	Rat Mongkhon	Semideterminate	42
44	Lee	Semideterminate	67
45	Bethel	Semideterminate	36
46	Cha Sengoku 81	Semideterminate	56
47	San Khiao	Semideterminate	38
48	Thua Nao	Semideterminate	34
49	TG 71	Semideterminate	37
50	Tainung 3	Semideterminate	41
51	Bossier	Semideterminate	37
52	Pha Bong 8	Semideterminate	59
53	IAC 13	Semideterminate	27
54	TG 61	Semideterminate	55
55	Acadian	Semideterminate	64

Table 4.3 Grouping of soybean accessions based on only growth habit showing plant height (continued)

No.	Accession name	Growth habit	Plant height (cm)
56	TG 65	Semideterminate	62
57	Doi Kham 1	Semideterminate	57
58	Don Chiang	Semideterminate	56
59	Sansai	Semideterminate	73
60	Pak chong	Semideterminate	79
61	TG 150	Semideterminate	43
62	Klang Dong	Semideterminate	61
63	TG 165	Semideterminate	66
64	SSR 8502-2-9	Semideterminate	58
65	Chosen Kuro daizu	Semideterminate	75
66	Ka La Dam	Semideterminate	45
67	Yot Son (165)	Semideterminate	66
68	Black Malla	Semideterminate	58
69	Forrest	Semideterminate	64
70	KKU 252	Semideterminate	77
71	Siam Riop	Semideterminate	64
72	Tadaeng	Semideterminate	44
73	Takhao Chiang Daot	Semideterminate	50
74	Zhongpin 661	Semideterminate	36
75	44 Lb-4E	Semideterminate	53
76	KKU 213	Semideterminate	45
77	KKU 74	Semideterminate	49
78	44 Ly-8	Semideterminate	48
79	KKU 223	Semideterminate	55
80	44 Ly-14 E	Semideterminate	54
81	KKU-5E	Semideterminate	54
82	KKU 282	Semideterminate	58
83	40 Ly-15	Semideterminate	55
84	74-T4	Semideterminate	56
85	Nakhon Sawan 1(set 2)	Semideterminate	35
86	Chiang Mai 60 (set 2)	Semideterminate	44

Table 4.3 Grouping of soybean accessions based on only growth habit showing plant height (continued)

No.	Accession name	Growth habit	Plant height (cm)
87	SJ 5 (set 2)	Semideterminate	54
88	KKU 35 (set2)	Semideterminate	97
89	Rahu Chiangfai	Indeterminate	59
90	TG 56	Indeterminate	85
91	Jupiter	Indeterminate	48
92	Thva Chao Khao	Indeterminate	119
93	OTOOTAN	Indeterminate	92
94	Fort lamy	Indeterminate	96
95	44 Ly-75	Indeterminate	104

*set 2 = the accession from Khon Kaen University

4.1.2 Days to maturity and yield production

The days to maturity varied significantly among the genotypes in table 4.4. The average days to maturity for all genotypes were 100 days. Based on the days to maturity, the genotypes were grouped into three categories as early (< 90 days), medium (90-105 days), and late (>105 days). The earliest maturity was observed in Nakhonswan 1 (85 days) and the highest in Pak chong (119 days). Similar results were reported by Diazcarrasco et al. (1986), Bowers (1990) and Tarasatyvathi et al. (2004), Manjaya and Bapat (2008) in soybean; Sudhakar et al. (2007) in sesame. Though the duration of the crop growth is a genetically controlled character, it is also influenced by the environmental and crop growth conditions such as soil moisture etc. (Govindarao, 2010)

In yield production, the genotypes were also grouped into three categories as low yielding (< 1,100 kg/ha), medium yielding (1,100-1,400 kg/ha), and

high yielding (>1,400 kg/ha). The average yield taken by the genotypes was 2,088.9 kg/ha. The genotype, TG 165 produced the highest yield (4,944.5 kg/ha) while the genotype (Korea 2007 no.70) produced the lowest yield (84.9 kg/ha). Similar results were reported by Reddy et al. (1989) in soybean. The difference in seed yield depends upon days to maturity; usually late and early maturity varieties gave high and low seed yield, respectively. This could be due to heritable characters of the varieties (Govindarao, 2010).

Table 4.4 Grouping of soybean accessions based on days to maturity and seed yield

No.	Accession name	Days to maturity	Maturity group	Seed yield (kg/ha)	Yielding group
1	Chiang Mai 60	101	Medium	1,340.8	Medium yield
2	Chiang Mai 5	104	Medium	2,611.9	High yield
3	Chiang Mai 6	102	Medium	2,374.2	High yield
4	Chiang Mai 1	105	Medium	2,435.7	High yield
5	SJ 2	105	Medium	1,923.9	High yield
6	SJ 4	102	Medium	2,151.8	High yield
7	SJ5	102	Medium	2,078.5	High yield
8	Sukhothai 2	89	Early	951.1	Low yield
9	Sukhothai 3	102	Medium	1,980.0	High yield
10	Srisamrong 1	87	Early	1,360.7	Medium yield
11	Nakhon Sawan 1	85	Early	719.7	Low yield
12	KKU 35	116	Late	3,255.7	High yield
13	Rat Mongkhon	97	Medium	1,838.9	High yield
14	G 2261	88	Early	1,212.4	Medium yield
15	Xanh tien tai	85	Early	1,849.8	High yield
16	Lee	103	Medium	2,802.7	High yield
17	Bethel	86	Early	636.5	Low yield
18	White Lion	96	Medium	511.7	Low yield
19	LV.Su 7	102	Medium	1,942.0	High yield
20	Austin	97	Medium	1,044.2	Low yield
21	Cha Sengoku 81	112	Late	1,593.9	High yield
22	Ogden USA	89	Early	420.4	Low yield
23	Alankar	102	Medium	981.0	Low yield
24	San Khiao	101	Medium	1,442.0	High yield
25	Thua Nao	116	Late	2,621.9	High yield
26	Rahu Chiangfai	86	Early	1,826.3	High yield
27	Clark 63	88	Early	1,009.9	Low yield
28	TG 71	102	Medium	2,376.0	High yield

Table 4.4 Grouping of soybean accessions based on days to maturity and seed yield (continued)

No.	Accession name	Days to maturity	Maturity group	Seed yield (kg/ha)	Yielding group
29	Tainung 3	89	Early	1,868.8	High yield
30	Bossier	103	Medium	3,347.9	High yield
31	Pha Bong 8	102	Medium	4,673.3	High yield
32	TG 56	112	Medium	2,349.8	High yield
33	Davis	102	Medium	1,728.6	High yield
34	IAC 13	104	Medium	1,188.9	Medium yield
35	TG 61	105	Medium	4,520.5	High yield
36	Acadian	100	Medium	1,329.9	Medium yield
37	TG 65	104	Medium	1,896.8	High yield
38	Doi Kham 1	102	Medium	1,408.6	High yield
39	Don Chiang	103	Medium	1,013.5	Low yield
40	Sansai	113	Late	3,823.4	High yield
41	Pak chong	119	Late	4,847.8	High yield
42	TG 150	116	Late	4,271.0	High yield
43	Klang Dong	118	Late	4,620.0	High yield
44	Jupiter	117	Late	4,192.3	High yield
45	TG 165	116	Late	4,944.5	High yield
46	GC 89001-B-33-B	102	Medium	1,829.9	High yield
47	CLOUD 190	97	Medium	644.6	Low yield
48	Kuro (dull)	87	Early	110.3	Low yield
49	Dam Tia	95	Medium	2,714.1	High yield
50	SSR 8502-2-9	102	Medium	2,394.1	High yield
51	Chosen Kuro daizu	102	Medium	2,055.9	High yield
52	Ka La Dam	102	Medium	1,495.4	High yield
53	Yot Son (165)	102	Medium	2,162.6	High yield
54	Leich hardt 5	102	Medium	4,414.7	High yield
55	Black Malla	102	Medium	2,163.5	High yield
56	Khai Mon	102	Medium	1,606.6	High yield
57	Forrest	103	Medium	3,690.5	High yield
58	OTOOTAN	118	Late	4,455.4	High yield
59	Fort lamy	112	Late	4,516.0	High yield

Table 4.4 Grouping of soybean accessions based on days to maturity and seed yield (continued)

No.	Accession name	Days to maturity	Maturity group	Seed yield (kg/ha)	Yielding group
60	KKU 252	115	Late	4,469.0	High yield
61	Black seed	86	Early	357.1	Low yield
62	Korea 5 (Bokwang kong)	100	Medium	1,284.7	Medium yield
63	Choe Chian (green)	89	Early	1,388.7	Medium yield
64	NC 103	102	Medium	688.02	Low yield
65	Prolina	102	Medium	1,446.6	High yield
66	Kao Song	96	Medium	345.4	Low yield
67	Siam Riop	117	Late	4,241.1	High yield
68	LV. Southern Shan 1	105	Medium	2,602.0	High yield
69	Tadaeng	101	Medium	3,341.6	High yield
70	Takhao Chiang Daot	101	Medium	2,422.9	High yield
71	Korea 2007 no.6	88	Early	867.0	Low yield
72	Korea 2007 no.47	95	Medium	550.6	Low yield
73	Korea 2007 no.70	96	Medium	84.9	Low yield
74	Korea 2007 no. 71	98	Medium	400.5	Low yield
75	Yezin	102	Medium	2,832.6	High yield
76	DS 1099-04-01	89	Early	1,142.8	Medium yield
77	China 1 Henyshoi-Heibei	87	Early	1,416.7	High yield
78	Dain 86-4	89	Early	867.9	Low yield
79	Zhongpin 661	89	Early	680.8	Low yield
80	44 Lb-4E	103	Medium	2,778.3	High yield
81	KKU 213	90	Medium	2,055.0	High yield
82	KKU 74	90	Medium	2,309.1	High yield
83	44 Ly-8	100	Medium	2,972.7	High yield
84	KKU 223	89	Early	2,996.2	High yield
85	44 Ly-14 E	101	Medium	1,967.3	High yield
86	KKU-5E	88	Early	1,454.7	High yield
87	KKU 282	101	Medium	1,308.2	Medium yield

Table 4.4 Grouping of soybean accessions based on days to maturity and seed yield (continued)

No.	Accession name	Days to maturity	Maturity group	Seed yield (kg/ha)	Yielding group
88	40 Ly-15	101	Medium	2,051.4	High yield
89	74-T4	106	Late	2,189.7	High yield
90	44 Ly-75	104	Medium	2,629.1	High yield
91	Nakhon Sawan 1 (Set 2)	85	Early	1,024.4	Low yield
92	Chiang Mai 60 (Set 2)	102	Early	1,608.4	High yield
93	SJ 5 (Set 2)	104	Early	1,931.2	High yield
94	KKU 35 (Set2)	118	Late	2,073.1	High yield
	Mean	100		2088.9	
	CV%	1.6		45.6	
	$LSD_{0.05}$	2.6		1533.91	
	F test	**		**	

** = highly significant, set 2 = the accession from Khon Kaen University

Note:

Early maturity : < 90 Days after sowing

Medium maturity : 90 – 105 Days after sowing

Late maturity : > 105 Days after sowing

Low yielding : <1,100 kg/ha

Medium yielding : 1,100 – 1,400 kg/ha

High yielding : > 1,400 kg/ha

(Govindarao, 2010)

4.1.3 Flower color at flowering

Based on flower color at flowering, the soybean accessions were classified into four groups (white, light purple, purple, and dark purple). White group consists of 32 soybean accessions, light purple of 15, purple of 44, and dark purple of 4. Those accessions with different groups are as shown in table 4.5. Similar results were observed by Edgar et al. (1970), Tarasatyavathi et al. (2004) in soybean; Muthiah (2006) in green gram. The actions of the genes were responsible for variations in the flower color of the genotypes. The genes determine the color of the petal by developing of blocking of anthocyanin pigmentation (Govindarao, 2010).

Table 4.5 Grouping of soybean accessions based on flower color

No.	Accession name	Flower color
1	Chiang Mai 60	white
2	Sukhothai 3	white
3	KKU 35	white
4	Bethel	white
5	White Lion	white
6	Austin	white
7	Alankar	white
8	Clark 63	white
9	Pha Bong 8	white
10	Davis	white
11	IAC 13	white
12	Don Chiang	white
13	Pak chong	white
14	TG 150	white
15	Klang Dong	white
16	TG 165	white
17	GC 89001-B-33-B	white
18	CLOUD 190	white

Table 4.5 Grouping of soybean accessions based on flower color (continued)

No.	Accession name	Flower color
19	Kuro (dull)	white
20	SSR 8502-2-9	white
21	Black Malla	white
22	KKU 252	white
23	Kao Song	white
24	Takhao Chiang Daot	white
25	Korea 2007 no.6	white
26	Korea 2007 no.47	white
27	Zhongpin 661	white
28	KKU 213	white
29	KKU 223	white
30	KKU 282	white
31	Chiang Mai 60 (Set 2)	white
32	KKU 35 (Set2)	white
33	G 2261	light purple
34	Ogden USA	light purple
35	Bossier	light purple
36	TG 56	light purple
37	TG 61	light purple
38	Doi Kham 1	light purple
39	Jupiter	light purple
40	NC 103	light purple
41	Korea 2007 no. 71	light purple
42	44 Lb-4E	light purple
43	KKU 74	light purple
44	44 Ly-8	light purple
45	44 Ly-14 E	light purple
46	74-T4	light purple

Table 4.5 Grouping of soybean accessions based on flower color (continued)

No.	Accession name	Flower color
47	44 Ly-75	light purple
48	Chiang Mai 5	purple
49	Chiang Mai 6	purple
50	Chiang Mai 1	purple
51	SJ 2	purple
52	SJ 4	purple
53	SJ 5	purple
54	Sukhothai 2	purple
55	Nakhon Sawan 1	purple
56	Rat Mongkhon	purple
57	Xanh tien tai	purple
58	Lee	purple
59	LV.Su 7	purple
60	Cha Sengoku 81	purple
61	San Khiao	purple
62	Thua Nao	purple
63	Rahu Chiangfai	purple
64	Tainung 3	purple
65	Acadian	purple
66	TG 65	purple
67	Sansai	purple
68	Thva Chao Khao	purple
69	Dam Tia	purple
70	Chosen Kuro daizu	purple

Table 4.5 Grouping of soybean accessions based on flower color (continued)

No.	Accession name	Flower color
71	Ka La Dam	purple
72	Yot Son (165)	purple
73	Leich hardt 5	purple
74	Khai Mon	purple
75	Forrest	purple
76	OTOOTAN	purple
77	Fort lamy	purple
78	Korea 5 (Bokwang kong)	purple
79	Choe Chian (green)	purple
80	Prolina	purple
81	Siam Riop	purple
82	LV. Southern Shan 1	purple
83	Tadaeng	purple
84	Yezin	purple
85	DS 1099-04-01	purple
86	China 1 Henyshoi-Heibei	purple
87	Dain 86-4	purple
88	KKU-5E	purple
89	40 Ly-15	purple
90	Nakhon Sawan 1(Set 2)	purple
91	SJ 5 (Set 2)	purple
92	Srisamrong 1	dark purple
93	TG 71	dark purple
94	Black seed	dark purple
95	Korea 2007 no.70	dark purple

*set 2 = the accession from Khon Kaen University

4.1.4 Pod color

Pod color varied among the soybean genotypes. Based on pod color, the soybean accessions were grouped into three groups (black, brown, and tin). Forty-four accessions were tin color, forty of brown and ten of black. The different groups were in table 4.6. The variation in pod number may be due to pod bearing ability of the genotype itself and varied in response to environmental conditions and nutritional status of the soil to some extent (Govindarao, 2010).

Table 4.6 Grouping of soybean accessions based on pod color

No.	Accession name	Pod color
1	Chiang Mai 1	tin
2	Srisamrong 1	tin
3	Nakhon Sawan 1	tin
4	Xanh tien tai	tin
5	Lee	tin
6	LV.Su 7	tin
7	San Khiao	tin
8	Thua Nao	tin
9	Rahu Chiangfai	tin
10	Clark 63	tin
11	Tainung 3	tin
12	Bossier	tin
13	TG 56	tin
14	IAC 13	tin
15	TG 61	tin
16	Acadian	tin
17	Don Chiang	tin
18	Sansai	tin
19	Pak chong	tin
20	TG 150	tin

Table 4.6 Grouping of soybean accessions based pod color (continued)

No.	Accession name	Pod color
21	Klang Dong	tin
22	Jupiter	tin
23	TG 165	tin
24	Dam Tia	tin
25	Ka La Dam	tin
26	Leich hardt 5	tin
27	Khai Mon	tin
28	Forrest	tin
29	Prolina	tin
30	LV. Southern Shan 1	tin
31	Korea 2007 no.6	tin
32	Korea 2007 no. 71	tin
33	Yezin	tin
34	DS 1099-04-01	tin
35	China 1 Henyshoi-Heibei	tin
36	44 Lb-4E	tin
37	KKU 74	tin
38	44 Ly-14 E	tin
39	KKU-5E	tin
40	40 Ly-15	tin
41	74-T4	tin
42	44 Ly-75	tin
43	Nakhon Sawan 1(set 2)	tin
44	Chiang Mai 60 (set 2)	tin
45	Chiang Mai 60	brown
46	Chiang Mai 5	brown
47	Chiang Mai 6	brown
48	SJ 2	brown
49	SJ 4	brown
50	SJ5	brown
51	Sukhothai 2	brown
52	Sukhothai 3	brown
53	KKU 35	brown
54	Rat Mongkhon	brown
55	G 2261	brown

Table 4.6 Grouping of soybean accessions based on pod color (continued)

No.	Accession name	Pod color
56	Bethel	brown
57	Austin	brown
58	Cha Sengoku 81	brown
59	Ogden USA	brown
60	TG 71	brown
61	Pha Bong 8	brown
62	Davis	brown
63	TG 65	brown
64	Doi Kham 1	brown
65	SSR 8502-2-9	brown
66	Chosen Kuro daizu	brown
67	Yot Son (165)	brown
68	Black Malla	brown
69	OTOOTAN	brown
70	Fort Lamy	brown
71	KKU 252	brown
72	Black seed	brown
73	NC 103	brown
74	Siam Riop	brown
75	Tadaeng	brown
76	Takhao Chiang Daot	brown
77	Dain 86-4	brown
78	Zhongpin 661	brown
79	KKU 213	brown
80	44 Ly-8	brown
81	KKU 223	brown
82	KKU 282	brown
83	SJ 5 (set 2)	brown
84	KKU 35 (set2)	brown
85	White Lion	black
86	Alankar	black
87	GC 89001-B-33-B	black
88	CLOUD 190	black
89	Kuro (dull)	black

Table 4.6 Grouping of soybean accessions based on pod color (continued)

No.	Accession name	Pod color
90	Korea 5 (Bokwang kong)	black
91	Choe Chian (green)	black
92	Kao Song	black
93	Korea 2007 no.47	black
94	Korea 2007 no.70	black

*set 2 = the accession from Khon Kaen University

4.1.5 Seed coat color

Based on seed coat color, the soybean accessions were classified into six groups (yellowish white, yellow, green, buff, reddish brown, and black). Yellowish white group consisted of 7 soybean accessions, yellow of 54, green of 8, buff of 7, reddish brown of 4, and black of 14. Those accessions with different groups are as shown in table 4.7.

Table 4.7 Grouping of soybean accessions based on seed coat color

No.	Accession name	Seed coat color
1	Rat Mmongkhon	yellowish white
2	Bethel	yellowish white
3	Acadian	yellowish white
4	Don Chiang	yellowish white
5	LV. Southern Shan 1	yellowish white
6	44 Ly-8	yellowish white
7	74-T4	yellowish white
8	Chiang Mai 60	yellow
9	Chiang Mai 5	yellow
10	Chiang Mai 6	yellow
11	SJ 2	yellow
12	SJ 4	yellow
13	SJ 5	yellow

Table 4.7 Grouping of soybean accessions based seed coat color (continued)

No.	Accession name	Seed coat color
14	Sukhothai 2	yellow
15	Srisamrong 1	yellow
16	Nakhon Sawan 1	yellow
17	G 2261	yellow
18	Lee	yellow
19	LV.Su 7	yellow
20	Ogden USA	yellow
21	San Khiao	yellow
22	Clark 63	yellow
23	TG 71	yellow
24	Tainung 3	yellow
25	Bossier	yellow
26	Pha Bong 8	yellow
27	TG 56	yellow
28	Davis	yellow
29	IAC 13	yellow
30	TG 61	yellow
31	TG 65	yellow
32	Doi Kham 1	yellow
33	Sansai	yellow
34	Pak chong	yellow
35	TG 150	yellow
36	TG 165	yellow
37	GC 89001-B-33-B	yellow
38	Ka La Dam	yellow
39	Leich hardt 5	yellow
40	Khai Mon	yellow
41	KKU 252	yellow
42	NC 103	yellow
43	Prolina	yellow
44	Takhao Chiang Daot	yellow
45	Korea 2007 no.6	yellow
46	Yezin	yellow
47	DS 1099-04-01	yellow
48	China 1 Henyshoi-Heibei	yellow

Table 4.7 Grouping of soybean accessions based seed coat color (continued)

No.	Accession name	Seed coat color
49	Dain 86-4	yellow
50	Zhongpin 661	yellow
51	44 Lb-4E	yellow
52	KKU 213	yellow
53	KKU 74	yellow
54	KKU 223	yellow
55	44 Ly-14 E	yellow
56	KKU-5E	yellow
57	KKU 282	yellow
58	40 Ly-15	yellow
59	Nakhon Sawan 1(Set 2)	yellow
60	Chiang Mai 60 (Set 2)	yellow
61	SJ 5 (Set 2)	yellow
62	Xanh tien tai	green
63	White Lion	green
64	Austin	green
65	Rahu Chiangfai	green
66	Jupiter	green
67	Choe Chian (green)	green
68	Kao Song	green
69	Korea 2007 no.47	green
70	Chiang Mai 1	buff
71	KKU 35	buff
73	Siam Riop	buff
72	Thua Nao	buff
74	Tadaeng	buff
75	44 Ly-75	buff
76	KKU 35 (Set2)	buff
77	Cha Sengoku 81	reddish brown
78	Klang Dong	reddish brown
79	Kuro (dull)	reddish brown
80	Korea 5 (Bokwang kong)	reddish brown
81	Sukhothai 3	black
82	Alankar	black

Table 4.7 Grouping of soybean accessions based on seed coat color (continued)

No.	Accession name	Seed coat color
83	CLOUD 190	black
84	Dam Tia	black
85	SSR 8502-2-9	black
86	Chosen Kuro daizu	black
87	Yot Son (165)	black
88	Black Malla	black
89	Forrest	black
90	OTOOTAN	black
91	Fort Lamy	black
92	Black seed	black
93	Korea 2007 no.70	black
94	Korea 2007 no. 71	black

*set 2 = the accession from Khon Kaen University

4.1.6 Correlation analysis for agro-morphological data

The correlation coefficients were computed to understand the nature and magnitude of relationship existing between yield and its component characters as well as the association among the component characters themselves. In the study, yield was highly and significantly associated with the tested traits except seeds per pod. The correlations among 9 characters are presented in table 4.8. It can be suggested that phenotypic selection could be made on the basis of these characters. Similar results were reported by Mukhekar et al. (2004), Chandel et al. (2005) and Turkec (2005).

4.1.6.1 Correlations of the characters with seed yield

Yield is a complex quantitative character governed by large number of genes and is highly influenced by environment. Therefore, the selection of superior genotypes based on yield is very difficult. For a rational approach towards improvement of yield, selection has to be made for the components of yield.

Association of yield components and yield may be assumed as special importance for the basis of indirect selection. Genetic correlation between different characters of plant often arises because of linkage or pleiotrophy (Harland, 1939).

Seed yield per hill had highly significant positive association with days to 50% flowering (0.62^{**}), days to pod formation (0.71^{**}), days to maturity (0.56^{**}), plant height (0.51^{**}), number of filled pods per hill (0.82^{**}), 100-seed weight (0.27^{**}) and harvest index % (0.62^{**}). Seed yield per hill had non-significant positive association with seeds per pod (0.09^{ns}) (table 4.8).

Amaranath et al. (1990) and Harer and Deshmukh (1992) reported that yield was positively correlated with days to maturity. Dixit and Patil (1982) and Perraju et al. (1982) indicated that yield was positively associated with number of pods per plant. Yield showed positive correlation with number of seeds per pod (Song et al., 1987 and Sahu and Mishra, 1988). In the correlation between yield and HI, Bhardwaj et al. (1990) and Yao et al. (1987) reported that these characters were positive associated. Basavaraja et al. (2005) determined the positive correlation of seed yield with 100-seed weight and harvest index.

4.1.6.2 Association among the characters

1) Days to 50% flowering

Days to 50% flowering showed positive and highly significant correlation with days to pod formation (0.90^{**}), days to maturity (0.64^{**}), plant height (0.54^{**}), number of filled pods per hill (0.63^{**}), yield per hill (0.62^{**}), and harvest index % (0.31^{**}). It was positively and non-significantly correlated with seeds per pod (0.01^{ns}) and negatively and non-significantly correlated with 100-seed weight (-0.43^{ns}) in table 4.8. The similar result on positive association between days to 50% flowering and plant height was reported by Harer and Deshmukh (1992), Ramgiry and Raha (1997) and Mehetre

et al. (1997). This result was in agreement with the association that days to 50% flowering was positive correlation with days to maturity reported by Ramana et al. (2000), Mukhekar et al. (2004) and Dev Vart (2005). This study was followed under the result that days to 50% flowering showed positive correlation with number of pods per plant reported by Ramgiry and Raha (1997), Bhandarkar (1999) and Nehru et al. (1999). Although Mehetre (1991), Ramgiry and Raha (1997) reported that there was negative correlation between days to 50% flowering and harvest index, this study showed positive correlation. This result is also in agreement with the research conducted by Harer and Deshmukh (1992), Mehetre et al. (1997), Ramgiry and Raha (1997).

2) Days to pod formation

Days to pod formation were positive and non-significantly correlated with number of seeds per pod (0.02^{ns}). However, it showed positive and highly significant correlation with days to maturity (0.74^{**}), plant height (0.58^{**}), number of filled pods per hill (0.70^{**}), seed yield per hill (0.71^{**}), and harvest index % (0.32^{**}). It was negative and highly significantly associated with 100-seed weigh (-0.48^{**}) in table 4.8.

3) Days to maturity

Only seeds per pod were positive and non-significantly associated with days to maturity, (0.04^{ns}). Other characters were positive and highly significantly correlated with days to maturity. They were plant height (0.56^{**}), number of filled pods per hill (0.50^{**}), 100-seed weight (0.31^{**}), yield per hill (0.56^{**}), and harvest index % (0.19^{**}) in table 4.8. The result was followed under the report that days to maturity was positive associated with number of pods per plant reported by Harer and Deshmukh (1992) and Mehetre et al. (1997). While Ramgiry and Raha (1997) and Nehru et al. (1999) reported that days to maturity showed positive

correlation with number of seeds per pod and Bhandarkar (1999), Dev Vart (2005) indicated that they were negative association, this result was followed the positive association phenomenon. Mehetre et al. (1997) and Ramgiri and Raha (1997) found that there was negative association between days to maturity and HI(%). However, this study showed positive association. Between number of pods per plant and number of seeds per pod, Ramgiry and Raha (1997), Nehru et al. (1999) reported that positive correlation, however, Harer and Deshmukh (1992), Bhandarkar (1997) observed that negative association. This study is followed under positive association.

4) Plant height

Plant height showed positive and highly significant correlation with other characters: number of filled pods per hill (0.53^{**}), number of seeds per pod (0.18^{**}), 100-seed weight (0.25^{**}), yield per hill (0.51^{**}) and harvest index % (0.23^{**}) in table4.8. Therefore, there was a trend to increase pods per plant with increased plant height as there were also positively correlated with each other. Malik et al. (2006) also revealed that pods per plant could be increased by selecting tall plants.

5) Number of filled pods per hill

Number of filled pods per hill had positive and non-significant correlation with seeds per pod (0.01^{ns}). However, it showed highly significant association with 100-seed weight (0.44^{**}), yield per hill (0.82^{**}), and harvest index % (0.48^{**}) in table 4.8. Mehetre et al. (1997) and Dev Vart (2005) reported the positive correlation between number of pods per plant and HI (%) while Ramgiry and Raha (1997) indicated negative association. This study was observed in positive association. Ramgiry and Raha (1997) and Dav Vart (2005) found that there was positive correlation between number of seeds per pod and HI (%). This study was also observed in positive correlation.

6) Seeds per pod

Seeds per pod were positive and non-significantly associated with 100-seed weight (0.01^{ns}), yield per hill (0.09^{ns}), and harvest index % (0.05^{ns}) in table 4.8.

7) 100-seed weight

100-seed weight showed positive and highly significant correlation with yield per hill (0.27^{**}), and harvest index % (0.18^{**}) in table 4.8.

8) Harvest index %

Harvest index % showed positive and highly significant association with the tested traits [(days to 50% flowering (0.31^{**}), days to pod formation (0.32^{**}), days to maturity (0.19^{**}), plant height (0.23^{**}), number of filled pods per hill (0.48^{**}), 100-seed weight (0.18^{**}), and seed yield per hill (0.62^{**}), except number of seeds per pod (0.05^{ns})].

Table 4.8 Correlation coefficients of 9 traits studied in ninety-four accessions of soybean

Characters	DF	DP	DM	PH	NF	SP	SW	PY
DP	0.90**							
DM	0.64**	0.74**						
PH	0.54**	0.58**	0.56**					
NF	0.63**	0.70**	0.50**	0.53**				
SP	0.01ns	0.02ns	0.04ns	0.18**	0.01ns			
SW	-0.43**	-0.48**	0.31**	0.25**	0.44**	0.01ns		
PY	0.62**	0.71**	0.56**	0.51**	0.82**	0.09ns	0.27**	
HI	0.31**	0.32**	0.19**	0.23**	0.48**	0.05ns	0.18**	0.62**

*=significant, **=highly significant, ns=non-significant, DF=days to 50% flowering, DP=days to pod formation, DM=days to maturity, PH=plant height, NF=number of filled pods per hill, SP=number of seeds per pod, SW=100 seed weight, PY=yield per hill, HI=harvest index (%)

4.1.7 Cluster analysis for morphological data

Figure 4.1 showed that the UPGMA procedure defined 7 clusters based on 7 morphological traits (flower color, leaf shape, plant type, pod color, pubescence color, seed coat color and helium color) at similarity coefficient, 0.52. The genetic similarity of the different genotypes ranged from 0.125 to 0.933. Mean and standard deviation for various characters in each cluster are presented in table 4.9. Cluster I consisted of 23 soybean accessions (24.5% of the total accessions), cluster II of 37 (39.4%), cluster III of 5 (5.3%), cluster IV of 4(4.3%), cluster V of 18 (19.1%),

cluster VI of 5 (5.3%) and cluster VII of 2 (2.1%). The accessions in group VI and VII showed low plant height. In this cluster analysis, the released varieties (Chiang Mai 60, Srisamrong 1, Chiang Mai 1, Nakhon Sawan 1), and local variety (Rhaimon) were in the same group (Group I). In Group II, released varieties (Chiang Mai 5, SJ 4, SJ 5, SJ 5 set 2, SJ 2, Chiang Mai 60 set 2, Nakhon Sawan 1 set 2, and Rat Mongkhon), local varieties (Kaladam, Donchiang, and Thuanao) were observed. Local varieties (Tadaeng and Siam Riop) were in the Group III. Released varieties (Sukhothai 2, Sukhothai 3, KKU 35, and KKU 35 set 2) and local varieties (Yotson 165 and Damtia) were in the Group V, respectively in figure 4.1 and table 4.10. The accessions SJ 4, SJ 5, SJ 2, and Acadian were in the same group II. The backgrounds of these accessions were: SJ was obtained by hybridization of Acadian and Tainung 4, SJ 5 was by Tainung 4 and SJ 2.

Cluster analysis grouped together having accessions with greater morphological similarity, thus representative accessions from a cluster of particular group could be chosen for hybridization program. Malik et al. (2011) reported that 98 soybean accessions were clustered into 3 groups (6-subgroups) by using the UPGMA procedure regarding of morphological data. In their study, means of the clusters showed that the accessions in cluster III were not only late maturing and high yielding but also had more pods per plant, branches per plant and 100 seed weight. They also reported that the accessions in cluster I showed some promise to earliness with high grain yield.

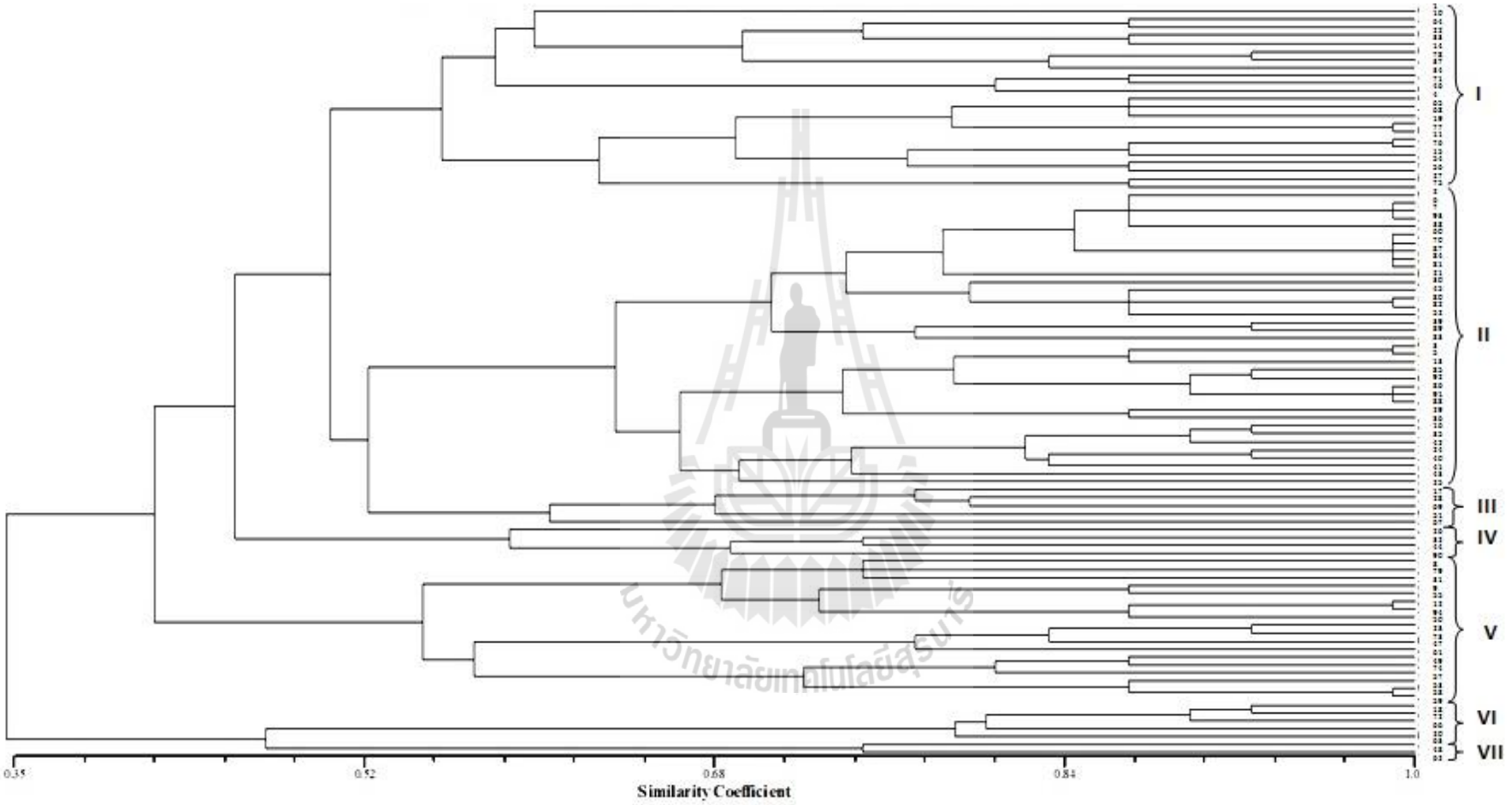

Figure 4.1 Dendrogram based on 7 morphological data of 94 soybean accessions by UPGMA method

Table 4.9 Cluster means and standard deviations of 9 morphological traits studied in 94 soybean accessions

No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	Days to 50% flowering	37 ± 11	39 ± 6	34 ± 6	42 ± 6	37 ± 6	31 ± 5	29 ± 3
2	Days to pod formation	47 ± 16	51 ± 10	43 ± 6	53 ± 9	48 ± 9	38 ± 6	36 ± 1
3	Days to maturity	96 ± 8	103 ± 9	102 ± 11	105 ± 14	102 ± 10	95 ± 3	94 ± 9
4	Plant height (cm)	33.81 ± 12.4	54.01 ± 10.9	51.28 ± 17.6	73.89 ± 25.5	50.75 ± 27.6	24.41 ± 10.5	20.90 ± 3.6
5	No. of filled pods per plant	45 ± 25	75 ± 36	58 ± 26	70 ± 23	69 ± 48	20 ± 5	8 ± 10
6	Seeds per pod	2 ± 1	2 ± 1	2 ± 1	3 ± 1	2 ± 0	2 ± 0	2 ± 0
7	Harvest index	59.51 ± 18.1	65.46 ± 13.4	61.91 ± 15.8	59.12 ± 10.7	56.26 ± 25.2	39.44 ± 16.8	35.14 ± 40.1
8	100 seed weight (g)	18.70 ± 4.9	16.51 ± 4.1	17.73 ± 3.5	17.60 ± 3.6	15.18 ± 4.8	23.76 ± 5.6	23.19 ± 6.1
9	Yield per hill (g)	17.71 ± 9.6	27.64 ± 12.5	27.99 ± 15.1	30.41 ± 11.3	23.46 ± 16.8	8.50 ± 4.8	7.72 ± 9.2

SD = standard deviation

Table 4.10 Distribution of 94 genotypes into different clusters by using 7 morphological traits

No.	Cluster No.	No. of accessions	%	Name of accessions
1	I	23	24.5	Chiang Mai 60, Srisamrong 1, NC-103, Ogden USA, Davis, G-62261, Dain 864, TG 65, IAC 13, Korea 2007(no. 6), GC 89001-B-33-B, Chiang Mai 1, Prolina, LV. Southern Shan 1, LV. Su 7, China 1 Henyshoi-Heibei, Nakhon Sawan 1, DS 1099-04-01, Xanh tien tai, Leich
2	II	37	39.4	Chiang Mai-5, SJ-4, SJ-5, SJ-5 (set2), Doi Kham 1, KKU 252, Takhao Chiang Daot, KKU 282, KKU 223, KKU 213, Cha Sengoku 81, Bossier, TG 165, 44 lb-4E, KKU 74, Ka La Dam, Don Chiang, 74-T4, 44 lb-8, CM-6, SJ 2, Rat Mongkhon, 44 lb-14E, Chiang Mai-60 (set2), KKU-5E, Nakhon Sawan 1(set2), 40 ly-15, Tainung 3, Acadian, Lee, TG 61, TG 150,
3	III	5	5.3	Bethel, TG 71, Tadaeng, Chosen Kuro daizu, Siam Riop
4	IV	4	4.3	Rahuchiangfai, TG 56, Jupiter, 44 ly-75
5	V	18	19.1	Sukhothai 2, Zhongpin 661, Phabong 8, Sukhothai 3, Blackmalla, KKU 35, KKU 35 (set 2), SSR 8502-2-9, Alankar, Korea 2007 (no.70), Cloud, Black Seed, Damtia, Korea 2007 (no.71), Forrest, Yot Son (165),
6	VI	5	5.3	White Lion, Korea 2007 (no.47), Kaosong, Austin, Choechian (green)
7	VII	2	2.1	Kuro (dull), Korea 5 (Bokwang Kong)

*set 2 = the accession from Khon Kaen University

4.2 Experiment II. Physiological identification

In physiological identification, SCMR and SLA were studied on 27 soybean accessions which had maturity date of more than 100 days after sowing. Among 27 soybean accessions, accession Thua Chao Khao could not give seed yield. Therefore, SCMR, SLA and yield were analyzed on 26 soybean accessions. In the study, SCMR, SLA and yield of 26 accessions were significant.

Among 27 soybean accessions studied on SCMR and SLA values, KKU 252 showed the highest SCMR (50.50) and the lowest SLA value (159.68 cm^2/g). Thva Chao Khao was observed that it had the lowest SCMR (36.39) and the highest SLA value, 376.67 cm^2/g in table 4.11.

Table 4.11 Mean values of SCMR and SLA of 27 soybean accessions

No.	Accession Name	SCMR	SLA (cm^2/g)
1	Chiang Mai 60	40.58	151.47
2	Chiang Mai 5	39.70	173.50
3	Chiang Mai 6	40.38	234.13
4	Chiang Mai 1	41.38	145.93
5	SJ 2	42.29	156.49
6	SJ 5	41.22	182.99
7	KKU 35	47.62	147.13
8	Thua Nao	40.00	246.62
9	TG 56	35.33	186.02
10	Sansai	40.84	240.89
11	Pak chong	42.00	224.45
12	TG 150	45.21	231.67
13	Klang Dong	38.72	234.78
14	Jupiter	41.61	216.84

Table 4.11 Mean values of SCMR and SLA values of 27 soybean accessions (continued)

No.	Accession Name	SCMR	SLA (cm^2/g)
15	TG 165	42.78	179.07
16	Thva Chao Khao	36.39	376.67
17	Leich hardt 5	48.13	185.19
18	Black Malla	43.96	160.97
19	Forrest	42.35	212.81
20	OTOOTAN	43.25	190.43
21	Fort Lamy	41.21	250.00
22	KKU 252	50.50	159.68
23	Siam Riop	41.49	200.09
24	74-T4	43.20	208.42
25	44 Ly-75	43.84	188.71
26	SJ 5 (Set 2)	38.21	260.21
27	KKU 35 (Set 2)	45.28	196.39
	Mean	42.13	205.24
	$LSD_{0.05}$	1.98	29.49
	CV%	4.62	16.94

* set 2 = the accession from Khon Kaen University

4.2.1 Correlation analysis for physiological data

When the physiological traits were studied for correlation analysis, there were negative and highly significant correlations between specific leaf area (SLA) and yield (-0.54^{**}), while SLA showed no association with SCMR (-0.39^{ns}). In the study, yield showed no association with SCMR (0.20^{ns}) in table 4.12. Ahmed (2011) indicated that SCMR showed significant positive correlation with chlorophylls at flowering stage. His result revealed that grain yield significantly correlated to the leaf chlorophyll.

Table 4.12 Correlation coefficients of physiological traits, yield and yield components studied in 26 soybean accessions

Characters	SCMR	SLA	No. of pods/hill	No. of seeds/pod	100 seeds wt.
SLA	-0.39^{ns}				
No. of pods/hill	-0.02^{ns}	0.35^{ns}			
No. of seeds/pod	0.30^{ns}	0.27^{ns}	0.74**		
100 seeds wt.	0.20^{ns}	-0.32^{ns}	-0.43*	-0.29^{ns}	
Yield	0.20^{ns}	-0.54**	-0.69**	-0.47*	0.20^{ns}

* = significant, **= highly significant, ns = non-significant

4.2.2 Cluster analysis for physiological data

In regarding of physiological traits (SCMR and SLA), 4 major groups were divided for 27 soybean accessions by using UPGMA method. Cluster I consisted of 11 soybean accessions (40.7%), cluster II of 5 (18.5%), cluster III of 9 (33.3%) and cluster IV of 2 (7.4%) in figure 4.2. In this cluster analysis based on physiological traits, released varieties Chiang Mai 60, Chiang Mai 1, SJ 5, and KKU 35 set 2 and local variety Klang Dong were in group I. Released varieties SJ 5 set 2 and local variety Thva Chao Khao were in group II. Released varieties Chiang Mai 5, Chiang Mai 6, and SJ 2 and local varieties Thva Nao and Pakchong 8 were in group III of table 4.14. Regarding of physiological traits, UPGMA procedure provided 4 groups in 27 soybean accessions. Similar results were found in peanut by others (Nageswara Rao et al., 2001; Nigam and Aruna, 2008).

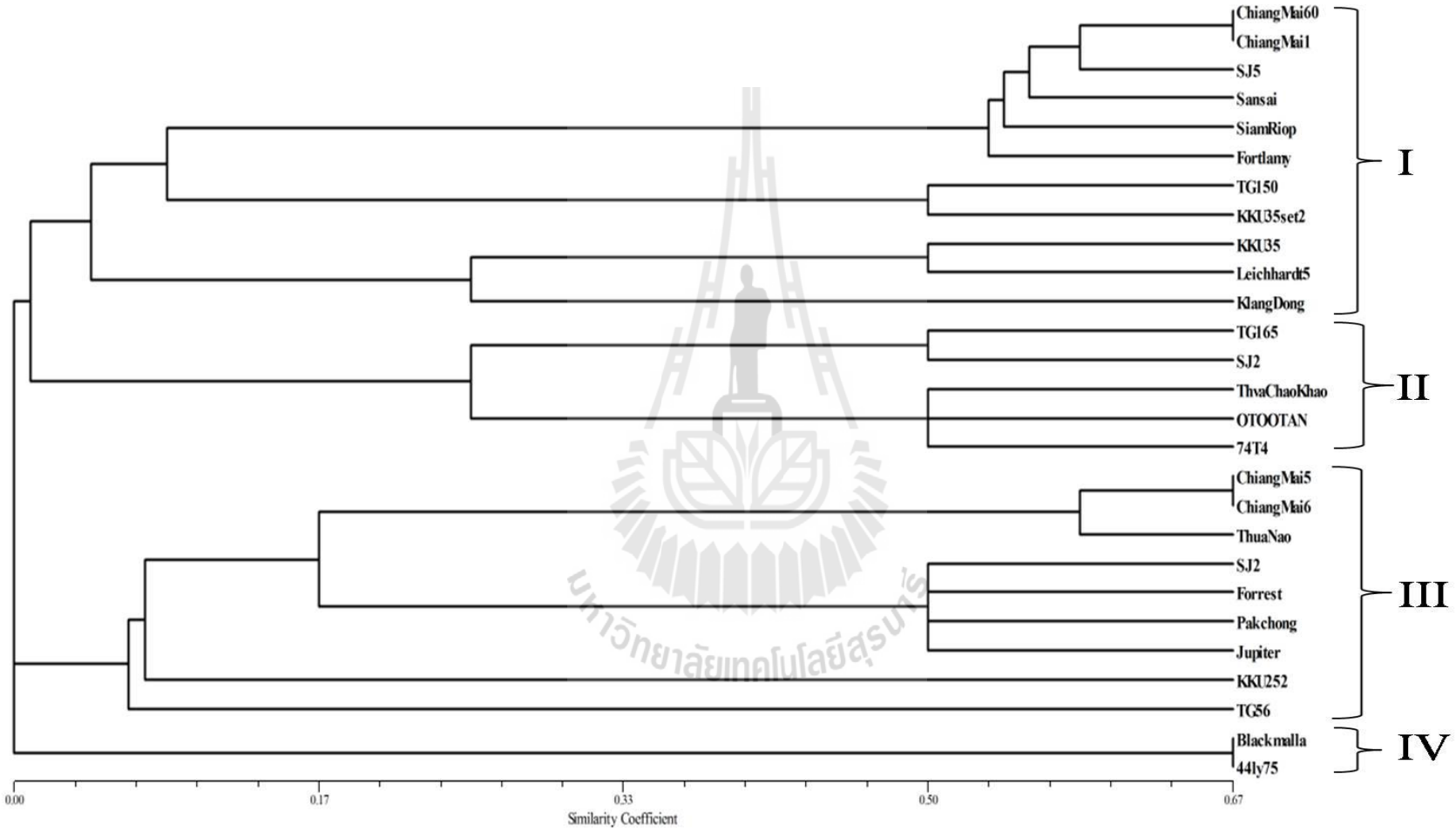

Figure 4.2 Dendrogram based on SCMR and SLA of 27 soybean accessions by using UPGMA method

Table 4.13 Cluster means and standard deviation of 2 physiological traits studied in 27 soybean accessions

No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
1.	Chlorophyll content	42.4 ± 2.9	40.8 ± 3.2	41.6 ± 3.9	43.9 ± 0.1
2.	Specific Leaf Area (cm^2/g)	201.9 ± 36.7	242.9 ± 80.9	201.2 ± 33.1	174.8 ± 19.6

Table 4.14 Distribution of 94 genotypes into different clusters based on 2 physiological traits (SCMR and SLA value)

No.	Cluster No.	No. of accessions	%	Name of accessions
1	I	11	40.7	Chiang Mai 60, Chiang Mai 1, SJ 5, Sansai, Siam Riop, Fort Lamy, TG-150, KKU-35 (set 2), KKU-35, Leich hardt 5,
2	II	5	18.5	TG-165, SJ 5(set 2), Thva Chao Khao, OTOOTAN, 74-T4
3	III	9	33.3	Chiang Mai-5, Chiang Mai-6, Thua Nao, SJ 2, Forrest, Pak chong 8, Jupiter, KKU 252, TG 56
4	IV	2	7.4	Black Malla, 44 ly-75

4.3 Experiment III. Molecular Identification

4.3.1. ISSR analysis of the tested 36 soybean accessions

For cultivar discrimination, genetic relationships among 36 soybean accessions were assessed using the Inter Simple Sequence Repeats (ISSRs) technique with seven primers.

Firstly, anneal temperature of each primer was examined to optimize the amplification condition for ten ISSR primers. Eventually, out of ten ISSR primers, only seven ISSR primers that produced clear and reproducible bands were selected for the amplification of tested 36 soybean DNA samples in table 4.15. Primer UBC-821, UBC-815, and UBC-812 did not give any amplification product. The remaining seven ISSR primers showed polymorphism and yielded 32 reliable fragments, of which only 23 fragments were polymorphic and revealed a high DNA polymorphism among the accessions. The range of polymorphism bands was 1 to 7 with the average polymorphism band was 3. The average per cent polymorphism of seven primers was 67.26. A large genetic diversity was detected among the samples based on estimation of DNA products amplified from seven selected ISSR primers, with the similarity coefficient varying from 0.5 to 1.0 in table 4.16. Therefore, the tested soybean individuals could be distinguished based on the differences in ISSR banding patterns.

The primer, UBC 822 showed the highest polymorphic percent, 87.50% while the primer, UBC 868 showed the lowest polymorphic percent, 33.33% among the 36 soybean accessions. The size of the scorable amplified fragments ranged from 250 to 900 bp. Most of the fragments were polymorphic among the tested DNA samples. The number of bands ranged from 3 by primer UBC 834, UBC 868 to 8 by primer UBC 822 with an average of 5 bands per primer and 3 bands per primer were

polymorphic. Example banding pattern of UBC 834 with the range from amplification product of 200 bp to 900 bp was shown in figure 4.4.

The highest molecular diversity (S_{ij} = 0.5) was observed between the accessions KKU 35 and Leich hardt 5, Kao Song, LV. Southern Shan 1, and Yezin, between Don Chiang and Forrest, OTOOTAN, Korea 2007 no.71, Dain 86-4, between Fort Lamy and Forrest and OTOOTAN, between Fort Lamy and Dain 86-4. High similarity with S_{ij} (1.0) was observed between Forrest and OTOOTAN and between Ka La Dam and Prolina in table 4.16.

A dendrogram based on UPGMA was constructed using a similarity matrix derived from 23 polymorphic ISSR fragments generated by seven ISSR primers in figure 4.3. The dendrogram constructed from the data revealed 6 major clusters at similarity coefficient level, 0.77. Cluster VI was the largest cluster with 22 varieties. Cluster IV was the smallest cluster with only one accession. The accessions contained in each cluster were in table 4.17. This laid a foundation for soybean variety evaluation and soybean germplasm exploitation. Our study based on DNA products amplified from the 7 selected ISSR primers demonstrated relatively high genetic variation in the tested 36 soybean accessions.

There are many successful examples for using ISSR markers to discriminate plant germplasm. Evaluation and identification of germplasm using ISSR markers are playing an important role in studies of genetics and breeding. Similar ISSR research was found in some cultivar identification analysis. About ISSR for cultivar identification, Monte-Corvo et al. (2001) studied ISSR markers for cultivar identification and for determination of the phenetic relationships among 24 pear cultivars. They observed that each of the eight ISSR primers tested was able to distinguish the 24 pear cultivars generating 337 markers (79.5% of which were polymorphic).

ISSR primers were also used to identify strawberry varieties. They can reveal the genetic relationships among the six strawberry varieties. In their observation, nine ISSR primers generated 102 total amplified fragments, of which 86 (84.3%) polymorphic fragments discriminated the varieties (Hussein et al., 2008).

Yan et al. (2003) observed that a large genetic diversity was detected among the tested soybean populations based on estimation of DNA products amplified from 15 ISSR primers with the similarity coefficient varying from 0.17 to 0.89.

The importance and need of soybean cultivars at global level requires evaluation of germplasm to assist the future breeding programs. Hence, it is essential to characterize soybean germplasm using markers like PCR-based marker such as ISSR, RAPD, etc.

Table 4.15 ISSR banding patterns generated using 7 primers for 36 different soybean accessions

No.	Primer	Primer sequence(3'-5')	Annealing temp. (°C)	Number of bands			
				Total	Polymorphic	Monomorphic	Percent of polymorphism
1	UBC-850	$(GT)_8YC$	54	6	5	1	83.33
2	UBC-836	$(AG)_8YA$	49	4	3	1	75.00
3	UBC-834	$(AG)_8YT$	56	3	2	1	66.67
4	UBC-822	$(TC)_8A$	53	8	7	1	87.50
5	UBC-828	$(TG)_8A$	56	4	3	1	75.00
6	UBC-868	$(GAA)_6$	56	3	1	2	33.33
7	UBC-857	$(AC)_8YG$	49	4	2	2	50.00
	Total			32	23	9	
	Average			5	3	1	67.26

Table 4.16 Similarity matrixes of 36 different soybean accessions based on ISSR profile

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	1.0																																			
2	0.6	1.0																																		
3	0.8	0.7	1.0																																	
4	0.9	0.6	0.9	1.0																																
5	0.7	0.7	0.9	0.7	1.0																															
6	0.8	0.8	0.8	0.8	0.8	1.0																														
7	0.8	0.7	0.9	0.9	0.8	0.9	1.0																													
8	0.7	0.6	0.8	0.8	0.8	0.8	0.8	1.0																												
9	0.9	0.7	0.9	0.9	0.7	0.9	0.9	0.8	1.0																											
10	0.7	0.8	0.7	0.7	0.7	0.8	0.8	0.7	0.8	1.0																										
11	0.8	0.8	0.8	0.7	0.7	0.9	0.8	0.7	0.9	0.9	1.0																									
12	0.7	0.9	0.8	0.7	0.8	0.9	0.8	0.7	0.8	0.9	0.9	1.0																								
13	0.9	0.7	0.9	0.8	0.8	0.9	0.9	0.7	0.9	0.8	0.9	0.9	1.0																							
14	0.7	0.6	0.7	0.6	0.6	0.6	0.7	0.7	0.7	0.8	0.8	0.7	0.7	1.0																						
15	0.8	0.8	0.9	0.7	0.8	0.8	0.8	0.7	0.9	0.7	0.8	0.8	0.9	0.6	1.0																					
16	0.9	0.7	0.9	0.8	0.8	0.8	0.8	0.7	0.9	0.7	0.8	0.8	0.9	0.6	0.9	1.0																				
17	0.9	0.6	0.9	0.8	0.7	0.7	0.8	0.8	0.8	0.7	0.8	0.7	0.8	0.7	0.7	0.9	1.0																			
18	0.8	0.8	0.8	0.8	0.8	0.9	0.9	0.7	0.9	0.8	0.8	0.9	0.9	0.6	0.8	0.8	0.7	1.0																		
19	0.8	0.6	0.8	0.8	0.7	0.7	0.8	0.8	0.8	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.8	0.7	1.0																	
20	0.8	0.5	0.8	0.7	0.6	0.7	0.7	0.8	0.7	0.7	0.7	0.6	0.7	0.8	0.7	0.8	0.9	0.7	0.8	1.0																
21	0.8	0.7	0.9	0.8	0.9	0.7	0.9	0.8	0.8	0.7	0.7	0.7	0.8	0.7	0.7	0.8	0.7	0.8	0.8	0.7	1.0															
22	0.7	0.7	0.8	0.8	0.9	0.9	0.9	0.8	0.8	0.8	0.8	0.9	0.9	0.6	0.8	0.8	0.7	0.9	0.7	0.7	0.9	1.0														
23	0.7	0.6	0.8	0.8	0.9	0.8	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.5	0.8	0.8	0.7	0.9	0.6	0.6	0.8	0.9	1.0													
24	0.7	0.6	0.8	0.8	0.9	0.8	0.8	0.7	0.8	0.7	0.7	0.7	0.8	0.5	0.8	0.8	0.7	0.9	0.6	0.7	0.8	0.9	1.0	1.0												
25	0.7	0.6	0.7	0.7	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.6	0.8	0.6	0.8	0.8	0.7	0.6	0.5	0.5	1.0											
26	0.7	0.7	0.9	0.8	0.9	0.8	0.9	0.8	0.8	0.7	0.8	0.8	0.9	0.6	0.8	0.8	0.7	0.9	0.7	0.7	0.9	0.9	0.9	0.9	0.6	1.0										
27	0.8	0.8	0.8	0.8	0.8	0.9	0.9	0.7	0.9	0.8	0.8	0.9	0.9	0.6	0.8	0.8	0.7	1.0	0.7	0.7	0.8	0.9	0.9	0.9	0.6	0.9	1.0									
28	0.8	0.5	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.8	0.9	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.7	0.8	0.8	1.0								
29	0.8	0.6	0.7	0.7	0.6	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.8	0.6	0.7	0.8	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.8	0.6	0.7	0.7	1.0							
30	0.9	0.5	0.8	0.8	0.6	0.7	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.8	0.7	0.8	0.9	0.7	0.8	0.9	0.7	0.7	0.6	0.6	0.8	0.7	0.7	0.8	0.8	1.0						
31	0.8	0.6	0.9	0.8	0.8	0.8	0.9	0.8	0.9	0.7	0.8	0.8	0.9	0.7	0.8	0.8	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.9	0.8	0.9	0.7	0.8	1.0					
32	0.7	0.8	0.8	0.8	0.9	0.8	0.8	0.8	0.8	0.6	0.7	0.7	0.8	0.5	0.9	0.8	0.7	0.9	0.7	0.6	0.8	0.9	0.9	0.9	0.6	0.9	0.9	0.7	0.6	0.6	0.8	1.0				
33	0.9	0.5	0.8	0.8	0.7	0.7	0.8	0.7	0.8	0.7	0.7	0.6	0.7	0.8	0.7	0.8	0.8	0.8	0.7	0.8	0.8	0.7	0.8	0.8	0.7	0.8	0.8	0.8	0.9	0.8	0.8	0.7	1.0			
34	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.7	0.7	0.7	0.8	0.6	0.8	0.8	0.7	0.8	0.8	0.7	0.9	0.8	0.8	0.8	0.7	0.8	0.8	0.7	0.7	0.7	0.7	0.9	0.8	1.0		
35	0.8	0.7	0.8	0.9	0.9	0.9	0.9	0.7	0.9	0.7	0.8	0.8	0.9	0.5	0.8	0.8	0.7	0.9	0.7	0.6	0.9	0.9	0.9	0.9	0.5	0.9	0.9	0.8	0.6	0.6	0.8	0.9	0.8	0.8	1.0	
36	0.7	0.7	0.8	0.8	0.9	0.9	0.9	0.7	0.8	0.7	0.7	0.8	0.8	0.6	0.9	0.9	0.7	0.9	0.6	0.6	0.8	0.9	0.9	0.9	0.6	0.9	0.9	0.8	0.6	0.6	0.9	0.9	0.8	0.8	0.9	1.0

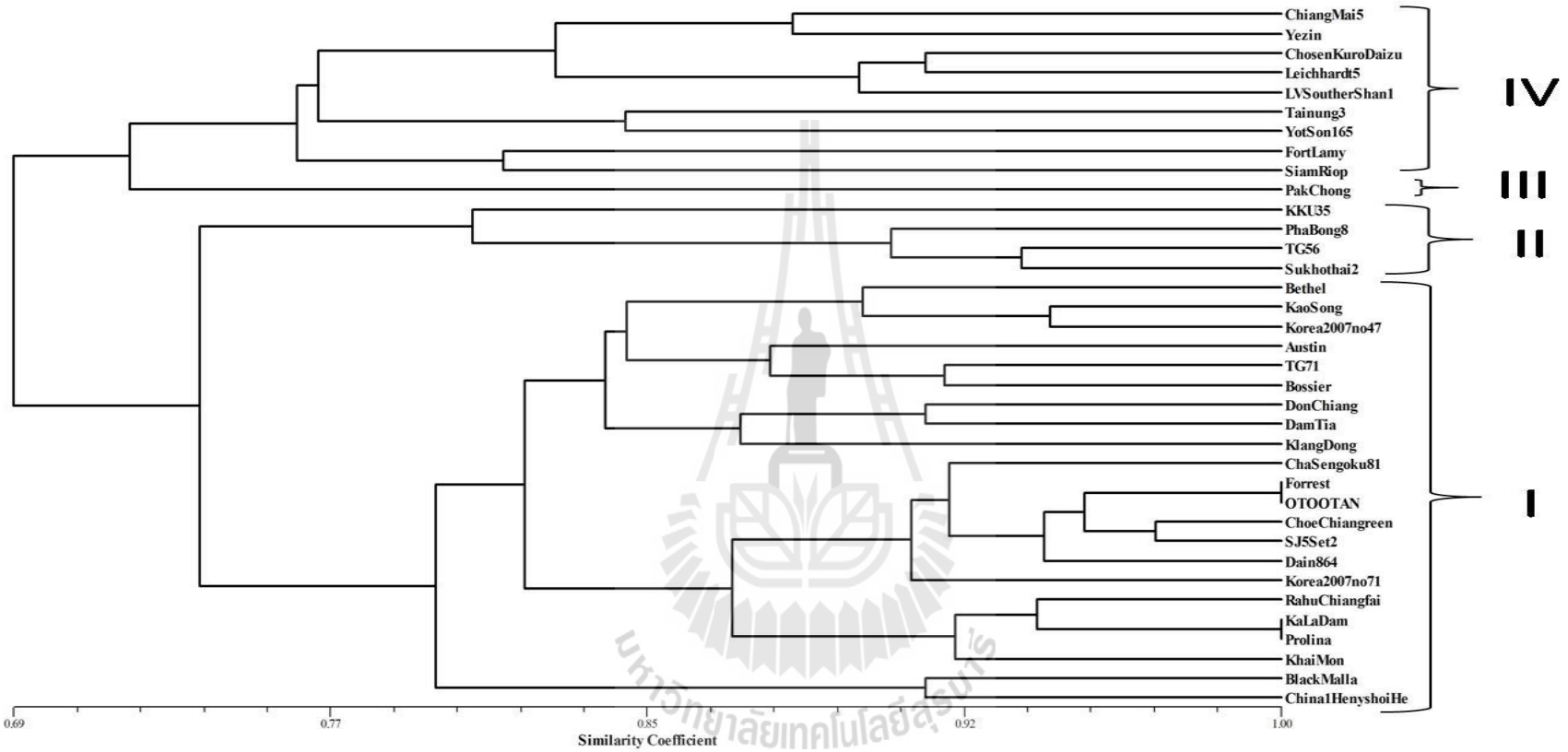

Figure 4.3 Dendrogram obtained from the data of ISSR profile of 36 soybean accessions by using UPGMA method

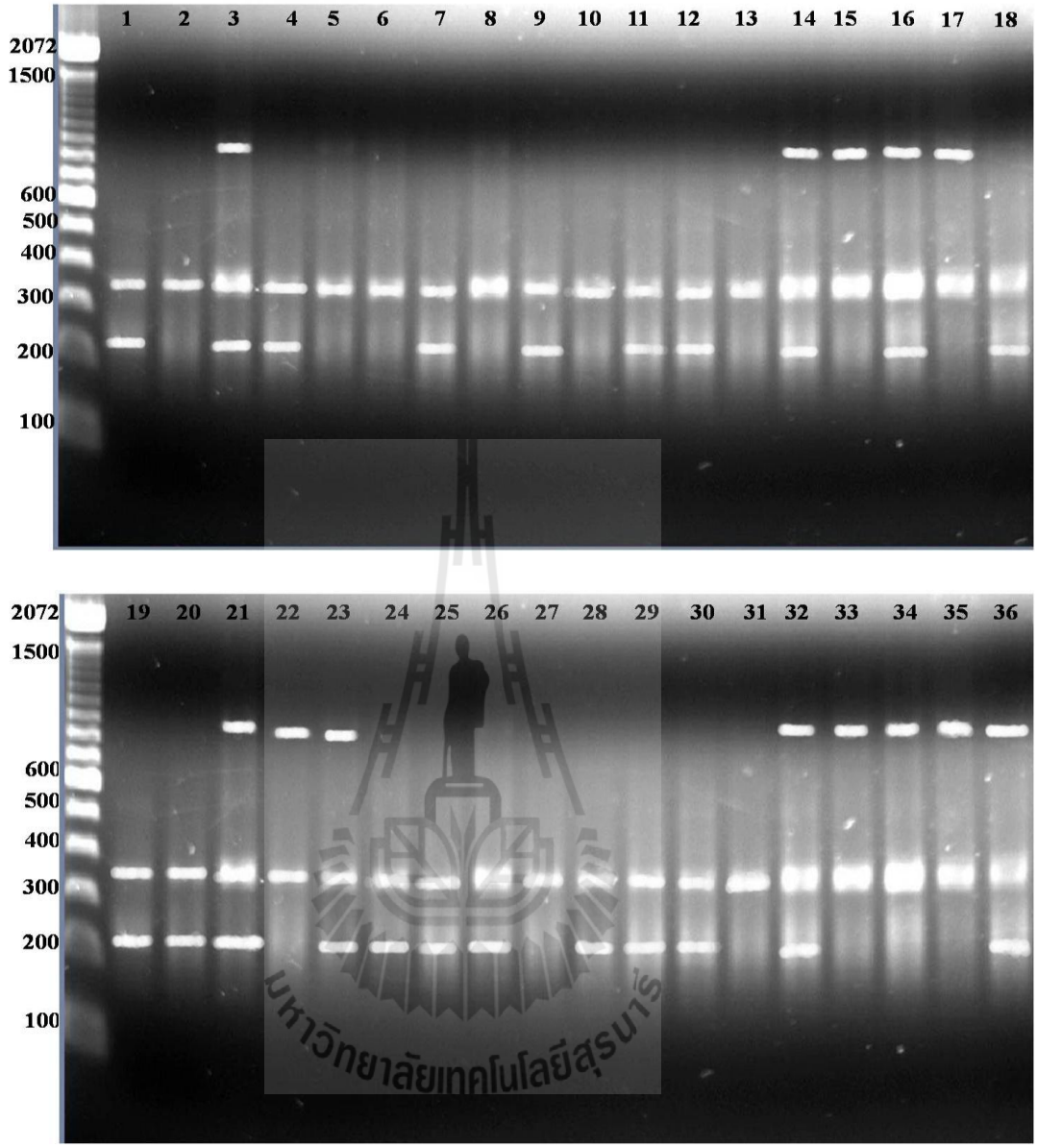

Figure 4.4 ISSR profile of genomic DNA from 36 soybean accessions amplified using primer UBC-834 $(AG)_8YT$ in a 2% agarose gel. Lane M contains 100 bp DNA ladder. Lanes 1 to 36 contain amplified soybean product [**lane 1**: Chiang Mai 5, **lane 2**: KKU 35, **lane 3**: Bethel, **lane 4**: Austin, **lane 5**: Cha Sengoku 81, **lane 6**: Rahu Chiangfai, **lane 7**: TG 71, **lane 8**: Tainung 3, **lane 9**: Bossier, **lane 10**: Pha Bong 8, **lane 11**: TG 56, **lane 12**: Su Khothai 2, **lane 13**: Don Chiang, **lane 14**: Pak Chong, **lane 15**: Klang Dong, **lane 16**: Dam Tia, **lane 17**: Chosen Kuro Daizu, **lane 18**: Ka La Dam, **lane 19**: Yot Son 165, **lane 20**: Leich hardt 5, **lane 21**: Black Malla, **lane 22**: Khai Mon, **lane 23**: Forrest, **lane 24**: OTOOTAN, **lane 25**: Fort Lamy, **lane 26**: Choe Chian (green), **lane 27**: Prolina, **lane 28**: TG 71, **lane 29**: Siam Riop, **lane 30**: LV.Southern Shan 1, **lane 31**: Korea 2007 no.47, **lane 32**: Korea 2007 no.71, **lane 33**: Yezin, **lane 34**: China 1 Henyshoi-Heibei, **lane 35**: Dain 86-4, **lane 36**: SJ 5 (set 2)]

Table 4.17 Distribution of 36 genotypes into different clusters based on DNA polymorphism by using ISSR profile.

No.	Cluster No.	No. of accessions	Name of accessions
1.	I	5	China 1 Henyshoi-Heibei, Black Malla, Khai Mon, Prolina, Kala Dam, Rahu Chiangfai, Korea 2007 no.71, Dain 86-4, SJ 5(set 2), Choe Chian (green), OTOOTAN, Forrest, Cha Sengoky 81, Klang Dong, Dam Tia, Don Chiang, Bossier, TG 71, Austin, Korea 2007 no.47, Kao Song, Bethel
2.	II	4	Sukhothai 2, TG 56, Pha Bong 8, KKU 35
3.	III	1	Pak Chong
4.	IV	9	Siam Riop, Fort Lamy, Yot Son 165, Tainung 3, LV. Southern Shan I, Leichhardt 5, Chosen Kuro

* set 2 = the accession from Khon Kaen University

4.3.2 Morphological identification of the tested 36 soybean accessions

Figure 4.5 illustrated the UPGMA procedure defined the tested 36 soybean accessions into 4 main groups based on 7 morphological traits (flower color, leaf shape, plant type, pod color, pubescence color, seed coat color and helium color) with the range of similarity coefficient from 0.30 to 1.00. The distribution of the accessions included in each group was shown in table 4.18. Cluster means and standard deviations of different characters in each group are illustrated in table 4.19.

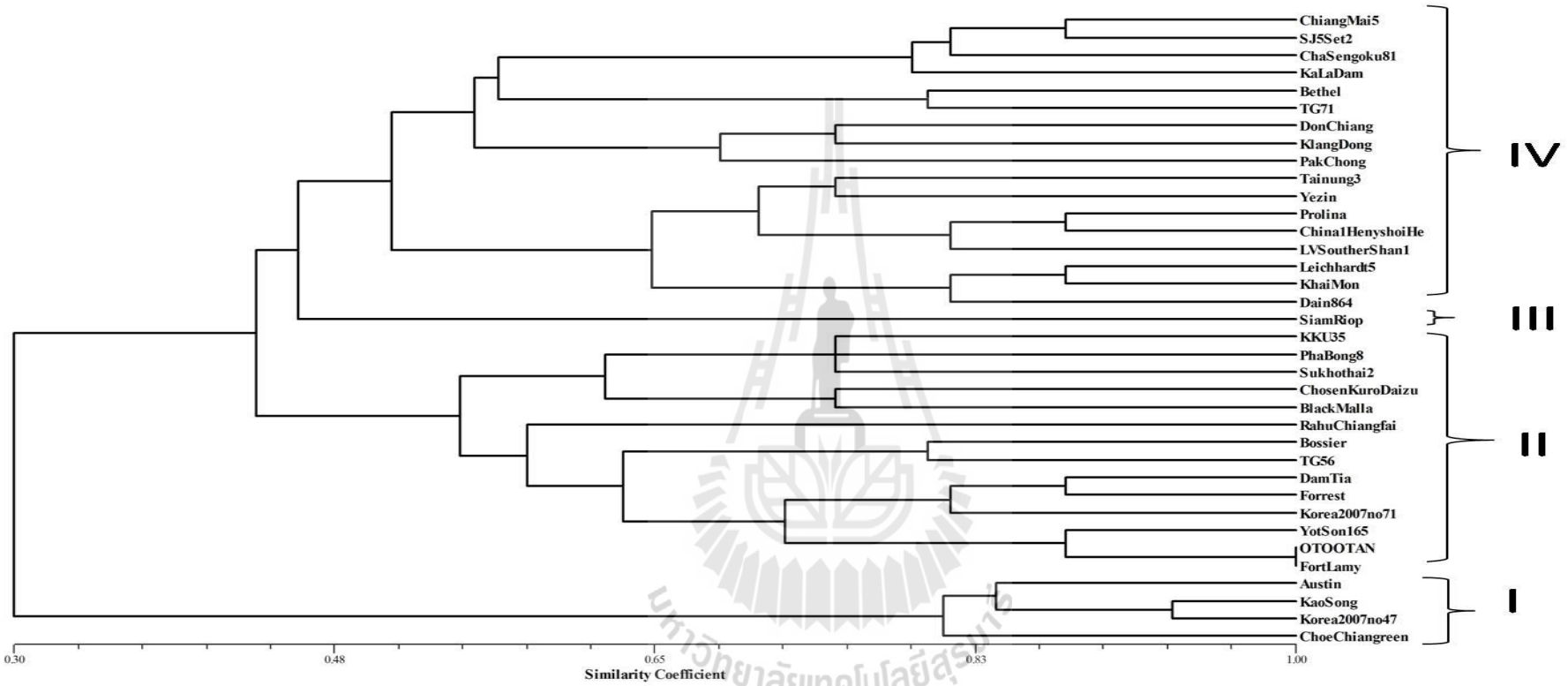

Figure 4.5 Dendrogram based on 7 morphological data of 36 soybean accessions by UPGMA method

Table 4.18 Distribution of 36 genotypes into different clusters based on 7 morphological traits

No.	Cluster No.	No. of accessions	Name of accessions
1.	I	4	Choe Chiangreen, Korea 2007 no.47, Austin
2.	II	14	Fort Lamy, OTOOTAN, Yot Son 165, Korea 2007 no.71, Forrest, Dam Tia, TG 56, Bossier, Rahu Chiangfai, Black Malla, Chosen Kuro Daizu, Sukhothai 2, Pha Bong 8,
3.	III	1	Siam Riop
4.	IV	17	Dain 86-4, Khai Mon, Leichhardt 5, LV. Sothern Shan 1, China 1 Henyshoi-Heibei, Prolina, Yezin, Tainung 3, Pak Chong, Klang Dong, Don Chiang, TG 71, Bethel, Ka La Dam, Cha Sengoku 81, SJ 5 (set 2), Chiang

Table 4.19 Cluster means and standard deviations of 9 morphological traits studied in 94 soybean accessions

No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	Days to 50% flowering	32 ± 4	39 ± 4	25 ± 0	37 ± 7
2	Days to pod formation	37 ± 5	50 ± 9	33 ± 0	49 ± 12
3	Days to maturity	94 ± 4	103 ± 9	102 ± 0	102 ± 10
4	Plant height (cm)	30.2 ± 9.6	61.4 ± 23.1	64.3 ± 0	43.6 ± 16.1
5	No. of filled pods per plant	24 ± 4	86 ± 41	89 ± 0	65 ± 47
6	Seeds per pod	2 ± 1	2 ± 1	2 ± 0	2 ± 0
7	Harvest index	49.8 ± 12.6	68.1 ± 17.6	87.7 ± 0	65.0 ± 19.1
8	100 seed weight (g)	19.8 ± 1.1	13.8 ± 2.6	17 ± 0	16.3 ± 3.8
9	Yield per hill (g)	11.0 ± 4.7	28.8 ± 13.6	46.9 ± 0	24.8 ± 14.3

SD = standard deviation

4.3.3 Comparison of morphological and molecular identification of the tested 36 soybean accessions

When the cluster analysis of the tested 36 soybean accessions were studied according to 7 morphological traits (flower color, leaf shape, plant type, pod color, pubescence color, seed coat color, and helium color) and the DNA polymorphisms by using 7 ISSR primers. The cluster analysis of 36 soybean accessions of molecular analysis and morphological analysis were in figure 4.3 and 4.5. The comparison of the distribution of 36 soybean accessions according to 7 morphological traits and ISSR polymorphism was shown in table 4.20. The accessions Choe Chian (green), Korea 2007 no.47, and Austin were observed in group I of both the morphological and molecular cluster analysis. OTOOTAN, Korea 2007 no.71, Dam Tia, Bossier, Rahu Chiangfai, and Black Malla were in the same group of group II of morphological clusters and group I of molecular clusters. Sukhothai 2, TG 56, Pha Bong 8, and KKU 35 were also observed in the same group II in not only the morphological cluster identification but also the molecular cluster identification. In the group IV of morphological cluster analysis and molecular cluster analysis, Leichhardt 5 and LV. Southern Shan 1 were observed together.

Table 4.20 The comparison of distribution of the tested 36 soybean accessions according to morphological traits and ISSR polymorphisms.

No.	Cluster no.	Name of accessions (Morphological cluster analysis)	Name of accessions (Molecular cluster analysis)
1.	I	Choe Chiangreen, Korea 2007 no.47, Austin	China 1 Henyshoi-Heibei, Black Malla, Khai Mon, Prolina, Kala Dam, Rahu Chiangfai, Korea 2007 no.71, Dain 86-4, SJ 5(set 2), Choe Chian (green), OTOOTAN, Forrest, Cha Sengoky 81, Klang Dong, Dam Tia, Don Chiang, Bossier, TG 71, Austin, Korea 2007 no.47, Kao Song, Bethel
2.	II	Fort Lamy, OTOOTAN, Yot Son 165, Korea 2007 no.71, Forrest, Dam Tia, TG 56, Bossier, Rahu Chiangfai, Black Malla, Chosen Kuro Daizu, Sukhothai 2, Pha Bong 8, KKU 35	Sukhothai 2, TG 56, Pha Bong 8, KKU 35
3.	III	Siam Riop	Pak Chong
4.	IV	Dain 86-4, Khai Mon, Leichhardt 5, LV. Sothern Shan 1, China 1 Henyshoi-Heibei, Prolina, Yezin, Tainung 3, Pak Chong, Klang Dong, Don Chiang, TG 71, Bethel, Ka La Dam, Cha Sengoku 81, SJ 5 (set 2), Chiang Mai 5	Siam Riop, Fort Lamy, Yot Son 165, Tainung 3, LV. Southern Shan I, Leichhardt 5, Chosen Kuro Daizu, Yezin, Chiang Mai 5

* set 2 = the accession from Khon Kaen University

CHAPTER V

CONCLUSIONS

Variability studies indicated that the characters filled pods per hill, yield per hill and harvest index had a high level of diversity among the accessions for these traits. Hence, selection on the basis of these traits can be useful. These characters can be exploited by direct selection for improving these characters or by involving them in hybridization work due to diversity. All tested agro-morphological characters were highly significant among the accessions.

In the correlation analysis, seed yield showed positive and highly significant association with days to 50% flowering, days to pod formation, days to maturity, plant height, number of filled pods per hill, 100-seed weight, and harvest index. It might be emphasized the importance of these characters to be considered for selection programs aimed for yield improvement. Diversity studies at morphological level suggest that there is a large amount of diversity present in the materials. Hence, diverse germplasm lines possessing desirable characters may be used in future breeding programs to get maximum spectrum of variability for wide range of characters and for broadening the genetic base of cultivars. In breeding programs, there is a need to utilize diverse germplasm lines to widen the genetic base in the crop.

The physiological study revealed that negative and highly significant correlations between SLA and yield (-0.54^{**}), while the SLA showed no association with SCMR (-0.39^{ns}). The yield was also not associated with SCMR (0.20^{ns}). The UPGMA method also divided 27 accessions into 4 groups.

A large genetic diversity was detected among the samples based on estimation of DNA products amplified from seven selected ISSR primers, with the similarity coefficient varying from 0.5 to 1.0. The primer UBC 822 showed the highest polymorphic percent (87.50%) while the primer UBC 868 showed the lowest polymorphic percent (33.33%) among the 36 soybean accessions. Molecular diversity also proved that crosses can be made between the accessions KKU 35 and Leich hardt 5, Kaw Song, LV. Southern Shan 1, and Yezin, between Don Chiang and Forrest, OTOOTAN, Korea 2007 no.71, and Dain 86-4, between Fort Lamy and Forrest, OTOOTAN, and Dain 86-4 which are the most diverse for identifying superior recombinants regarding similarity coefficient level, 0.5.

Diversity studies at both morphological and molecular levels suggest that there is high amount of diversity present in the materials. Hence, diverse germplasm lines possessing desirable characters may be used in future breeding programs to get maximum spectrum of variability for wide range of characters and for broadening the genetic base of cultivars. Results obtained from this study can make better choice for soybean breeders for selection of genotypes among large number of accessions because there is a need to utilize diverse germplasm lines in breeding programs to widen the genetic base in the crop. The obtained correlation information of the tested traits can also provide for the soybean breeders in the indirect selection programs of the future breeding program.

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APPENDICES

APPENDICES

ATTACHED FIGURES

Attached figure 1 : General view of the experimental size

Attached figure 2 : Determinate plant type

Attached figure 3 : Semi-determinate plant type

Attached figure 4 : Indeterminate plant type

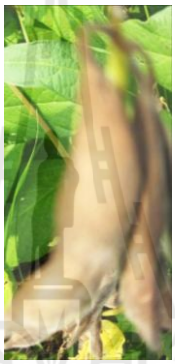
Attached figure 5 : White coloured flower

Attached figure 6 : Light purple coloured flower

Attached figure 7 : Purple coloured flower

Attached figure 8 : Dark purple coloured flower

Attached figure 9 :
Tin coloured pod



Attached figure 10 :
Brown coloured pod

Attached figure 11 :
Black coloured pod

Attached figure 12 :
Yellowish white coloured seed

Attached figure 13 :
Yellowish coloured seed

Attached figure 14 :
Green coloured seed

Attached figure 15 :
Buff coloured seed

Attached figure 16 :
Reddish brown coloured seed

Attached figure 17 :
Black coloured seed

Attached table 1 Mean squares obtained from the analysis of variance for the tested 10 characters in 94 soybean accessions

Source of Variation	Degree of Freedom	Days to emer - gence	Days to 50% flowering	Days to pod formation	Days to maturity	Plant height	No. of filled pods/hill	No. of seeds/ pod	100 seed weigh t	Yield/hill	Harvest index
Replication	2	25.8	11.8	6.9	2.6	11.0	2722.1	1.1	2.2	421.2	4107.3
Genotypes	93	4.3**	109.7**	276.8**	249.3**	1200.9**	4285.9**	0.4**	71.3**	555.4**	1073.7**
Error	186	2.4	4.6	8.5	2.7	7.9	578.6	0.2	1.0	110.9	370.4
Total	281	3.2	39.4	97.2	84.3	402.8	1820.9	0.3	24.3	260.3	629.8
CV%		24.9	5.8	6.1	1.6	6.0	39.4	18.9	5.7	45.6	32.2

** = highly significant

Attached table 2 Mean squares obtained from the analysis of variance for SCMR, SLA and yield in 26 soybean accessions

Source of Variation	Degree of freedom	SCMR	SLA	yield
Replication	1	1.41	45.27	0.520
Genotypes	25	20.58**	2368.93*	505.72**
Error	25	3.94	1154.61	60.64
Total	51	12.05	1728.12	277.64
CV%		4.69	17.11	20.22

* = significant, ** = highly significant

BIOGRAPHY

Ms. Sandar Moe was born on March 12, 1980 in Wundwin, Myanmar. She attended Yezin Agricultural University, Myanmar and received her Bachelor Degree of Agricultural Science in March, 2004. She has joined as a research technician at Foodlegumes Section, Department of Agricultural Research, Yezin, Nay Pyi Taw on July 27, 2004.

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