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THESIS

MAP CONSTRUCTION AND IDENTIFICATION OF MAJOR AGRONOMIC TRAITS IN MUNGBEAN (*Vigna radiata* (L.) Wilczek)

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A Thesis Submitted in Partial Fulfillment of
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Mungbean (*Vigna radiata*) is an important annual legume in Asia. It is widely grown in South and Southeast Asia. The aim of this research was to use SSR markers to identify chromosome regions controlling agronomic traits. The first mungbean genetic linkage map was successfully constructed from 186 F_2 plants, derived from a cross between an annual cultivated mungbean line 'KUML29-1-3' (*Vigna radiata* var. *radiata*) and an Australian wild perennial mungbean accession 'W021' (*Vigna radiata* var. *sublobata*). A total of 150 SSR primers were composted into 11 linkage groups, each containing at least 5 markers. The map spans 1,019.1 cM with the average distance between markers of 7.4 cM. QTLs controlling major agronomic characters, viz. days to first flowering (D_1), days to first pod maturity (D_2), days to harvesting (D_3), 100-seed weight (100sw), number of seeds per pod (Sp), number of pods per plant (Pp), pod length (Pl), pod width (Pw) and seed yield per plant (Yp) were mapped onto this map. Fifty three QTLs associated with these traits were identified in which each QTL explained 0.8% to 29.6% of the phenotypic variation of the traits. The amount of phenotypic variation explained by QTLs of each trait ranged from 8.0% to 60.3%.

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LIST OF ABBREVIATIONS

µl	=	microliter(s)
AFLP	=	amplified fragment length polymorphisms
avg	=	average
bp	=	base pairs
CTAB	=	cetyltrimetyl ammonium bromide
dNTPs	=	deoxynucleotide triphosphate
ISSR	=	inter simple sequence repeats
MCMC	=	Monte Carlo Markov Chain
ng	=	nanogram(s) (10^{-9}g)
PCR	=	polymerase chain reaction
RAPD	=	random amplified polymorphic DNA
RFLP	=	restriction fragment length polymorphisms
SNP	=	single nucleotide polymorphisms
SSR	=	simple sequence repeats
STS	=	sequence tagged site

MAP CONSTRUCTION AND IDENTIFICATION OF MAJOR AGRONOMIC TRAITS IN MUNGBEAN (*Vigna radiata* (L.) Wilczek)

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the most important annual legumes. It is native to India and widely grown in Southeast Asia, Africa and South America. Mungbean seed is consumed as a protein source for human and animals. Mungbean plants can also be made into hay and green manure. It is often cultivated in cropping systems. The production of mungbean grain in the world is around 3.5 to 4 million tons per year (Weinberger, 2003). Products from mungbean seed are rich in vitamins, minerals and easily digested protein. However, the average yield of mungbean is still low due to susceptibility to pests and diseases such as cowpea weevil, powdery mildew and *Cercospora* leaf spot diseases, its indeterminate growth habit and photoperiod sensitivity (Fernandez and Shanmugasundaram, 1988).

All mungbean cultivars are annual crop with 2 broad growth stages, vegetative (V) and reproductive (R). V stage is determined by counting the number of developed nodes on the main stem, beginning with the unifoliolate nodes as the first nodes (stage V_1) and the final node is the node which has fully developed leaf (stage V_n) when the leaf at the node above is unrolled sufficiently. R stages are determined form R_1 (beginning bloom), R_2 (beginning pod), R_3 (beginning seed), R_4 (full seed), R_5 (beginning maturity), R_6 (first harvest), and R_7 (second harvest) (Promkham *et al*., 1988 and Pookpakdi *et al*., 1992). Difference in number of dates specifying to each growth stage may affect yield. This opens another dimension of improving mungbean crop for higher yields.

To perform the breeding process effectively, inheritance of dates specifying different growth stages should be investigated in order to manipulate developmental stages of mungbean through selection. In addition, molecular markers associated with

the traits should be determined in order to save time through the use of marker-assisted selection.

OBJECTIVES

1. To investigate the inheritance of yield components and dates specifying growth stages in mungbean.

2. To locate molecular markers linking to yield components and dates specifying growth stages in mungbean.

LITERATURE REVIEW

1. Crop description

Mungbean belongs to family Leguminosae. It is commonly called green gram or golden gram depending on seed coat colors. Both wild and cultivated mungbeans are naturally annual plants. Growth habits are either erect, sub-erect, or viny types. Mungbean germination is of epigeal type which the cotyledons are lifted up above the soil with the true leaves. Mungbean has trifoliate leaves as in soybean with the leaf size and shape depending on genotypes. Mungbean leaves are arranged alternately on the stem with the plant height ranging between 50 and 75 cm. Mungbean is a self-pollinating crop because the pollen pollinates the stigma of the same flower before opening of the flower. Flowering of cultivated varieties takes place between 30 and 50 days after planting. The growth duration from planting to maturity varies from 60 to 120 days depending on variety. Young pods are all green, while mature pods can have different colors ranging from yellow-brown to black. There are 8 to 15 seeds per pod with the pod length of 7 to 15 cm. There are 10 to 25 pods per plant. The pods are set at leaf axils with varying in shape and size. Mature seed colors are green, yellow, brown or black depending on variety. Seed coat surface is either shiny or dull with flat and long hilum (Duke, 1983).

Mungbean seed is used for several purposes like other food legumes. It is a good source of protein, vitamins and minerals. Whole seed has high protein (20-25%). In India, dried beans are boiled, eaten in whole seed or consumed as dahl (seed coat removed) or mixed in soup or sweet (Thomas Jefferson Agricultural Institute, 2007). The green pods are used as a vegetable. Mungbean sprout is an essential ingredient in noodle and salad. Mungbean starch can be made into noodle or flour which is used in various Indian and Chinese dishes (Akpapunam, 1996). The whole plants contain high calcium, phosphorus and vitamin A, and thus can be used as hay, green manure, cover crop and forage. Cracked seed and cake from starch industry are used as animal feeds.

Mungbean can be grown in a warm climate at the latitude between 0^o to 30^o north or south. The optimum growing temperature is ranging between 20^oC and 45^oC. High humidity and rainfall during growing season can cause diseases and yield loss. Mungbean can be grown from the elevation at sea level to approximately 2000 m, preferably in sandy-loam soils but not in wet or poorly drained soils (Akpapunam, 1996). It is drought tolerant but susceptible to frost, water-logging and salinity. The optimum soil pH for mungbean growing is between 6.2 and 7.2. Mungbean requires high levels of phosphorus, potassium, calcium, magnesium and sulfur in the soil, but does not require much nitrogen fertilizer as it can fix N_2 from the air by rhizobium bacteria living in the nodules developed from root hairs and can directly supply N to the plant and eventually to the soil upon harvesting.

Mungbean seed is a source of protein, essential minerals, and vitamins as can be summarized in Table 1. Mungbean amino acids (as presented in mg per 100 g of seed) include aspartic acid (716), cystine (44), threonine (209), serine (296), glutamic acid (865), proline (229), glycine (210), alanine (242), valine (259), methionine (33), isoleucine (233), leucine (441), tyrosine (156), phenylalanine (306), lysine (504), histidine (182) and arginine (345) (Duke, 1983 ; Salunkhe *et al*., 1985).

Mungbean is usually harvested when at least two-thirds of the pods are mature. Harvesting should be performed with care to reduce damage to the produce. The seed should be dried down to 13–15% moisture content before storage. All mungbean cultivars possess indeterminate growth habit, which causes uneven maturity. Field harvesting can be done either by hand or machine depending on area, planting method and labor availability.

2. Growth stages of mungbean

Growth and development of annual legumes are commonly included vegetative and reproductive stages. The vegetative stage begins with V_E stage which shows the emergence of the cotyledon from the soil surface. The hook-shaped hypocotyls spread out and stop growing. The vegetative and elongation processes are

included in the stages V_C through $V_{(n)}$, where (n) represents the last stage before R_1 (beginning bloom). V_C stage is defined when the cotyledons are fully expanded above the soil surface and the unifoliolate leaves above unrolled. V_1 (first node) stage is classified when the unifoliolate leaves are fully expanded and the 1st trifoliolate leaf above the node starts unrolling. The unifoliolate leaves are located on the opposite sides of the stem. The trifoliolate leaves (true leaves) are produced singularly on each node. V_2 stage is defined when the leaflets or the 1st trifoliolate leaf is fully expanded and the 2nd trifoliolate leaf above the node starts unrolling. At this stage, the mungbean plant is 15-20 cm tall. Symbiotic nitrogen fixation usually begins at this stage and continues until R_5 and R_6 stages. The unifoliolate leaf node is the first node or reference point to count the number of leaf nodes. V_3 stage is classified when four nodes have leaves with completely unfolded leaflets, and the 2nd trifoliolate leaf is fully expanded. The plant is 18-23 cm tall. The axillary bud of the 1st trifoliolate leaf node is initiated and may develop into a branch or a flower cluster, or may remain dormant. V_4 stage is defined when the 3rd trifoliolate leaves on the 4th node are fully expanded, and the 4th trifoliolate leaf above the node starts to unroll. Thus, in general, $V_{(n)}$ stage is assigned to the vegetative stage where the nth node on the main stem has completely unfolded leaflets. At the reproductive stage, R_1 is the beginning bloom stage when the first flower blooms at any node on the main stem. R_2 is the beginning pod stage, one pod of 1.0 cm in length appear between 4th node to 6th node of the main stem. R_3 is the beginning seed stage, one pod of 5.0 cm in length appears on the top three nodes on the main stem. R_4 is the full pod stage, one pod on the top three nodes constricted between seeds. R_5 is the beginning maturity stage, the color of one pod on the main stem turns to brown, dark brown or black. R_6 is the first harvest stage, when fifty percent of pods are mature. R_7 is the second harvest stage, when the remaining pods from R_6 stage are mature. (Tesar, 1984; Promkham *et al*., 1988; Pookpakdi *et al*., 1992 and Kaiser, 1995).

Table 1 Nutritional contents of mungbean seed in 100 g of edible portion

Nutritional contents	Amount per 100 g seed
Protein	22-25 g
Fat	1-1.5 g
Ash	3-4 g
Crude fiber	4-6 g
Carbohydrate	55-70 g
Starch	45-55 g
Vitamin A	0.5-0.6 mg
Vitamin B1	0 .52- 0.66 mg
Vitamin B2	0.29 - 0.30 mg
Niacin	2.4 - 3.1 mg
Vitamin C	0 – 10 mg
Potassium	850 – 1450 mg
Sodium	30 – 170 mg
Magnesium	65 – 125 mg
Phosphorus	280 – 580 mg
Iron	5.43-7.1 mg
Calcium	80- 330 mg

Source: Duke (1983) and Salunkhe *et al.* (1985)

3. Correlation between yield components and date specifying growth stage in mungbean

Khattak *et al.* (2002) assessed a half diallele cross among six mungbean genotypes (NM92, 6601, NM89, VC 1560D and VC 3902A). They found that NM92 x NM89 was the best specific combiner among all crosses. Both additive and dominant gene effects controlled the inheritance of plant height at first pod and to 90% pod maturity, degree of indetermination of plant height (DDh) from first flower to first pod maturity (DDh_1), DDh from first flower to 90% pod maturity (DDh_2) and DDh from first pod maturity to 90% pod maturity (DDh_3).

Khattak *et al.* (2004) studied on gene action for synchrony in pod maturity and indeterminate growth habit in mungbean. Six populations (both parents, F_1, BC_1, BC_2, and F_2) in two crosses, 6601 X NM92 and ML-5 X NM54 were used to detect all types of gene effects. The results indicated that both additive (d) and dominant (h) gene effects controlled days to first flower, days to first pod maturity, days to 90% pod maturity, plant height at first flower, plant height at first pod maturity, plant height at 90% pod maturity, degree of indetermination of pod maturity from first flower to 90% pod maturity and degree of indetermination of plant height from first flower to 90% pod maturity, except days to first flower and first pod maturity in ML-5 X NM54.

Gayen and De (2005) studied on genetic variability of growth characters of pod and seed in 11 cultivars/varieties of mungbean at different developmental stages. Variations due to developmental stages viz. moisture content of husk, seeds and pods and genotypes were significant. However, genotypic difference contributed highly to pod length. The broad-sense heritability was very high for all the characters except for the moisture content of seeds. This indicates stability in genetic variability over different developmental stages.

4. Estimation of heritability

Heritalility (h^2) is the most useful statistics that can be derived from the phenotypic variance components. Generally, there are two of types heritability. The first is based on the ratio of genetic variation to the total phenotypic variation and is called broad-sense heritability, h^2_b. The second is more important because it measures the breeding values of a population which is due to the additive effects of genes in the population and is called narrow-sense heritability, h^2_n (Kearsey and Pooni, 1996).

The broad-sense heritability of F_2 generation can be estimated following this formula

$$h^2_b = V_G/(V_G+V_E)$$
$$= (F_2 \text{ variance} - V_E)/F_2 \text{ variance}$$

Where V_G = genotypic variance

V_E = environmental variance

Similarly, the narrow-sense heritability, h^2_n can be estimated as

$$h^2_n = V_A/(V_A+V_D+V_E)$$

Where V_A = additive variance

V_D = dominant variance

V_E = $V_A + V_D$

V_E = environmental variance

5. Molecular markers and their uses

There are three types of markers used so far by plant breeders (1) morphological markers (phenotypic traits) employing the visible characters such as plant height and canopy, colors of flower and seed. (2) Biochemical markers, which are called isozymes. Isozymes are enzymes that differ in amino acid sequence but catalyze the same biochemical reaction. These enzymes usually displayed the difference in regulatory properties. Isozymes are coded by homologous genes that represent enzymes from different alleles of the same gene. Isozyme markers may be

influenced by environmental effects or different in the growth stages of the plant. (3) DNA markers or molecular marker are genes or DNA sequences on the chromosomes. It can be described as variation due to mutation or alteration in the genomic loci. DNA markers may be a short or long DNA sequences such as a single nucleotide polymorphism, microsatellites or minisatellites (Taji *et al.*, 2002). In plants, molecular markers have been used for paternity analysis, varietal identification, phylogenetic analysis and marker assisted-selection (MAS). High quality molecular markers can be used as a potential tool in plant breeding (Gonzalez, 1999 and Dudley, 2002). Molecular markers can increase accuracy in selection of an interesting trait, usually at any stages of plant growth. According to Knapp (1998), MAS can be more efficient than phenotypic selection in selecting a segregation population, especially when the markers are tightly linked to the genes controlling the trait. There are many types of molecular markers such as RFLP (restriction fragment length polymorphisms), RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphisms), microsatellite or SSR (simple sequence repeat), and SNP (single nucleotide polymorphism).

To determine RFLP markers, DNA samples are cut by restriction enzymes and visualised on photographic film using radioactively labeled reagents. The RFLP has an advantage that it can give 5-6 loci of co-dominance markers in each reaction. However, the reaction requires large amount (2-10 ug) of high quality DNA and thus results in high cost. RFLP analysis is slow and requires 3-4 weeks of laboratory work, while the level of polymorphism is medium.

RAPD are DNA fragments amplified by a polymerase chain reaction (PCR) using short (10 bp) synthetic primers of random sequence. The reannealing temperature in the PCR must be low (35-40 ^{o}C) for the primer to bind. Both forward and reverse primers are able to amplify the fragments which are separated by gel-electrophoresis and polymorphism is detected as the presence or absence of bands of particular size. The polymorphism occurs from variation in the primer annealing sites. The RAPD technique does not required sequence data for primer construction, easy and quick to test with low cost. However, RAPD requires purified and high molecular

weight DNA to begin with. The experimental procedures are rather sensitive and thus often result in low degree of repeatability. Band profiles cannot be interpreted in terms of loci and alleles. A considerable amount of DNA (10-50 ng) is required. The alleles are complete dominant with low reproducibility and medium level of polymorphism (Twyman, 1998).

Nowadays microsatellite or SSR (simple sequence repeat) is probably the most popular molecular marker. The markers are formed from a small cluster of short tandem repeated nucleotide units ranging from 1-4 bp up to 200 bp. The most common repeats are $(A)_n$, $(TAT)_n$ and $(GATA)_n$. SSR are co-dominant markers with medium cost of development. These markers are highly polymorphic and distributed throughout the genome (Twyman, 1998). It does not requires high quality DNA (50-100 ng). It is believed that different sequence repeats occurred from the length of repeated nucleotides among cultivars. Mutation rates affected microsatellite variability. They can be explained by slipped strand mispairing (slippage) during DNA replication on a single DNA strand.

Amplified Fragment Length Polymorphism (AFLP) is a polymerase chain reaction (PCR) based marker. It was developed in the early 1990 and widely used in genetic fingerprinting technique and identification of genetic variation. The procedure of this technique comprises three steps. Firstly, total DNA is digested with one or more restriction enzymes to cut the genomic DNA. Then all restriction fragments are ligated with specific adaptors which complemented double stranded adaptors to the ends of the restriction fragments. A subset of the restriction fragments is amplified using 2 primers that are complementary to the adaptor and restriction site fragments. The selectively amplified fragments are separated by gel electrophoresis and visualized autoradiographically (Vos *et al*., 1995). The advantages of AFLP are medium cost of development, giving high polymorphism as both dominant and co-dominant markers. The disadvantages are that the technique requires 1-2 ug of purified DNA.

Ouédraogo *et al*. (2001) identified AFLP markers linked to resistance of cowpea (*Vigna unguiculata* (L.) Walp.) to parasitism by witch weed (*Striga gesnerioides)*. The F_2 progeny derived from the cross between Tvx3236 (a susceptible line) and IT82D-849 (a resistant line) resisted to parasitism of race 1. The trait was controlled by a single dominant gene, *Rsg2–1*. Three AFLP markers were identified to tightly link with *Rsg2–1*. They were E-AAC/M-CAA300 (2.6 cM away), E-ACT/M-CAA524 (0.9 cM) and E-ACA/M-CAT140/150 (0.9 cM) which all showed codominant. Genetic analysis of F_2 population derived from the cross between IT84S-2246–4 (a susceptible line) and Tvu 14676 (a resistant line) resulted in identification of another dominant gene, *Rsg4-3* controlling resistance to *S. gesnerioides* race 3. Six AFLP markers linked to *Rsg4–3* were identified as E-ACA/M-CAG120 (10.1 cM away), E-AGC/M-CAT80 (4.1 cM), E-ACA/M-CAT150 (2.7 cM), E-AGC/MCAT150 (3.6 cM), E-AAC/M-CAA300 (3.6 cM), and EAGC/M-CAT70 (5.1 cM).

Millan *et al*. (2003) evaluated a chickpea population of $F_{6:7}$ lines against a blight disease caused by *Ascochyta rabiei*. The disease severity was measured by area under the disease progress curve (AUDPC) in the field, and as the linear infection index (LII) in the greenhouse. At least two genes were found controlling reaction to the disease with the resistance alleles were reported in the parent ILC3279. Analysis of the whole polymorphic marker data revealed 39 markers associated with blight resistance, viz. 34 RAPD, 2 ISSR and 3 STMS. Among them, 20 markers indicated clear association with resistance or susceptibility. Then, 15 markers were formed into a linkage group covering a map distance of 18.8 cM with a high concentration of loci in a 7.4 cM region at one end of the linkage group.

Kelly *et al*. (2003) studied on gene mapping, QTL, and molecular marker-assisted selection for traits of economic importance in common bean (*Phaseolus vulgaris* L.) and cowpea (*V. unguiculata*). The bean map expanded approximately 1200 cM with 500 markers, while the cowpea map was 2670 cM with over 400 markers. Map of major disease resistance genes in bean are on linkage groups B1, B4,

B7, and B11 carrying resistance to bean rust, anthracnose, common bacterial blight and white mold.

Stackelberg *et al*. (2003) identified AFLP and STS markers closely linked to the *def* locus in pea. F_1 plants derived from a cross between 'DGV' (*def* wild-type) and 'PF' (*def* mutant) showed the wild-type phenotype. In the F_2, the *def* phenotype segregated 355:121 (wild type:mutant) following a ratio of 3:1. F_3 analysis of 355 wild type F_2 plants showed 115 homozygous and 240 heterozygous *def* genotypes, thus gave the genotypic segregation of 115:240:121 in the F_2. The mutant phenotype was restricted to the seed funiculus and did not influence other visible phenotypic characteristics. Linkage of 38 AFLPs to the *def* locus was analyzed from 60 F_2 plants. Among them,15 loci showed the distances of less than 5 cM, five were less than 2 cM and two were less than 1 cM from the *def* gene.

Pooprompan *et al.* (2006) identified SSR markers associating with days to flowering (DTF) in RILs from the cross between vegetable soybean cultivar "AGS292" and the grain soybean line "K3". The narrow-sense heritability values for DTF in the late rainy and dry seasons were 94.2 and 91.6 %, respectively. The 63 polymorphic SSR markers revealed that two major and nineteen minor QTLs were involved in controlling DTF. The QTL near SSR markers Satt132 and Satt431 in molecular linkage group J had the greatest effect on DTF.

Somta *et al.* (2006) studied on interspecific Vigna linkage map between *V. umbellata* (Thunb.) Ohwi & Ohashi and *V. nakashimae* (Ohwi) Ohwi & Ohashi and used in analysis of bruchid resistance. The parents *V. umbellata* was completely resistant to *C. chinensis* and *C. maculatus*, whereas *V. nakashimae* was completely susceptible to both bruchids. F_1 seeds showed resistance to both species. Out of 131 F_2 seeds planted, 57 (43.5%) showed abnormalities and were excluded from further study. Among them, 20 died at the vegetative stage, six failed to flower, eight dropped all their pods and 23 produced abnormal seeds. Finally, 74 F_2 individuals were used to develop a genetic linkage map of 11 linkage groups from a total of 175 DNA marker loci (74 RFLPs and 101 SSRs), spanning a total length of 652 cM.

Soehendi *et al*. (2006) studied on inheritance and AFLP tagging of leaflet mutant in mungbean. The cross between large heptafoliate leaflet with small pentafoliate leaflet mutants gave all F_1 with normal trifoliate leaflets. The F_2 segregated in a 9:3:3:1 ratio of large-trifoliate: large-heptafoliate: small-pentafoliate: small-heptafoliate plants, showing that the genes controlling leaflet size and number were 2 independent loci. Three AFLP markers were found linked to number of leaflets per leaf and the N_1 allele of the small-pentafoliate parent.

6. Mapping population

In molecular mapping, four types of mapping populations are commonly used. They are F_2, backcross (BC), double haploid (DH) and recombinant inbred line (RIL) populations.

An F_2 population is developed by selfing (or intermating for cross pollinated species) of F_1 hybrid plants. The F_1 individuals are developed by crossing between two parents that show significant polymorphism for different types of loci. The F_2 population segregates with the expected 1:2:1 ratio for co-dominant markers and 3:1 for dominant markers. This mapping population is the best population for preliminary mapping and requires less time to generate.

A backcross (BC) population is formed from crossing between F_1 hybrids with either of the parents. The population can be developed from both self and cross-pollinated crops. A advantage of BC population is that it is powerful in categorizing a dominant character with a segregation ratio of 1:1. (Lynch and Walsh, 1998; Acquaah, 2007).

A double haploid (DH) population can be developed by doubling the chromosome number in pollen mother cells (PMC) of F_1 plants, followed by regeneration of the diploid PMC. Each DH plant produces a complete homozygosity of each locus. The success in producing double haploid plants depends on the efficiency of regeneration *in vitro* through colchicine treatment. The advantages of

DH populations are the speed to attain complete homozygosity in short time. The DH plants can be tested in replicated trials in different locations and years in the same manner as a population of inbreds or pure lines (Acquaah, 2007).

A recombinant inbred line (RIL) or a single seed descent population can be constructed by taking an F_1 line through multiple generations of selfing. RILs is derived from a self-pollinated of individual F_2 plants through at least F_6 generation. The major advantages of RIL are nearly the same as DH that it can produce a population of homozygous plants for yield testing in many locations. The disadvantage of RIL is that it takes time to develope the population (Lynch and Walsh, 1998; Acquaah, 2007).

7. Linkage map construction

Linkage analysis was one of the primary approaches used for mapping genes to specific chromosomal regions. Linkage analysis is based on the principle that two genes are linked on the same chromosome. A linkage map represents a group of marker positions in the chromosome. The map can be used for identifying gene or QTLs controlling the traits of interest. It can allow the calculation of the distance between two or more alleles, based on the probability of crossing over events occurring between them during meiosis (Walter and Rapley, 1997; Lynch and Walsh, 1998). Linkage map construction involves the ordering of loci and the distance between them. The regions between markers were estimated from the recombination frequencies. Interference of double cross-over between loci affects recombination frequencies when new loci are added to the map. The mapping assigns specific positions within genomes or individual chromosomes. The construction of mapping indicates the distance between genes or genetic markers (Walter and Rapley, 1997).

Mapping functions were presented by Haldane (1919) and Kosambi (1944) to convert recombination frequencies into genetic map distance. Genetic distance between markers is measured in centiMorgans (cM). Haldane mapping function assumes that crossing over occurs randomly without an interference, while Kosambi

mapping function also accounts for the interference between loci (Lynch and Walsh, 1998; Phillips and Vasil, 2001). Genetic mapping software used for constructing a map is based on the log of odds ratio or LOD score. Linkage is statistically supported if the LOD score is equal to or greater than 3.0. This theoretically indicates that there is a 1000 times higher probability to have linkage than non-linkage events. LOD value or LOD score are threshold value estimating all pairs of possible linkages to produce a grouping of the markers. If two markers are significantly linked at a high LOD value, they are considered to located on the same linkage group. (Walter and Rapley, 1997; Phillips and Vasil, 2001)

8. QTL analysis

Characters that are controlled by multiple genes and segregate in continuous phenotypic variation are referred to as quantitative traits. The locations of these genes on chromosome are called polygenes or quantitative trait loci (QTL) (Brown and Caligari, 2008). QTL analysis predicates associations between quantitative trait inheritance and genetic markers (Kearsey and Farquhar, 1998; Liu, 1998) in three steps: (1) genes considered as quantitative traits can be mapped on the genomic linkage like simple genetic markers. (2) if the markers span a large portion of the genome, then there is a good chance that some of the genes controlling the quantitative traits are related to some of the genetic markers. (3) if the genes and the markers alleles are segregating in the population, then the linkage relationships among them may be considered by looking at the association between trait variation and marker segregation pattern. Three methods have been widely used in identifying QTLs. They are single marker, simple interval mapping and composite mapping analysis.

Single marker analysis is the statistical method based on comparing the phenotypic means for each of the genotypic classes at each marker by means of ANOVA, likelihood ratio test, or simple regression (Kearsey and Hyne, 1994). A significant difference between markers and genotypic classes which consider as the

marker is related with the QTL. The main disadvantage of single marker analysis is the confounding estimates of QTL effect and locations.

Simple interval mapping (SIM) is the mathematic method using a different statistical approach such as likelihood, regression and combination between likelihood and regression (Lander and Bostein, 1989; Kearsey and Hyne, 1994). The method estimates the likelihood of the effect on single QTL flanked by a pair of markers along a chromosome. Simple interval mapping involves two main steps, i.e. detecting the presence of a QTL and then locating it (Lander and Botstein, 1989). The adjacent of QTL is located by maximum likelihood residual variance on the interval of markers. The main advantage of this method is that it can overcome problems of single marker analysis and their effect can be more precisely determined.

Composite interval mapping is a method used in improving the effectiveness of the simple interval mapping by considering bias estimation of the location and effect of the QTL when other QTLs exist on the same region in the chromosome. The method combines interval mapping with multiple regression by including the other markers in the statistical models so that they are unaffected by QTLs located outside flanking markers (Zeng, 1994). The major advantage of this method is that the defined QTL interval is independent of external QTL effect (Zeng, 1993).

9. Comparative mapping

Comparative mapping was developed from Dunn in 1920 (Lyon, 1990). His idea was to compare the genetic linkage maps of the co-linear genome, i.e. comparing the arrangements of homologous genes that are conserved among the co-linear species. Comparative mapping revealed gene locations, gene order, location of types of sequence repeats including function of genes in the genome. It can identify families of genes that play in the organism comparative genomics can predicted the relative locations of related genes in separate genomes that may be functional or regulatory of the gene in the genome. Comparative mapping studies can confirm the phylogenetic

relationship and patterns of chromosome evolution such as inversion, deletion, duplication and translocation between related genomes.

A new application in legume genomics is to integrate studies in biological and agronomic in the crop species. It is important to know genomic features in plant families based on QTL construction on linkage map. The comparative maps can be used to study through its rearrangement and repeated sequences. Comparative map was prepared for studies in map based cloning that is easy for QTL study in other plants (Phillips and Vasil, 2001; De Vienne, 2003).

Gupta *et al.* (2008) compared SSR linkage maps between black gram and azuki bean. There was a high level of co-linearity between three maps. The order of SSR markers was highly conserved, with some were revealed in order among the three maps.

Isemura *et al.* (2010) reported the comparative genome analysis of rice bean with the other members of subgenus *Ceratotropis*. The result revealed highly related genome between rice bean and azuki bean from QTL comparison between species. Moreover, they found that major QTLs were located on LG4 in rice bean while they were on LG9 in azuki bean.

MATERIALS AND METHODS

1. Mapping population

A population used in this study was developed from an inter-subspecies cross between an annual cultivated mungbean line 'KUML29-1-3' (*V. radiata* var. *radiata*) and a wild perennial mungbean accession 'W021' (*V. radiata* var. *sublobata*). The KUML29-1-3 was developed from the Project on Genetics and Breeding of Field Legumes for Thailand under the support of the Thailand Research Fund, Kasetsart University, Kampheang Saen Campus. The line has high and stable seed yield (Sriphadet *et al*., 2010). 'W021' or 'ACC13' was obtained from the National Institute for Agrobiological Sciences (NIAS), Tsukuba, Japan. It is a small-seeded wild perennial mungbean with long vegetative and reproductive growth stages. The population was advanced to the F_2 generation using a single seed descent method. The F_2 seeds were grown for evaluation, and 186 F_2 plants were used as the mapping population in this study.

2. Phenotyping and data analysis

The F_1 seeds were grown to observe the number of days required in each growth stage, agronomic characters and then allowed to set the F_2 seeds. Random F_2 seeds were sown and the F_2 plants were extracted for DNA from young leaves. Observation were made on agronomic characters, growth habit and number of days required in each stage. The F_3 seeds from each F_2 plant sown in replicated trials at the spacing of 25×50 cm. and observed on agronomic characters together with their parents.

Data were recorded on days to first flowering (D_1), days to first pod maturity (D_2), days to harvesting (D_3), pod width (Pw) in mm., pod length (Pl) in cm., number of seeds per pod (Sp), number of pods per plant (Pp), 100 seed weight (100sw) in g., and seed yield per plant (Yp) in g.

Each trait was observed and the data were analyzed by an analysis of variance (ANOVA) following a completely randomized statistical design (CRD). Mean comparison was done using Duncan's Multiple Range Test (DMRT). The mean and standard deviation were calculated from 20 plants from each of the parents, F_1, and F_{1r}, while 306 plants were observed on the F_2 population.

Broad-sense heritability (h^2) of each trait was estimated according to Fehr (1987), using the equation $h^2 = \sigma^2_g/\sigma^2_p$. Where σ^2_g is the genotypic variance component and σ^2_p is the phenotypic variance component. In this experiment, σ^2_g was estimated from $V_{F2} - (V_{P1}+V_{P2}+V_{F1}+V_{F1r})/4$; where V_{F2}, V_{P1}, V_{P2}, V_{F1} and V_{F1r} are the variation between plants within the specified genotypes, and σ^2_p was estimated from V_{F2}. Normal distribution of all traits was tested by normality tests of distribution frequencies following W-test method (Royston, 1992).

Phenotypic correlation coefficients between traits were calculated according to Johnson and Kuby (2004). The observed traits were analyzed for simple correlation by R-stat software v. 2.8.1 (http://www.r-project.org/).

3. DNA extraction

The parental lines and F_2 plants were determined for polymorphic markers. Young leaves from each plant were extracted for DNA using CTAB method (Doyle and Doyle, 1990). The extraction buffer consists of 100 mM TrisHCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% PVP (Polyvinylpyrolidone), 2% CTAB and 2% 2-mercaptoethanol, liquid nitrogen, 5 M KoAc (potassium acetate), absolute EtOH, 70% ethanol, chloroform:isoamyl (24:1), and TE buffer. The equipment used were water bath, hot air oven, centrifuge, shaker, PCR machine, fluorescent spectrophotometer, electropholysis chamber. The SSR analysis was done in a sub-population by randomizing 186 individual F_2 population. Young leaves of around 7 days old were frozen in liquid nitrogen and mixed with 700 µl extraction buffer and 2% PVP. The samples were incubated in water bath at 65.5^{o}C for 30 min, added with 300 µl 5 M

KoAc, incubated on ice for 30-60 min, and spun at 12,000 rpm/min for 10 min. The upper liquid was transferred into a new tube and added with 700 µl chloroform:isoamyl (24:1), shaked with a rocker for 20 min and spun for 10 min. The upper liquid was transferred to a new tube and added with 700 µl frozen absolute EtOH. The sample was centrifuged with 12,000 rpm/min for 10 min and the lower part with DNA was kept. The DNA was washed twice with 70% ethanol, then diluted with 50 µl TE buffer. The DNA concentration was estimated by agarose gel electrophoresis using 50 ng/µl and 100 ng/µl λ DNA standard. Photos were taken with a camera at a suitable sharpness and contrast. The DNA concentration was adjusted to approximately 10 ng/µl.

4. SSR analysis

Nine hundred and seventy-one SSR markers were screened to detect polymorphism between the two parents. Of these, 595 SSR markers were developed from mungbean (Kumar *et al.*, 2002a and 2002b; Gwag *et al.*, 2006; Somta *et al.*, 2008 and 2009; Seehalak *et al.*, 2009 and Tangphatsornruang *et al.*, 2009), 191 were from azuki bean (Wang *et al.*, 2004), 119 were from common bean (Blair *et al.*, 2003 and Buso *et al.*, 2006) 7 were from cowpea (Li *et al.*, 2001) and 5 were from blackgram and 30 were from others. PCR reaction cycling time was performed by initial denaturation at 94^{o}C for 2 min, further denaturation at 94^{o}C for 30s (DNA loses its structure or melt to single strands), annealing at 55-60^{o}C for 30 s (primers bind to target DNA), extension at 72^{o}C for 60 s (primers extend to make a double stranded DNA). The reactions were carried out for 36 cycles in a 10 µl reaction. The separated DNA was electrophoresed in 5% acrylamine gel with 0.5x TE buffer for 30-40 min. The banding patterns were visualized according to the silver staining method. The DNA fragment sizes were compared with 1 kb ladder, which was used as a DNA marker. Finally, the DNA bands were scored for the presence or absence (Soehendi *et al.*, 2006). The polymorphic bands were scored as A; when bands were similar to P_1, B; when bands were similar to P_2 and H; when bands were similar to both of the

parents. All polymorphic markers were used for interval QTL mapping to detect the major QTLs locating on the genome.

5. Linkage mapping

The genotypic data from SSR markers were analyzed by JOINMAP 4.0-EVALUATION LICENSE program (Van Ooijen, 2009) to construct the linkage map of mungbean. For grouping the markers, a minimum LOD score of 3.0 were used as a threshold value in analysis. The linkage map was constructed with the Kosambi map function (Kosambi, 1944). Linkage groups were named after azuki bean linkage groups (Han *et al*., 2005).

6. QTL analysis

The QTL analysis for each character was performed using composite interval mapping (CIM) by WinQTL Cartographer 2.5 (Wang *et al.*, 2007). The analysis was done on the data of F_2 plant means. The thresholds for QTL detection were estimated using 2,500 permutation tests to declare a significant QTL. A P <0.01 level of significance was used as an evidence that there was a linkage between a marker locus and a QTL. The amount of phenotypic variation explained by all QTLs for each trait was determined by multiple regressions. The marker variation was explained by the coefficient of determination (R^2) value using R-stat software v. 2.8.1 (http://www.r-project.org/).

RESULTS AND DISCUSSION

RESULTS

1. Phenotypic data and broad-sense heritability

Mean and standard deviation of the parents and F_2 population were presented in Table 2. All traits were different among the parents with no maternal effect between F_1 and F_{1r}, revealing no maternal effect conditioning these traits. 'KUML29-1-3' showed determinate, while W021 showed indeterminate in growth habit. Days to first flowering (D_1) of 'KUML29-1-3' was only 31 days while 'W021' took 65 days. The same relationship was also found in days to first pod maturity (D_2) (47 vs 82 days) and days to harvesting (D_3) (76 vs 139 days). Pod length (Pl) of 'KUML29-1-3' was longer than W021 (8.2 vs 4 cm), while pod width (Pw) was also wider (4.7 vs 3.1 mm). Number of pods per plant (Pp) of 'KUML29-1-3' was lower than 'W021' (109 vs 25), while they were not different in number of seeds per pod. The F_2 population can be classified into different classes according to days to first flowering, days to first pod maturity, days to harvesting, 100 seed weight, number of seeds per pod, number of pods per plant, pod length, pod width and seed yield per plant (Fig. 1). All traits, except 100 seed weight showed transgressive segregation. Days to flowering, pod maturity and harvesting of the F_2 population ranged from 29-76 days, 44-96 days and 79-178 days, showed skewing toward 'KUML29-1-3'. They showed positive segregation when compared with 'W021' (Fig. 1a, 1b and 1c). Yield components such as number of seeds per pod, seed yield per plant and pod length showed transgressive segregation. On the other hand, the 100 seed weight of F_2 population fell between parents, at 1.0 g to 3.0 g per 100 seeds, while 'W021' and 'KUML29-1-3' were 0.6 and 4.2 g/100 seeds, respectively (Fig. 1d), when compared with 'KUML29-1-3', seed yield per plant and pod width showed positive transgressive segregation but number of seeds per pod showed negative segregation. Number of pods per plant showed positive transgressive segregation when compared with W021.

Broad-sense heritability (h^2) as calculated from the F_2 data of each traits were presented in Table 2. The broad-sense heritability of flowering dates, viz. D_1, D_2 and D_3 were 88.6%, 91.2% and 86.8%, respectively, which were considered highly heritable. The heritabilities of yield components were high in Pl (92.36%), Pw (97.5%), Sp (91.2%) and 100sw (90%) and medium-high in Pp (77.0%), Yp (65.1%).

2. Correlation analysis

The phenotypic correlation coefficients among 9 quantitative traits were revealed in Table 3. D_1 showed highly positive correlation with D_2 ($r = 0.966^{**}$) and D_3 ($r = 0.693^{**}$). However, these traits had negative correlations with yield components such as 100 seed weight (-0.373**) (Table 3). D_2 showed nearly the same result as D_1 with negative correlation with 100 seed weight (-0.395**). On the other hand, D_3 showed no significant correlation with seed yield per plant. In addition, 100 seed weight revealed positive correlation with seed yield per plant (0.535**) pod length (0.574**) and Pw (0.376**). Correlation between yield components were high in number of seeds per pod and pod length (0.781**), and 100 seed weight and pod length (0.574**). This result indicated that yield depends on seed size and pod size, such as pod length, pod width, number of seeds per pod, and 100 seed weight. In this experiment, pod length, pod width and 100 seed weight are important for seed yield improvement in new varieties. However, negative correlation was also found between days to flowering and yield. The optimum number of days to flowering should be included as a selection criterion together with yield components.

3. Map construction

Nine hundred and forty-seven SSR markers were screened to detect polymorphism between the two parents. One hundred and fifty-two markers were found polymorphic between the parents, representing 16.05% of the detected polymorphism. However, 150 markers could be assigned into 11 linkage groups of mungbean chromosomes plus a small linkage group, with the total coverage of 1,019.1 cM, giving the average chromosome length of 84.9 cM. The average

distanced between SSR loci on the map is 7.4 cM (Fig. 2). Microsatellite markers were place on the 11 chromosomes, each tagged with at least five or more SSR markers.

4. QTL Analysis

Fifty-three putative QTLs of agronomic traits were detected by CIM with LOD of ≥ 2.0 (Fig. 2 and Table 4). The amount of phenotypic variation in each trait explained by its respective QTLs were carried out by multiple regression and found ranging from 8.01 to 60.34%. Five of Fifty-three QTLs can individually explain more than 20% of the phenotypic variation of the traits. The number of QTLs per trait range between 2 and 12 loci with the average of 5.9 QTLs per trait. D_1, D_2, D_3, 100sw, Sp, Yp, Pl, Pp and Pw traits were explained by 4, 5, 5, 12, 9, 5, 8, 2 and 3 QTLs, respectively (Table 4). The cumulative regression of composite interval markers related to the above traits explain 54.8%, 54.5%, 28.7%, 60.3%, 35.3%, 21.3%, 40.6%, 9.1% and 8.0% of the total phenotypic variations, respectively. The QTLs were spanned on all linkage groups. Some QTLs appeared to co-locate on the map. For example, CEDG026 marker on LG2 linked to *Dtf2-1* and *Dfm2-1*. VR0364 marker on LG2 linked to *Dtf2-2*, *Dfm2-2* and *Dh2-2*. All QTLs clusters relating to flowering dates were on LG2 and LG4. Two clusters were identified, viz. VR0364 markers tightly linked to *Dtf2-2-Dfm2-2-Dh2-2*, while CEDG107 maker tagged *Dtf4-1-Dfm4-1-Dh4-1*. The yield component QTLs comprises nine clusters. CEDG104-CEDG024 marker tagged *Sp1-1- Yp1-Pl1-1*, VR0200-VR17 markers located with *Pl2-Swt2-2*, SSR-IAC63-CEDG107 markers were associated with *Pl4-Yp4-1-Swt4-1*, VR035-VR0366 markers related to *Swt4-2-Sp4*, CEDG020 markers associated with *Sp5-Pl5-Swt5-1*, GBssr-MB7 marker tagged *Sp6-Pl6*, CEDG111-VR0126 markers located with *Swt7-Pl7*, VR-SSR005 marker was associated with *Yp8-Pl8* and CEDG198 marker associated with *Swt10-Sp10-Yp10*. The interesting neighborhoods QTLs were the clusters of QTLs for flowering dates and yield components on LG4, which comprised 2 sub-groups. One cluster was located between SSR-IAC63 to CEDG107 (*Pl4-Yp4-1-Swt4-1-Dh4-1-Dtf4-1-Dfm4-1*), and another was found between VR035 to VR0366 (*Swt4-2-Sp4-Dtf4-2-Dfm4-2*). The highlight of the results

were that this work is the first to construct SSR mungbean linkage map that resolves the 11 linkage groups and tagged the composite interval markers related to four major agronomic traits (D_1, D_2, 100sw and Pl), together explaining ≥ 40 % of the total phenotypic variation (Table 4).

5. Comparative linkage map between mungbean, azuki bean and black gram

The mungbean linkage map was compared with azuki bean linkage map (Han *et al*., 2005) and black gram linkage map (Chaitieng *et al.*, 2006). Mungbean linkage map was co-linear with azuki bean and black gram linkage map. The azuki-developed markers were revealed on all mungbean linkage groups ranging from 1 to 6 markers, comparing between azuki bean linkage map and mungbean linkage map. While, our map compared with black gram linkage map ranging between 1 (LG 1, 2, 4, 6, 8, 10) to 4 (LG 5 and 9) markers. Of 62 azuki-developed SSR marker loci on the mungbean linkage, 42 marker loci were common with azuki bean linkage map, while 19 loci with black gram linkage map (Fig. 3). Markers order was 42 (68%) marker loci on mungbean linkage were co-linear with azuki bean and 19 (90%) marker loci of 21 marker loci from black gram linkage map (see list similar markers in Chaitieng *et al*., 2006).

The maximum numbers of similar loci were 6 loci on LG 5, 6 and 8 when compared with azuki bean linkage map, and 4 loci on LG 5 and 9 when aligned with black gram linkage map. This research found highly reverse regions between mungbean and azuki bean. Five internal inversions between mungbean linkage map and azuki bean linkage map were on LG 1, 2, 4, 5 and 9 where tagged CEDG051 and CEDG263, CEDG026 and CEDG065, CEDG154 and CEDG107, CEDG268 and CEDG114 and CEDG056 and CEDG259, respectively (Fig. 3). Moreover, on LG 3, 6, 7, 8, 11 were affected by insertion/deletion. For comparing between mungbean linkage map and black gram linkage map, two inversion regions were tagged by CEDG010 and CEDG043 on LG 3 and CEDG166 and CEDG056 on LG 9. Other linkage groups were also affected by insertion/deletion. Co linearity of genetic linkage

found every linkage groups in all comparison. Our result implied that in/del of comparative linkage map affected to evolve divergence of Asian *Vigna* species.

Table 2 Mean and standard deviation of major agronomic traits observed from parents and progenies from the cross between an annual cultivated mungbean line 'KUML29-1-3' and a wild perennial mungbean accession 'W021', including their variances and corresponding heritability

	D_1	D_2	D_3	Pl (cm)	Pw (mm)	Sp	Pp	100sw (g)	Yp
P_1(29-1-3)	31.2c ± 1.6	47.4c ± 1.1	76.4b ± 0.5	8.2a ±0.1	4.7a ±0.2	12.0a ±0.4	25.0c ±6.5	4.2a ±0.1	9.6a ±2.7
P_2(W021)	65.5a ±6.5	81.8a ±6.5	139.6a ±19.2	4.0b ±0.2	3.1c ±0.1	9.7ab ±0.9	109.5ab±14.8	0.6c ±0.04	1.4b ±0.2
F_1	42.5b ±2.2	57.0bc ±1.5	124.0a ±6.0	5.3ab ±0.3	4.0ab ±0.1	8.8b ±3.0	149.6a ±21.2	1.9b ±0.1	12.0a ±4.1
F_{1r}	47.8b ±2.1	62.7b ±2.0	123.5a ±5.4	5.3ab ±0.4	4.0b ±0.1	8.4b ±1.0	151.4a ±29.1	2.0b ±0.2	12.1a ±6.1
F_2	47.7b ±11.0	64.2b ±12.0	123.4a ±28.6	5.7ab ±2.7	3.9bc ±0.7	10.1ab ±2.6	61.2bc ±41.2	1.8b ±0.4	7.7ab ±6.6
$LSD_{.05}$	11.1	12.1	28.1	3.0	0.7	2.9	52.9	0.4	8.1
V_{F2}	120.3	143.6	818.2	1.1	0.4	6.8	1695.9	0.2	43.8
V_E	13.7	12.6	108.3	0.1	0.01	0.6	390.1	0.02	15.3
h^2(%)	88.6	91.2	86.8	92.36	97.5	91.2	77.0	90.0	65.1

Means of the same trait followed by the same letter are not different at $P \leq 0.05$

D_1 = days to first flowering, D_2 = days to first pod maturity, D_3 = days to harvesting, Pl = pod length (cm), Pw = pod width (mm), Sp = number of seeds per pod, Pp = number of pods per plant, 100sw = 100-seed weight (g) and Yp = seed yield (g) per plant

Table 3 Correlation between number of days in each growth stage and yield components

	D_2	D_3	Pw	Pl	Sp	Pp	100sw	Yp
D_1	0.966**	0.693**	-0.152*	-0.286**	-0.244**	-0.278**	-0.373**	-0.293**
D_2		0.700**	-0.162*	-0.349**	-0.314**	-0.319**	-0.395**	-0.347**
D_3			-0.146*	-0.303**	-0.272**	-0.021^{ns}	-0.312**	-0.118^{ns}
Pw				0.328**	0.190**	0.134*	0.376**	0.212**
Pl					0.781**	0.232**	0.574**	0.522**
Sp						0.235**	0.226**	0.472**
Pp							0.295**	0.822**
100sw								0.535**

ns,** Non significant and significant at 0.01 level of probability, respectively.

D_1 = days to first flowering, D_2 = days to first pod maturity, D_3 = days to harvesting, Pl = pod length (cm), Pw = pod width (mm), Sp = number of seeds per pod, Pp = number of pods per plant, 100sw = 100-seed weight (g) and Yp = seed yield (g) per plant.

Table 4 Genetic information of significant QTLs conditioning morphological traits in the F_2 population of the cross between an annual cultivated mungbean line 'KUML29-1-3' and a wild perennial mungbean accession 'W021'

Trait	QTL name	Linkage group	Marker	Position (cM)	LOD	Composite interval mapping results		
						Additive (a)%	Dominance (d)%	R^2(%)
Days to first flowering (D_1)	*Dtf2-1*	2	CEDG026	16.6	7.13	-5.59	6.1	12.26%
	Dtf2-2	2	VR0364	33.03	10.83	-7.46	4.21	21.30%
	Dtf4-1	4	CEDG107	20.2	13.98	-8.49	-2.62	29.02%
	Dtf4-2	4	VR035-VR0366	49.12	14.08	-11.47	-4.56	29.59%
Cumulative R^2								54.80%
Days to first pod maturity (D_2)	*Dfm2-1*	2	CEDG026	16.6	6.83	-6.25	5.88	12.49%
	Dfm2-2	2	VR0364	33.03	9.09	-7.85	4.96	18.43%
	Dfm4-1	4	CEDG107	21.2	13.11	-8.93	-2.55	27.68%
	Dfm4-2	4	VR0366	49.12	13.48	-12.14	-4.84	13.48%
	Dfm4-3	4	CEDG241	69.87	6.54	-6.87	2.37	23.17%
Cumulative R^2								54.49%
Days to harvesting (D_3)	*Dh2-1*	2	CEDG026	20.68	4.03	-10.59	12.19	7.01%
	Dh2-2	2	VR0364	32.03	6.68	-13.38	13.31	10.97%
	Dh4-1	4	CEDG107	18.2	4.38	-10.9	4.17	10.05%
	Dh4-2	4	VR0366-VR0313	61.24	5.32	-12.1	11.95	12.33%
	Dh4-3	4	VR20-CEDG241	69.87	4.59	-10.91	9.72	9.67%
Cumulative R^2								28.72%

Table 4 (Continued)

Trait	QTL name	Linkage group	Marker	Position (cM)	LOD	Composite interval mapping results		
						Additive (a)%	Dominance (d)%	R^2(%)
100-seed weight (100sw)	*Swt2-1*	2	CEDG108-CEDG050	24.29	2.81	0.14	0.04	7.02%
	Swt2-2	2	VR0200-VR17	81.2	4.42	0.18	0.013	10.83%
	Swt3	3	CEDG296-CEDAAG001	89.2	3.59	0.17	0.02	8.90%
	Swt4-1	4	SSR-IAC63-CEDG107	14.24	3.98	0.2	-5.47	10.04%
	Swt4-2	4	VR035-VR0366	48.12	3.28	0.2	0.01	7.62%
	Swt5-1	5	CEDG020	11.36	2.11	0.15	-0.04	4.24%
	Swt5-2	5	GATS11B	81.95	2.44	0.12	-0.03	5.62%
	Swt7	7	CEDG111-VR0126	50.56	3.97	0.14	0.13	8.15%
	Swt8	8	VR-SSR031	41.8	2.51	0.13	0.02	6.26%
	Swt9	9	CEDG127-BMd-27	40.97	3.08	0.14	0.07	7.12%
	Swt10	10	CEDG198	63.92	2.55	0.13	0.14	3.66%
	Swt11	11	MB-SSR104 - VR-SSR011	44.89	4.29	0.2	0.02	10.51%
Cumulative R^2								60.34%
Number of seeds per pod (Sp)	*Sp1-1*	1	CEDG104-CEDG024	39.67	4.66	1.91	0.26	12.17%
	Sp1-2	1	VR0198	75.56	4.93	1.65	0.71	10.77%
	Sp1-3	1	CEDG263-VR-SSR015	96.18	4.14	1.81	0.34	10.45%
	Sp4	4	VR035-VR0366	48.12	2.5	1.03	0.58	5.53%
	Sp5	5	CEDG020	7	2	0.63	0.64	3.87%
	Sp6	6	GBssr-MB7	43.1	2	-0.86	-0.24	4.85%
	Sp8	8	DMB-SSR79	2	2.66	0.72	0.64	4.76%
	Sp9	9	SSR-IAC47	45.3	2.3	0.58	0.65	3.71%
	Sp10	10	CEDG198	70.4	3.54	0.92	0.34	8.08%
Cumulative R^2								35.30%

Table 4 (Continued)

Trait	QTL name	Linkage group	Marker	Position (cM)	LOD	Composite interval mapping results		
						Additive (a)%	Dominance (d)%	R^2(%)
Pod length (Pl)	*Pl1-1*	1	CEDG104-CEDG024	40.7	3.09	0.54	-0.07	7.83%
	Pl1-2	1	CEDG048-VR0248	52.2	2.62	0.41	0.2	6.36%
	Pl2	2	VR0200-VR17	80.2	3	0.32	0.24	7.88%
	Pl4	4	SSR-IAC63 - CEDG107	13.2	3.41	0.41	0.24	8.03%
	Pl5	5	CEDG020	9.4	2	0.26	0.1	4.16%
	Pl6	6	GBssr-MB7	45.36	2.03	-0.22	-0.3	2.51%
	Pl7	7	CEDG111-VR0126	52.6	4.53	0.43	-0.06	10.83%
	Pl8	8	VR-SSR005	40.2	3.1	0.33	0.19	6.58%
Cumulative R^2								40.58%
Number of pods per plant (Pp)	*Pp4*	4	CEDG154-VR035	40.1	2.8	19.3	7.54	6.72%
	Pp6	6	CEDG118	52.6	2.3	10.58	14.04	2.69%
Cumulative R^2								9.10%
Pod width (Pw)	*Pw2*	2	VR078	89.2	2	0.13	0.35	1.19%
	Pw3	3	VR0468	25.6	2.7	-0.4	-0.6	0.78%
	Pw4	4	SSR-IAC63	8.2	2.6	0.3	-0.08	5.88%
Cumulative R^2								21.26%
Yield per plant (Yp)	*Yp1*	1	CEDG104-CEDG024	40.7	2.6	3.02	-2.24	6.79%
	Yp4-1	4	SSR-IAC63-CEDG107	13.25	3.27	3.53	0.73	8.12%
	Yp4-2	4	VR20-CEDG241	68.87	3.86	2.4	-1.4	9.48%
	Yp8	8	VR-SSR005	40.2	2.1	2.1	1.4	4.42%
	Yp10	10	CEDG198	70.4	2	2.1	0.43	4.49%
Cumulative R^2								8.01%

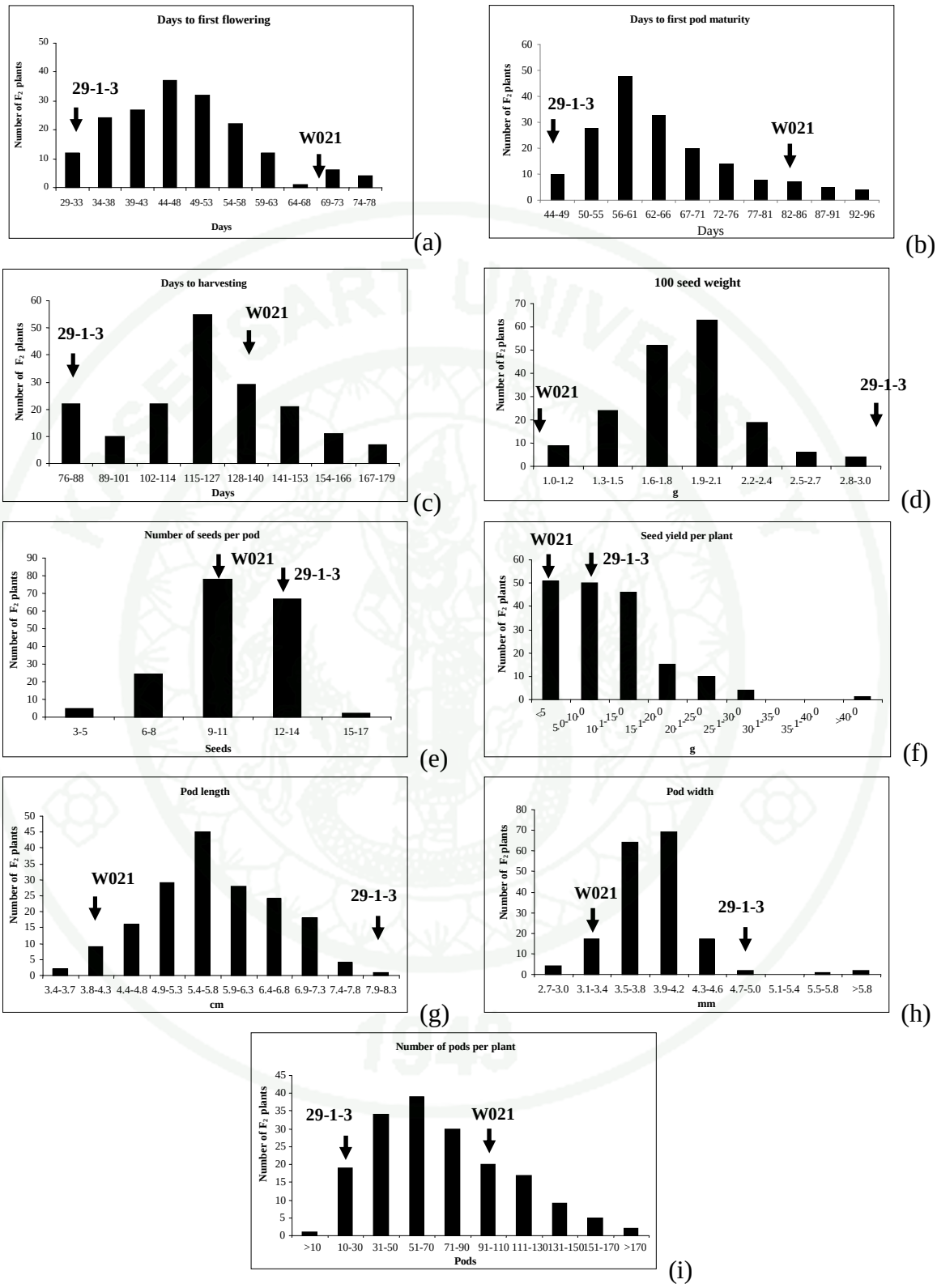


Figure 1 Frequency distribution of the F_2 population, derived from the cross 'KUML29-1-3 x W021': (a) D_1, (b) D_2, (c) D_3, (d) 100sw, (e) Sp, (f) Yp, (g) Pl, (h) Pw and (i) Pp

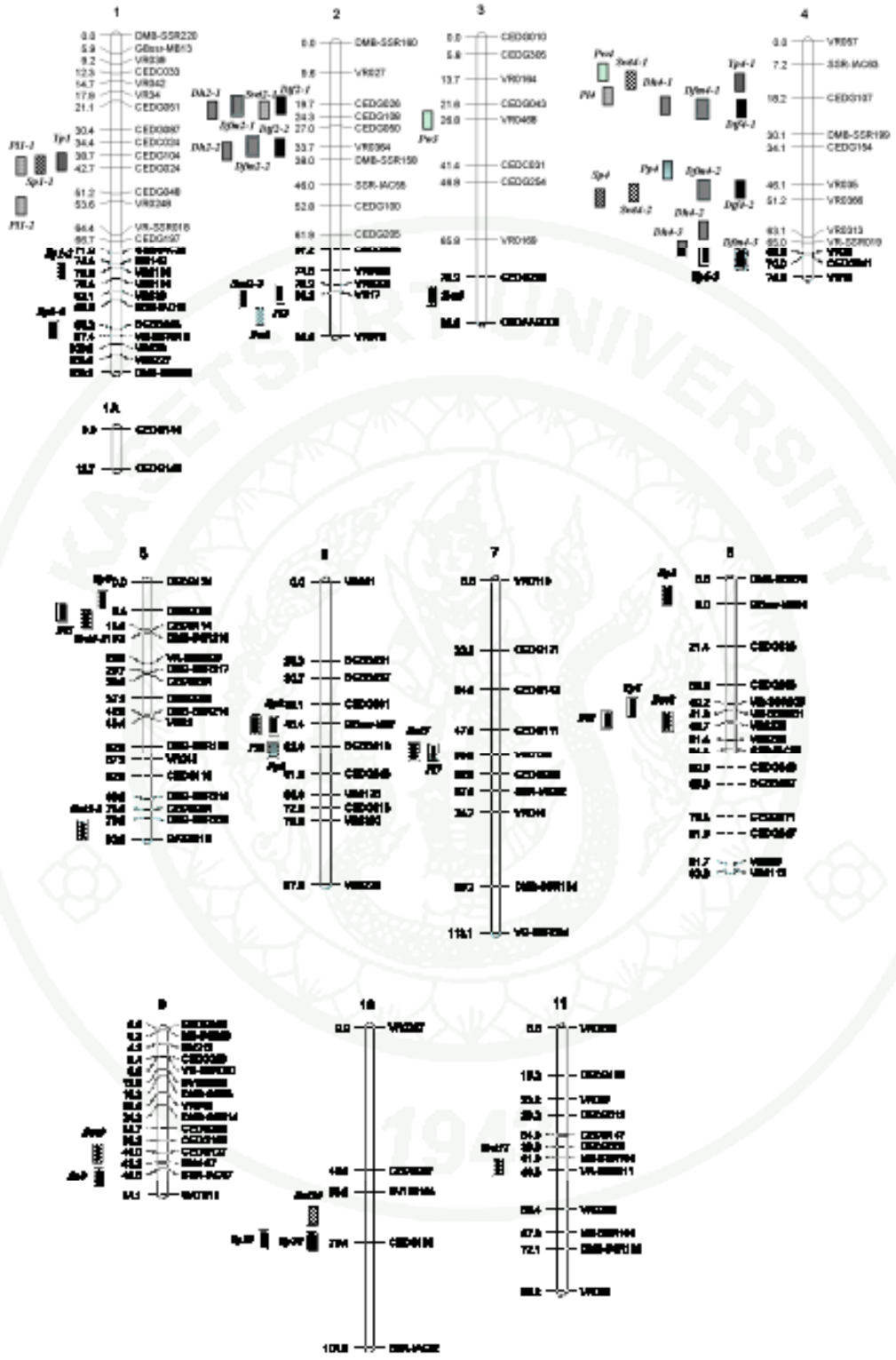


Figure 2 SSR linkage map of mungbean constructed from the F_2 population. Cumulative distances in centimorgans (Kosambi's) and markers name are shown on the left and right sides of the linkage group. QTL intervals detected at LOD ≥2.0 are presented as boxes on the left of the linkage groups

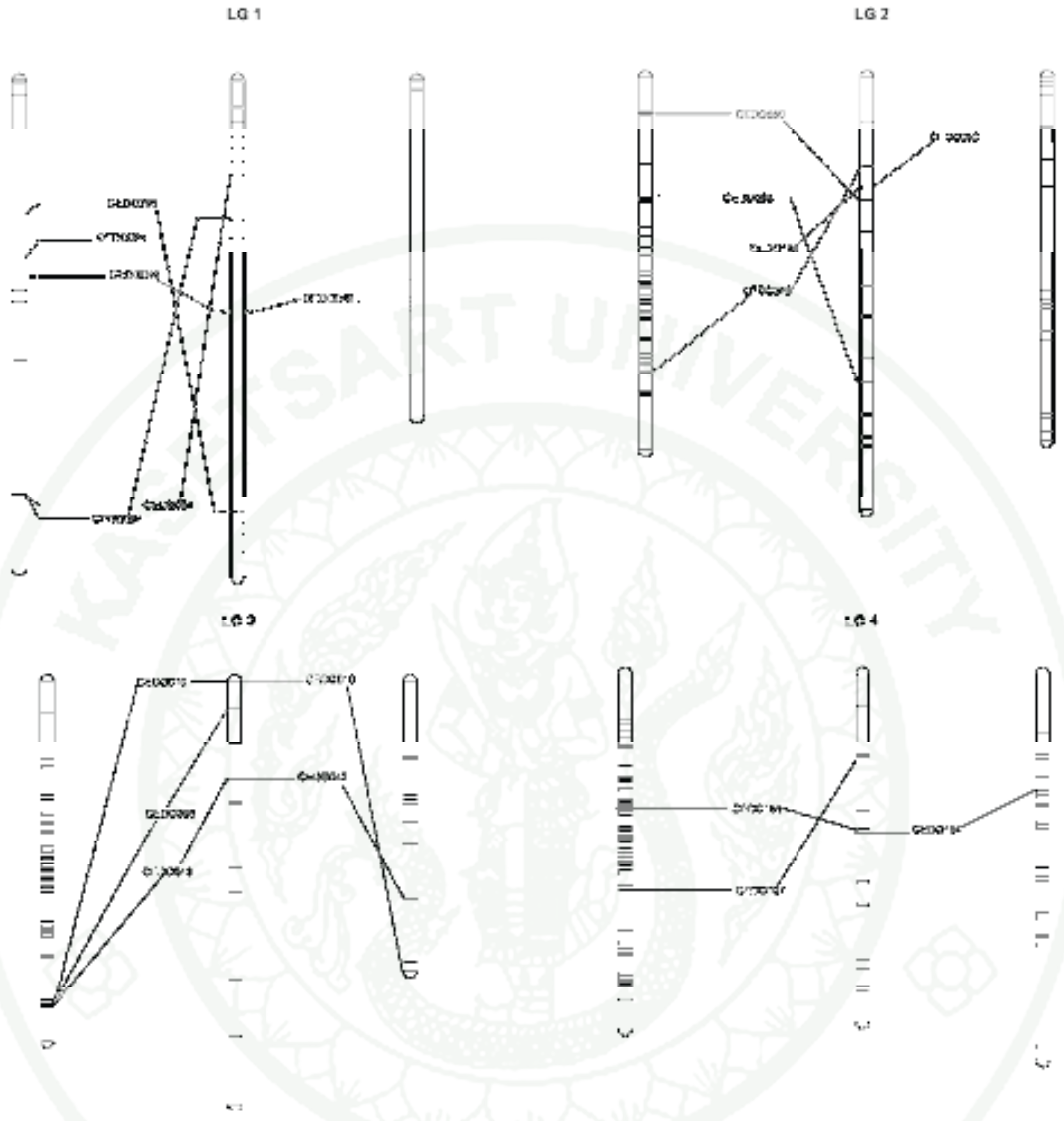


Figure 3 A comparative linkage map between mungbean from this study vs azuki bean (left) (Han *et al.*, 2005) and black gram (right) (Chaitieng *et al.*, 2006), based on SSR azuki common markers

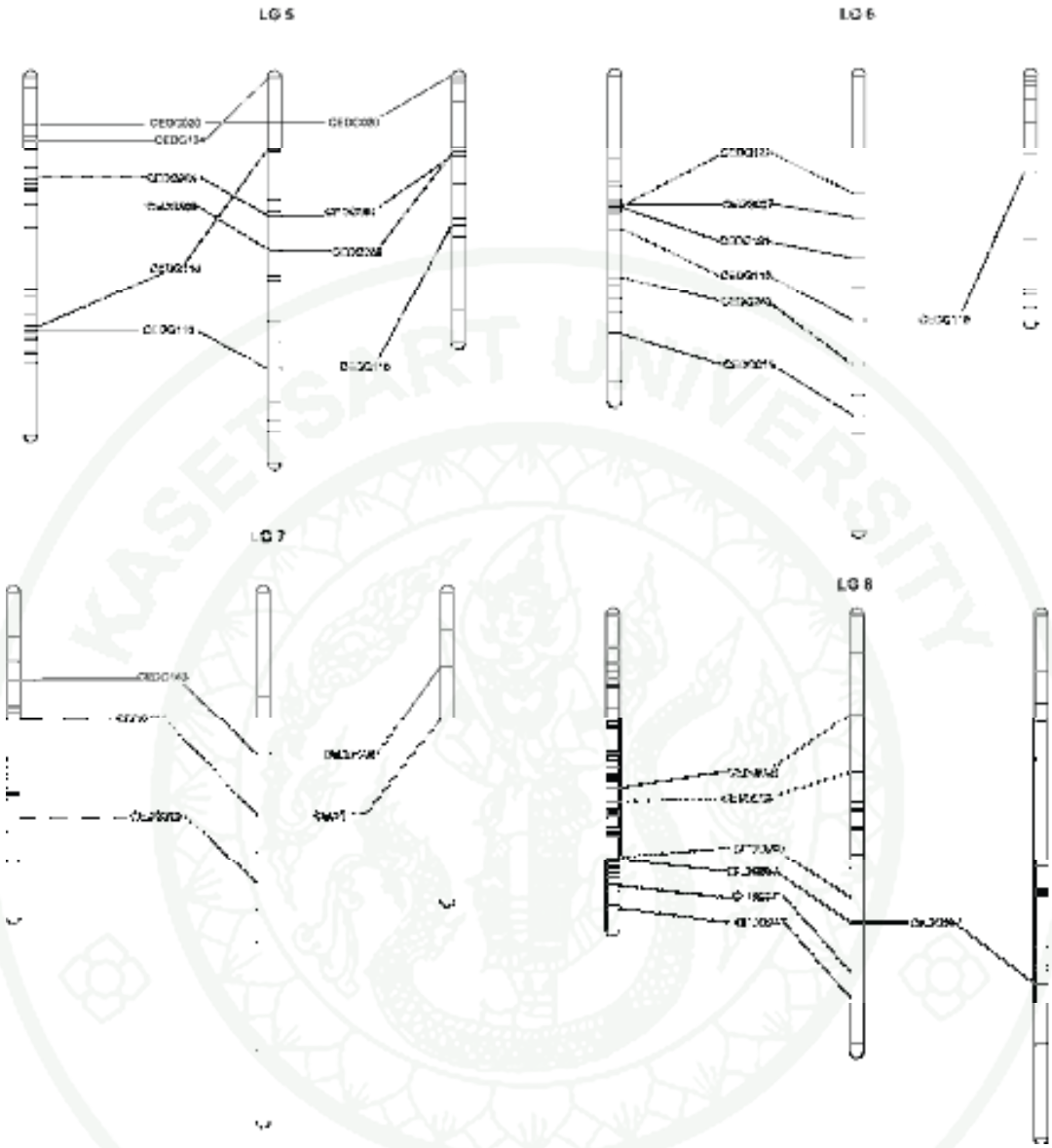


Figure 3 (Continued)

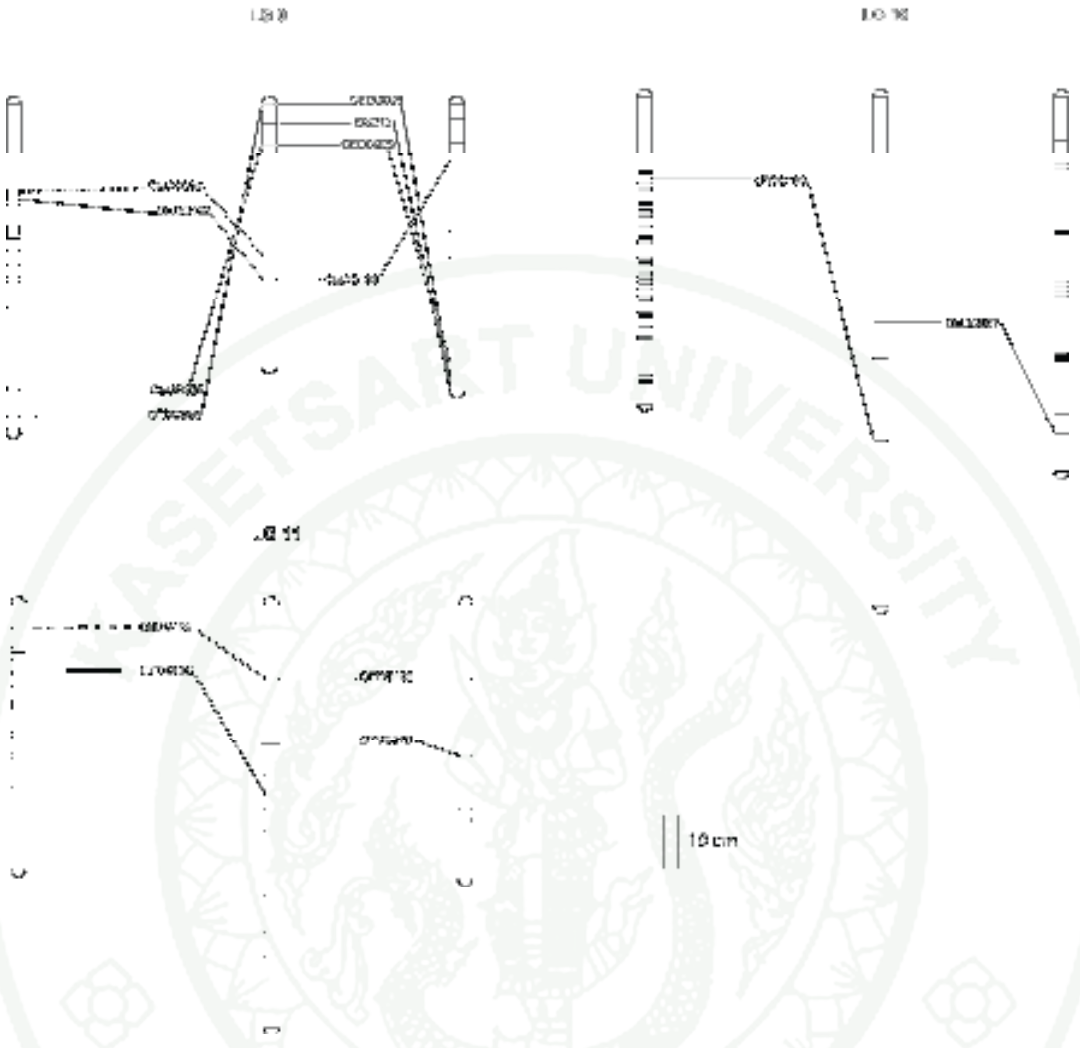


Figure 3 (Continued)

DISCUSSION

Phenotypic data and broad-sense heritability

In this study, broad-sense heritability of days to first flower (D_1), days to first pod maturity (D_2) and days to harvesting (D_3) were high (88.6, 91.2 and 86.8%, respectively). Sriphadet *et al.* (2007) studied inheritance of agronomic traits and their interrelationship in RIL mungbean lines obtained from the cross between wild mungbean 'ACC 41' and the cultivated 'Berken'. They found that flowering date skewed towards ACC 41, but the narrow-sense heritability was high at 88.0 %. They also reported an abnormal distribution in D_1, D_2 and D_3 data. Similar results were also reported by Siddique *et al.* (2006) that there were high heritabilities in days to first flower and days to harvesting. Rohman *et al.* (2003) reported that days to first flower, days to harvesting, 100 seed weight and plant height had high heritability, while number of pods per plant and number of seeds per pod were low in heritability. Dursun (2007) studied in common bean and found that heritability was high in pod width, but low in number of seeds per pod.

Correlation analysis

Correlation analysis in this study revealed that days to flowering showed positive correlation with days to first pod maturity and days to harvesting. Days to flowering and days to maturity presented negative correlation with yield components such as 100 seed weight. According to Khattak *et al*. (1995), days to flowering was positively correlated with days to maturity, but negatively correlated with number of pods per plant and total seed weight. Days to maturity was negatively correlated with total seed weight. In contrast to Rohman *et al*., (2003), days to flowering presented negative correlation with days to maturity but positively correlated with 100 seed weight and total seed weight. Yucel *et al.* (2006) studied path analysis in chickpea and found that days to flowering, number of branches per plant and 1000 seed weight had direct effect on total seed weight. Turk *et al.* (2008) reported correlation analysis in narbon bean (*Vicia narbonensis*) that seed yield was negatively correlated with days to

flowering. Rajan *et al.* (2000) worked in mungbean and found similar result to ours that seed yield had positive genotypic correlation with pods per plant, seeds per pod and one hundred grain weight. Thus the genetic of grain yield can be improved by selecting characters having positive correlation.

Map construction

One hundred and fifty SSR markers were assigned into 11 linkage groups, corresponding to the haploid number of mungbean chromosomes. In the previous research by Humphry *et al.* (2002), 65 RFLP probes were clustered into 13 linkage groups, with the total length of the map spanned 737.9 cM at an average distance between markers of 3.0 cM and a maximum distance between linked markers of 15.4 cM. While our map has covered 1,019.1 cM, with the average distance between SSR loci of 7.4 cM. Han *et al.* (2005) analyzed azuki bean genetic linkage map from a backcross population of (*V. nepalensis x V. angularis*) x *V. angularis*. They used 486 markers comprising 205 SSR, 187 AFLP and 94 RFLP. Their linkage map covered altogether 11 linkage groups as our results. Azuki bean map spanned 832.1 cM with an average marker distance of 1.85 cM. Our result showed longer genome coverage than both maps, with the longer average marker distance.

QTL analysis

Ogundiwin *et al.* (2005) studied QTLs for morphological and agronomic traits in F_2 population of *V. vexillata* using RAPD, AFLP and SSR markers. We found the same QTLs conditioning pod length on LG 2 and 7, number of seeds per pod on LG 1 and 100 seed weight on LG 4 and 11. Our results also agree with Fatokun *et al.* (1992) who located major QTLs for seed weight in cowpea and mungbean and found that genomic region with the greatest effect on seed weight were linked to the RFLP markers on LG 2. Kelly *et al.* (2003) tagged pod length in bean on LG 2 and 7, and seed weight on LG 4 as our result but different in the QTLs for days to flowering and to maturity that they found locating on LG 1 and 8, respectively.

Comparative linkage map between mungbean, black gram and azuki bean.

Azuki bean SSR marker can useful for construct the black gram linkage maps (Chaitieng *et al.*, 2006) and used for mungbean linkage map construction in this study. Our linkage map has been success because mungbean, black gram and azuki bean were co-linear genome in same sub group. Our result was carried out 68% related common loci between azuki bean and 90% related common loci between mungbean and black gram. The common markers were present more highly conserve loci between mungbean and black gram than mungbean and azuki bean. This result supported Tomooka *et. al.* (2002) publication. They reported that mungbean and black gram were in the same section *Ceratotropis,* while azuki bean was assigned in section *Angulares.* However, Chaitieng *et al.* (2006) reported highly co linearity between black gram and azuki bean (88%). So, the useful of related genetic marker can be use for closely genome.

In this study, the mungbean linkage map was compared with an azuki bean linkage map (Han *et al.*, 2005) and black gram (Chaitieng *et al.*, 2006) by the azuki bean SSR markers. Our result found 42 marker loci common to azuki bean SSR markers. Chaitieng *et al.*, (2006) reported that 80 marker loci from black gram were commom to these from azuki bean. Gupta *et al*. (2008) compared a SSR marker linkage map of black gram with azuki bean linkage map (Han *et al.*, 2005) and found 41 co-linear loci. Moreover, Isemura *et al.*, (2010) reported that 129 loci from azuki bean map can be used in rice bean.

Our results found internal inversions between mungbean linkage map and azuki bean linkage map, insertion/deletion on LG 3, 6, 7, 8, 11 and inversion regions, insertion/deletion between mungbean linkage map and black gram linkage map. These results indicated that in subgenus *Ceratotropis* genomes have accumulated a number of insertions/deletions. The mutation in chromosomes detected between mungbean, black gram and azuki bean linkage maps played a role in the phylogenetic of these genomes.

The comparative map revealed the gene position that related with the important QTL traits. Our results presented mungbean QTL having important trait such as DTF, seed size, pod length that may be useful for mungbean improvement program all transfer to other *Vigna.* Efficient mungbean linkage map will get contribute usefulness for marker assisted selection.

CONCLUSION AND RECOMMENDATION

Conclusion

The first mungbean genetic linkage map that resolves subspecific 11 linkage groups was constructed with 186 F_2 plants derived from an inter-subspecific cross between a heptafoliate mutant mungbean line 'KUML29-1-3' (*Vigna radiata* var. *radiata*) and an Australian wild perennial mungbean accession 'W021' or 'ACC13' (*Vigna radiata* var. *sublobata*). A total of 150 SSR primers were located on 11 linkage groups, each containing at least 5 markers. There are also 2 unlinked markers. The map spans 1,019.1 cM with the average distance between markers was 7.4 cM. QTLs of days to first flowering (D_1), day to first pod maturity (D_2), days to harvesting (D_3), 100-seed weight (100sw), number of seeds per pod (Sp), number of pods per plant (Pp), seed yield per plant (Yp), pod length (Pl) and pod width (Pw) were located on this map. Totally 53 QTLs associated with nine traits were detected. Each QTL explained 0.8% to 29.6% of the phenotypic variation for the traits. The composite phenotypic variation explained by QTLs of each trait ranged from 8.0% to 60.3%.

Recommendation

The results from this study provided useful information on QTLs of major agronomic traits in mungbean. It can be used as information for selecting parents and progenies in mungbean breeding project aiming at increasing regions of candidate loci in novel breeding lines.

LITERATURE CITED

Acquaah, G. 2007. **Principle of Plant Genetics and Breeding**. Blackwell publishing, UK.

Akpapunam, M. 1996. Mungbean (*Vigna radiata* (L.) Wilczek), pp. 209-215. *In* E. Nwokolo and J. Smartt, eds. **Food and Feed from Legumes and Oilseeds**. Chapman and Hall, London.

Blair, M., F. Pedraza, H. Buendia, E. Gaitán-Solís, S. Beebe, P. Gepts and J. Thome. 2003. Development of a genome-wide anchored microsatellite map of common bean (*Phaseolus vulgaris* L.). **Theor. Appl. Genet.** 107:1362-1374.

Brown J. and P. Caligari. 2008. **An Introduction to Plant Breeding**. Blackwell Publishing, Oxford.

Buso, G., Z. Amaral, R. Brondani and M. Ferreira. 2006. Microsatellite markers for the common bean *Phaseolus vulgaris*. **Mol. Ecol. Notes** 6: 252-254.

Chaitieng, B., A. Kaga, N. Tomooka, T. Isemura, Y. Kuroda and D. Vaughan. 2006. Development of a black gram [*Vigna mungo* (L.) Hepper] linkage map and its comparison with an azuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi] linkage map. **Theor. Appl. Genet.** 113: 1261-1269.

de Vienne, D. 2003. **Molecular Markers in Plant Genetics and Biotechnology.** Science Pulishers, Inc., USA.

Doyle, J. and J. Doyle. 1990. Isolation of plant DNA from fresh tissue. **Focus**. 12: 13-15.

Dudley, J. 2002. Integrating molecular techniques into quantitative genetics and plant breeding, pp. 65-83. *In* M.S. Kang, ed. **Quantitative Genetics Genomics and Plant Breeding**. CABI International. Wallingford.

Duke, J. 1983. **Handbook of Legumes of the World Economics Importance**. Plenum Press, New York.

Dursun, A. 2007. Variability, heritability and correlation studies in bean (*Phaseolus vulgaris* L.) genotypes. **World J. Agric. Sci.** 3: 12-16.

Fatokun, C., D. Menancio-Hautea, D. Danesh and N. Young. 1992. Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. **Genetics** 132: 841-846.

Fehr, W. 1987. **Principles of Cultivar Development, vol. 1. Theory and Technique**. McGraw-Hill Inc, New York.

Fernandez , G. and S. Shanmugasundaram. 1988. The AVRDC mungbean improvement program: The past, present and future. *In*: Shanmugasundaram, S. and B.T. McLean (eds.) **Mungbean: Proceeding of the Second International Symposium**. AVRDC, Shanhua, Taiwan, pp. 58-70.

Gayen, P. and D. De. 2005. Genetic variability of pod and seed characters in mungbean as influenced by genotype and development stages. **Crop Research (Hisar)** 30: 59-62.

Gonzalez, J. 1999. Molecular markers and heterosis, pp. 257-268. *In* J.G. Coors and S. Pandey, eds. **The Genetics and Exploitation of Heterosis in Crops**. American Society of Agronomy, Inc., Wisconsin.

Gupta, S., J. Souframanien and T. Gopalakrishna. 2008. Construction of a genetic linkage map of black gram, *Vigna mungo* (L.) Hepper, based on molecular markers and comparative studies. **Genome.** 51: 628-637.

Gwag, J., J. Chung, H. Chung, J. Lee, K. Ma, A. Dixit, Y. Park, E. Cho, T. Kim and S. Lee. 2006. Characterization of new microsatellite markers in mungbean, *Vigna radiata* (L.). **Mol. Eco. Note.** 6: 1132-1134

Haldene, J. 1919. The combination of linkage values and the calculation of distances between the loci of linked factors. **J. Genet**. 8: 299-309.

Han, O., A. Kaga, T. Isemura, X. Wang, N. Tomooka and D. Vaughan. 2005. A genetic linkage map for azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi). **Theor. Appl. Genet.** 111: 1278-1287.

Humphry, M.E., V. Konduri, C.J. Lambrides, T. Magner, C.L.McIntyre, E.B. Aiken and C.J. Liu. 2002. Development of a mungbean (*Vigna radiata*) RFLP linkage map and its comparison with lablab (*Lablab purpureus*) reveals a high level of colinearity the two genomes. **Theor. Appl. Genet.** 105:160-166

Isemura, T., A. Kaga, N. Tomooka, T. Shimizu and D. Vaughan. 2010. The genetics of domestication of rice bean, *Vigna umbellata*. **Ann. Bot.** 106: 927-944.

Johnson, R. and P. Kuby. 2004. **Elementary Statistics.** Thomson Learning, Inc., U.S.A.

Kaiser, C. 1995. Vegetative and elongation growth stages, pp. 169-175. *In* M. Pessarakli, eds. **Handbook of Plant and Crop Physiology**. Marcel Dekker, Inc. New York.

Kearsey, M. and A. Farquhar, 1998. QTL analysis in plants; where are we now?. **Heredity**. 80: 137-142.

_____ and H. Pooni. 1996. **The Genetical Analysis of Quantitative Traits**. Chapman and Hall, London.

_____ and V. Hyne. 1994. QTL analysis: a simple 'marker-regression' approach. **Theor. Appl. Genet.** 89: 698-702.

Kelly, J., P. Gepts, P. Miklas and D. Coyne. 2003. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. **Field Crop Res**. 82: 135-154.

Khattak, G., P. Srinives and D. Kim. 1995. Yield partitioning in high yielding mungbean (*Vigna radiata* (L.) Wilczek). **Kasetsart J. (Nat. Sci.)** 29: 494-497.

_____, M. Ashraf, M. Haq, T. Mcneilly and E. Rha. 2002. Genetic basis of plant height and its degree of indetermination in mungbean (*Vigna radiata* (L.)Wilczek). **Hereditas** 137: 52-56.

_____, _____, _____ and R. Zamir. 2004. Gene action for synchrony in pod maturity and indeterminate growth habit in mungbean (*Vigna radiata* (L.) Wilczek). **Pak. J. Bot.** 36: 589-594.

Knapp, J. 1998. Marker-assisted selection as a strategy for increasing the probability of selection superior genotypes. **Crop Sci**. 38: 1164-1174.

Komatsu, K., S. Okuda, M. Takahashi, R. Matsunaga and Y. Nakazawa. 2007. Quantitative trait loci mapping of pubescence density and flowering time of insect-resistant soybean (*Glycine max* L. Merr.). **Genet. Mol. Biol.** 30: 635-639.

Kosambi, D. 1944. The estimation of map distances from recombination values. **Ann. Eugen.** 12: 172-175.

Kumar, S., S. Tan, S. Quah and K. Yusoff. 2002a. Isolation of microsatellite markers in mungbean, *Vigna radiata*. **Mol. Eco. Note**. 2: 96-98.

_____,_____,_____and_____. 2002b. Isolation and characterization of seven tetranucleotide microsatellite loci in mungbean, *Vigna radiata*. **Mol. Eco. Note**. 2: 293-295.

Lander, E.S. and B.D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. **Genetics** 121: 185-199.

Li, C., C. Fatokun, B. Ubi, B. Singh and G. Scoles. 2001. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. **Crop Sci.** 41:189-197.

Liu, H.B. 1998. **Statistical Genomics: Linkage, Mapping, and QTL Analysis.** CRC Press, Washington, D.C.

Lynch, M. and B. Walsh. 1998. **Genetics and Analysis of Quantitative Traits.** Sinauer Associates, Inc., Massachusetts.

Lyon, M.F. 1990. Dunn and mouse genetic mapping. **Genetics** 125: 231-236.

Millan, T., J. Rubio, M. Iruela, K. Daly, J. I. Cubero and J. Gil. 2003. Markers associated with *Ascochyta* blight resistance in chickpea and their potential in marker-assisted selection. **Field Crop Res**. 84: 373-384.

Ogundiwin, E., G. Thottappilly, M. Aken'Ova, M. Pillay and C. Fatokun. 2005. A genetic linkage map of *Vigna vexillata*. **Plant Breed.** 124 : 392-398.

Ouédraogo, J., V. Maheshwari, D. Berner, C. St-Pierre, F. Belzile and M. Timko. 2001. Identification of AFLP markers linked to resistance of cowpea (*Vigna unguiculata* L.) to parasitism by *Striga gesnerioides*. **Theor. Appl. Genet**. 102: 1029-1036.

Phillips, R. and I. Vasil. 2001. **DNA-Based Markers in Plants.** Kluwer Academic Publishers, Dordrecht.

Pookpakdi, A., V. Promkham, C. Chuangpetchinda, S. Pongkao, C. Lairungrueng and C. Tawornsuk. 1992. Growth stage identification in mungbean (*Vigna radiata* (L.) Wilczek). **Kasetsart J. (Nat. Sci.)** 26: 75-80.

Pooprompan, P., S. Wasee, T. Toojinda, J. Abe, S. Chanprame and P. Srinives. 2006. Molecular marker analysis of days to flowering in vegetable soybean (*Glycine max* (L.) Merrill). **Kasetsart J. (Nat. Sci.)** 40: 573-581.

Promkham, V., C. Lairungrueng and C. Tawornsuk. 1988. Study on growth stages development of mungbean, pp 179-186. *In* **the proceedings of 26th Kasetsart University Annual Conference**. Kasetsart University, Bangkok.

Rajan, R., D. Wilson, K. Vijayaraghava. 2000. Correlation and path analysis in the F_2 generation of green gram (*Vigna radiata* (L.) Wilczek). **Madras Agri. J.** 87: 590-593.

Rohman, M., A. Iqbal Hussain, S. Arifin, Z. Akhter and M. Hasanuzzaman. 2003. Genetic variability, correlation and path analysis in mungbean. **Asian J. Plant Sci.** 2: 1209-1211.

Royston, P. 1992. **Approximating the Shapiro-Wilk W-test for non-normality**. Stat. Comput. 2: 117-119.

Salunkhe, D., S. Kadam and J. Chavan. 1985. **Postharvest Biotechnology of Food Legumes**. CRC Press, Florida.

Seehalak, W., P. Somta, W. Sommanas and P. Srinives. 2009. Microsatellite markers for mungbean developed from sequence database. **Mol. Eco. Res.** 9: 862-864.

Siddique M., M. Faisal, A. Malikand S. Awan. 2006. Genetic divergence, association and performance evaluation of different genotypes of mungbean (*Vigna radiata*). **Int. J. Agri. Biol**. 6: 793-795.

Soehendi, R., S. Chanprame, T. Toojinda and P. Srinives. 2006. Inheritance and AFLP tagging of leaflet mutants in mungbean (*Vigna radiata* (L.) Wilczek). **Kasetsart J. (Nat. Sci.)** 40: 566-572.

Somta, P., A. Kaga, N. Tomooka, K. Kashiwaba, T. Isemura, B. Chaitieng, P. Srinives and D. A. Vaughan. 2006. Development of an interspecific Vigna linkage map between *Vigna umbellata* (Thunb.) Ohwi & Ohashi and *V. nakashimae* (Ohwi) Ohwi & Ohashi and its use in analysis of bruchid resistance and comparative genomics. **Plant Breed**. 125: 77-84.

_____, W. Musch, B. Kongsmai, S. Chanprame, S. Nakasathein, T. Toojinda, W. Sojjapinun, W. Seehalak, S. Tragoonrung and P. Srinives. 2008. New microsatellite markers isolated from mungbean (*Vigna radiata* (L.) Wilczek). **Mol. Eco. Res.** 8: 1155-1157.

_____, W. Sommanas and P. Srinives. 2009. Molecular diversity assessment of AVRDC-The World Vegetable Center elite-parental mungbeans. **Breed. Sci.** 59: 149-157.

Sriphadet, S., J. Lambrides and P. Srinives. 2007. Inheritance of agronomic traits and their interrelationship in mungbean (*Vigna radiata* (L.) Wilczek). **J. Crop Sci. Biotech.** 10: 249-256.

_____, P. Kasemsap and P. Srinives. 2010. Effect of leaflet size and number on agronomic and physiological traits of mungbean. **J. Agric. Sci**. 148: 353-361.

Stackelberg, M., S. Lindemann, M. Menke, S. Riesselmann and H. Jacobsen. 2003. Identification of AFLP and STS markers closely linked to the *def* locus in pea. **Theor. Appl. Genet**. 106: 1293-1299.

Taji, A., P. Kumar and P. Lakshmanan. 2002. **In Vitro Plant Breeding**. Food Product Press (an Imprint of the Haworth Press), Inc., New York.

Tangphatsornruang, S., P. Somta, P. Uthaipaisanwong, J. Chanpraset, D. Sangsrakru, W. Seehalak, W. Sommanas, S. Tragoonrung and P. Srinives. 2009. Characterization of microsatellites and gene contents from genome shotgun sequences of mungbean (*Vigna radiata* (L.) Wilczek). **BMC Plant Biology** 9: 137.

Tasma, I., L. Lorenzen, D.E. Green and R. Shoemaker. 2001. Mapping genetic loci for flowering time, maturity, and photoperiod insensitivity in soybean. **Mol. Breed**. 8: 25-35.

Tesar, M. 1984. **Physiological Basis of Crop Growth and Development**. American Society of Agronomy Crop Science Society of America Madison, Wisconsin.

Thomas Jefferson Agricultural Institute. 2007. **Mungbean**. Available Source : http://www.jeffersoninstitute.org/mungbean.php#plant_description, July 27, 2007.

Turk, M., N. Celik., G. Bayram and E. Budakli. 2008. Relationships between seed yield and yield components in narbon bean (*Vicia narbonensis* L.) by path analysis. **Bangladesh J. Bot.** 37: 27-32.

Twyman, R. 1998. **Advanced Molecular Biology: A Concise Reference**. Redwood Books, Trowbridge.

van Ooijen, J. 2009. **JoinMap®3.0, software for the calculation of genetic linkage maps.** Plant Research International, Wageningen, Netherlands.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. **Nucleic Acids Res.** 23: 4407-4414.

Walter, M. and R. Rapley. 1997. **Route Maps in Gene Technology.** Blackwell Science Ltd., United Kingdom.

Wang, X., A. Kaga, N. Tomooka and D. Vaughan. 2004. The development of SSR markers by new method in plants and their application to gene flow studies in azuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi). **Theor. Appl. Genet.** 109: 352-360

Wang, S., C. Basten and Z. Zeng. 2007. **Windows QTL Cartographer 2.5.** Department of Statistics, North Carolina State University, Raleigh, NC.

Weinberger, K. 2003. **Impact Analysis on Mungbean Research in South and Southeast Asia.** Final report GTZ Eigenmassnahme no. 99.9117.5, AVRDC, Shunhua, Taiwan.

Yucel, D.O., A. E. Anlarsal and C. Yucel. 2006. Genetic variability, correlation and path analysis of yield, and yield components in chickpea (*Cicer arietinum* L.). **Turk J. Agric. For.** 30: 183-188.

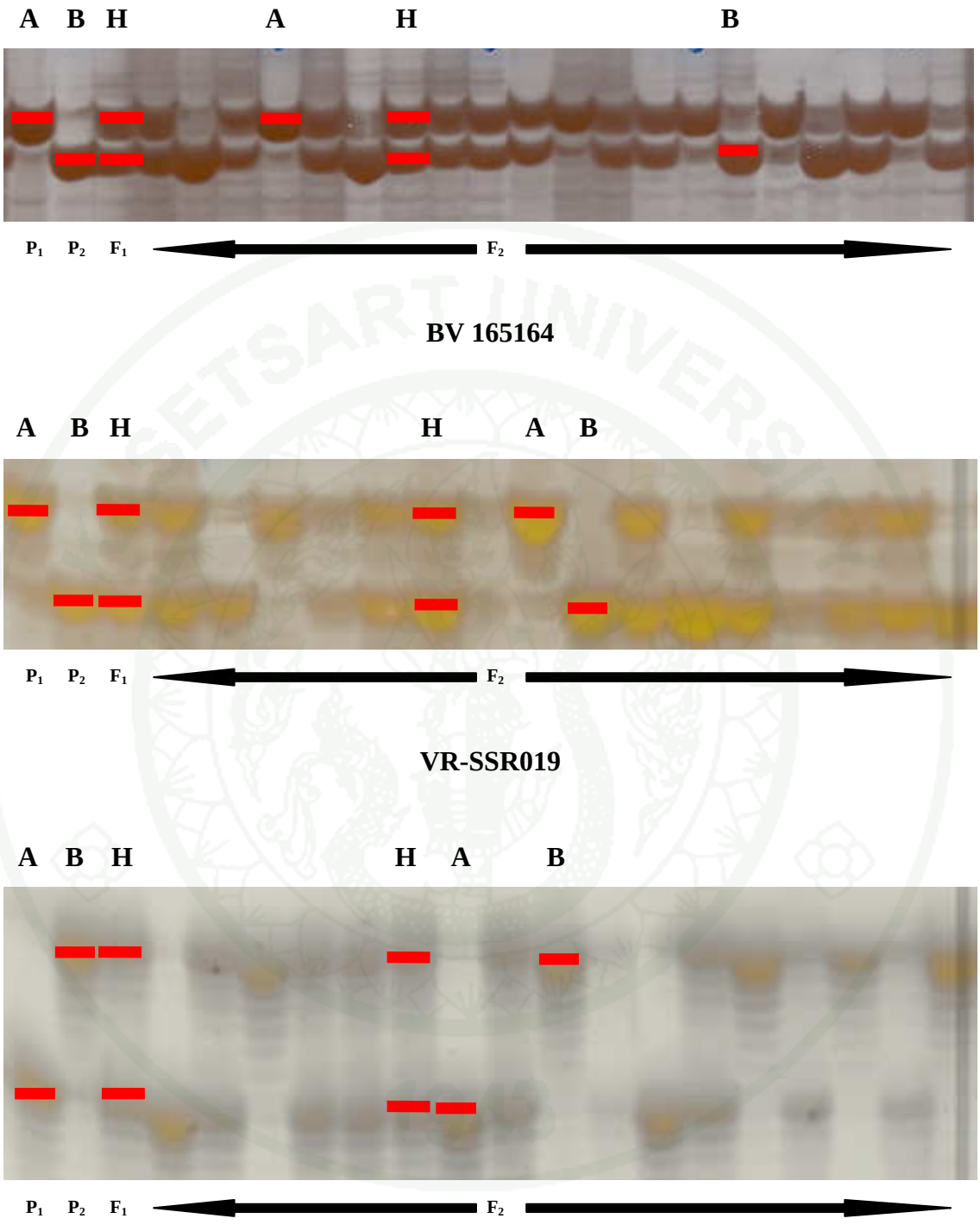
Zeng, Z-B. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. **Proc. Natl. Acad. Sci. USA** 90: 10972-10976.

_____. 1994. Precicision mapping of quantitative trait loci. **Genetics** 136:1457-1468.

APPENDIX

Appendix Figure 1 Parental mungbeans used in this study; the annual cultivated mungbean line ‘KUML29-1-3’ (right) and the wild perennial mungbean accession ‘W021’ (left)

Appendix Figure 2 F_2 plants derived from the cross between the annual cultivated mungbean line ‘KUML29-1-3’ and the wild perennial mungbean accession ‘W021’



Appendix Figure 3 Band patterns of parents, F_1 and some F_2 plants between the annual cultivated mungbean line 'KUML29-1-3' and the wild perennial mungbean accession 'W021'

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