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THESIS

GENETIC, AGRONOMIC, AND MOLECULAR STUDY OF MULTIPLE LEAFLET MUNGBEAN (*Vigna radiata* (L.) Wilczek)

RUDY SOEHENDI

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The increase in mungbean production so far comes mainly from the increase in cultivated area. A possible breakthrough for this production limitation is to exploit hybrid vigor of the F_1 for possible production of hybrid varieties. Heterosis over mid and better parent were estimated in four cross combinations involving 3 diverse mungbean genotypes, using data of plant height, number of leaves per plant, leaf area/plant, no. of pods/plant, pod length, no. of seeds/pod, 100-seed weight, and yield/plant. All crosses showed significant yield heterosis over mid-parent and better-parent. Crosses showing heterosis for grain yield also showed heterosis for pod length, number of seeds per pod, and plant height. However, only plant height expressed heterobeltiosis over the high parent. Superiority over mid parent for grain yield ranged from 52.2 to 95.7 %, and that over better parent ranged from 31.8 to 78.5 %. The highest heterosis over mid and better parent was shown in the cross S-5 x L-7.

Mungbean plants generally have a relatively close canopy, thus a large amount of self-shading can reduce yield due to poor light penetration. Modification of leaflet type can affect leaf canopy and probably alter seed yield. Two new multiple leaflet mutants were obtained from gamma-rays irradiation and used in studying its mode of inheritance, tagging with AFLP markers, and evaluating for agronomic characters related to it. The cross between 7-leaflet mutant with 5-leaflet mutant gave all F_1 plants with normal trifoliate leaflets. The F_2 plants segregated in number of leaflets per leaf and leaflet size into a 9:3:3:1 ratio of large-trifoliate: large-heptafoliate: small-pentafoliate: small-heptafoliate plants, suggesting that the genes controlling leaflet size and leaflet number are independent loci. In number of leaflets per leaf, the F_2 population can be classified into trifoliate, pentafoliate, and heptafoliate at the dihybrid ratio of 9:3:4. The gene symbols N_1n_1 and N_2n_2 were proposed for leaflet number, and *Ss* for leaflet size. There are 3 AFLP markers linked to number of leaflets per leaf and all of them corresponded to n_1 locus only. The effect of multifoliate leaflet on yield and yield components were evaluated in 4 mungbean families each with 4 leaflet isolines under 3 environments. Averaging across the families and environments, the normal trifoliate and large heptafoliate lines gave higher yield than small pentafoliate and heptafoliate lines. These two large leaflet lines also had higher leaf area per plant than the other multifoliate lines. It was clear that the mungbean lines with greater leaf area, which was likely to intercept more sun light, gave greater yield.

R Soehendi
Student's signature

Peerasak Srinives
Thesis Advisor's signature

20 / March / 2006

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March 2006

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INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is a widely-grown, short-duration grain legume crop in South and Southeast Asia. It is an important source of inexpensive protein in most Asian diets and a significant component of various cropping systems. However, the average yields in the farmers' fields are still low, ranging between 500 to 600 kg/ha. One reason is due to the use of traditional cultivars and low management inputs by most farmers.

Current mungbean cultivars are all pure lines. One way to increase the yield is to utilize the phenomenon of heterosis or hybrid vigor manifested in F_1 hybrids. The hybrid is a plant type resulting from the fusion of dissimilar gametes or those having heterozygous gene pairs for a particular character. Hybrid varieties have contributed greatly worldwide to the production of many crop species, including the most important food crops such as maize and rice. The commercial exploitation of heterosis has been one of the driving forces behind the rapid and extensive development of seed production. Heterosis breeding has allowed yield breakthrough in several crops, including cross-pollinated, often cross-pollinated, and self-pollinated species. The exploitation of heterosis to raise productivity in grain legumes, as in any other crops, depends on three major factors: the magnitude of heterosis; feasibility of large-scale production of hybrid seeds; and type of gene action involved. Heterosis may take the form of an increase in yield, size, number of plant parts, chemical components, and disease resistance.

Mungbean plant generally has a relatively close canopy compare to the other grain legume species. The large amount of self-shading can reduce seed yield due to poor light penetration. Leaflet type is a canopy characteristic related to light interception. Thus modification of leaflet can influence the plant canopy and probably alter seed yield. A new mungbean variety 'Samgang' was released in Korea

with lobed leaflets that can intercept more sun light than the control variety 'Seonhwanogdu' (Lee *et al*., 2004). Wells *et al*., (1993) stated that greater photosynthesis per unit leaf area was related to a more uniform distribution of light in the canopy or a greater proportion of leaves actually involved in photosynthesis. Hicks *et al*. (1969), and Sung and Chen (1989) also reported greater light penetration to lower canopy strata in narrow leaflet canopies of soybean. However, only a few studies have focused on leaf architecture influencing photosynthesis in mungbean.

The genetic variability in mungbean species is considered low as compared to the other crops. Thus induced mutation can produce a useful complementary genetic resource for this crop. Wongpiyasatid *et al.* (1999) tested mungbean mutant lines showing potential for development into new varieties, viz. M5-10 and M5-25 for resistance to powdery mildew; M5-22 and M5-25 for resistance to Cercospora leaf spot; M5-16 and M5-29 for resistance to cowpea weevil, and M4-2, M5-1, M5-5, M5-15 and M5-28 for high yielding ability. In addition, Sandhu and Saxena (2003) reported high variability found in 34 mungbean mutants for yield per plant and nutritional quality traits such as the contents of protein, methionine, tryptophan, sulfur, phenol, and total sugars.

Multiple leaflet mutants express a potential in altering mungbean yield and thus worth a more detail investigation on inheritance of the trait, gene tagging using molecular markers, and studying on the effect of the trait on yield and yield components. The use of certain mutants can help breaking the yield limit encountered in the available mungbean germplasm. Dwivedi and Singh (1985) reported that narrow leaf character in mungbean is governed by two recessive genes symbolised by *nl1* and *nl2*. Whereas Bhadra (1991) reported that a nine-foliate leaflet character was monogenic recessive to normal trifoliate leaf. He proposed the symbols *tf* and *Tf* for the genes regulating these two characters.

Molecular markers which are free from environment effect can be used to tag genes controlling traits of interest and to form into a partial linkage group. This is particularly useful as a starting point in constructing a more informative molecular

linkage group for mungbean crop that molecular marker technology is at the beginning stage. AFLP marker is chosen in this study because of its excellent reproducibility, which is essential if screening protocols are to be established (Jones *et al*., 1998 and Matthes *et al*., 1998). AFLP screens high number of loci for polymorphism and simultaneously detects a greater number of DNA markers than any other polymerase chain reaction based detection system (Vos *et al*., 1995). Linkage map has recently been developed in some crops including genus *Vigna* (Tomooka *et al*., 2002; Somta *et al*., 2006).

OBJECTIVES

The objectives of this study are:

1) to study on heterosis and heterobeltiosis indicating the increase of the F_1 over mid- and better- parents from the cross between multifoliate leaflet mutants.

2) to study on the inheritance of multifoliate leaflet mutants in mungbean.

3) to identify AFLP markers associated with the multifoliate leaflet character.

4) to study the effect of multifoliate leaflet character on yield and its components.

LITERATURE REVIEW

Mungbean utilization and classification

Mungbean is used in several food products, both as whole seed and in processed form. In India and Pakistan, it is consumed as *dhal*; cooked into curries; sweet and salty soups; boiled and toasted with onion, chili and salt; in sweet and salt *pongal* (rice preparation); and patties and sweets of different kinds (Thirumaran and Seralathan, 1988; Singh *et al*., 1988). In Thailand, mungbean is used for making a wide range of Thai foods, viz. desserts, snacks, noodles, and bean sprouts (Prabhavat, 1988).

Like most legumes, mungbean is rich in starch and protein. Mungbean sprout is high in protein (21%–28%), calcium, phosphorus, amino acid lysine, and several vitamins (Lawn and Russell, 1978; Oplinger *et al.*, 2005). The amino acid profile of mungbeans, similar to other beans, is complementary to cereal grains. They are easily digested, and thus can replace scarce animal protein in human diets in tropical areas of the world.

Mungbean, *Vigna radiata* (L.) Wilczek (2n = 2x = 22), is a short duration grain legume (Lawn and Ahn, 1985; Siemonsma and Na Lampang, 1989). It is also known as green gram or golden gram. Current taxonomy places mungbean in the family Fabaceae (synonym: Leguminoseae), subfamily Papilionoideae, tribe Phaseoleae and in the genus *Vigna Savi* (Lawn and Russell, 1978), although it was formerly assigned to the genus *Phaseolus* L. Studies by Verdcourt (1970) and others led to the extensive reorganization of the *Vigna* spp., which now places mungbean in the subgenus *Ceratotropis* (Piper) Verdc., with several other Asiatic *Vigna* species including adzuki bean (*Vigna angularis* Willd.), black gram (*Vigna mungo* (L.) Hepper), and rice bean (*Vigna umbellata* Thunb.). *V. radiata* has been further divided into three subspecies: subsp. *radiata,* which includes the green grams and cultivated mungbean, subsp. *sublobata,* a wild form with lobed leaves, and subsp. *glabra* (Verdcourt, 1970; Lawn and Russell, 1978).

Heterosis and hybrid varieties

Heterosis or hybrid vigor is manifested by improved performance of F_1 hybrids generated from crossing two inbred or pure line parents. Heterosis is expressed based on the criteria used to compare the performance of a hybrid. The term heterosis is used to verify the performance of F_1 progeny over mid-parent, or a standard variety. However, the term heterobeltiosis was coined when the hybrid is compared with its better parent (Fanseco and Peterson, 1968). High parent heterosis is preferred in some circumtances particularly in self-pollinated crops, for which the goal is to find a better hybrid than both of the parents.

Heterosis breeding has allowed yield breakthrough in several crops, including cross-pollinated, often cross-pollinated, and self-pollinated. Basically, the exploitation of heterosis to raise productivity in grain legumes, as in any other crops, depends on three major factors, viz. magnitude of heterosis, feasibility of large scale production of hybrid seeds, and type of gene action involved. Heterosis may take the form of an increase in yield, size, number of plant parts, chemical components, disease resistance, etc. The hybrid is a plant type resulting from fusion of dissimilar gametes or those having heterozygous gene pairs for a particular character. Utilization of heterosis has become a major strategy for increasing productivity of plants. Hybrid varieties have contributed greatly worldwide to the production of many crop species, including the most important food crops such as maize and rice. The commercial exploitation of heterosis has been one of the driving forces behind a rapid and extensive development of seed production.

The phenomena of heterosis are important in evolutionary and applied genetics. Increased fitness or yield of heteroygotes contributes to high yield in many crop species and maintain genetic variation in natural populations. Despite their importance, the factors causing hybrid breakdown are unknown at the genetic and molecular level (Mitchell-Olds, 1995). Heterosis is a widely documented phenomenon in diploid organisms that undergo sexual reproduction. It was first observed in animals more than 1400 years ago and later in plants from the

experiments of hybridization in the 19th century (Xiao *et al*., 1995). However, the underlying genetic basis for the phenomenon has not been satisfactorily explained (Stuber *et al*., 1992). The genetic basis of heterosis has been debated for more than 80 years and is still not resolved (Xiao *et al*.,1995).

There has also been considerable interest in the genetic basis of heterosis. Two hypotheses, the dominance and the overdominance, were proposed early last century to explain these phenomena (Crow, 1999). The genetic causes of heterosis are not completely understood, but possible explanation have been around dominance and overdominance gene action.

A) The dominance theory

The theory was proposed by Davenport in 1908 that heterosis is due to cancelling of deleterious recessives contributed by one parent, by dominant alleles contributed by the other parent in the heterozygous F_1 (Xiao *et al.,* 1995 and Crow, 1999). Dominance (masking of deleterious recessives) causes heterosis due to the fact that inbred lines become fixed for recessive or partially recessive deleterious alleles. Thus, crosses beween such inbred lines, fixed for deleterious alleles at different loci, produce genotypes which are superior to the parents because the phenotype of the dominant nondeleterious alleles is seen in the F_1 (Jonhson and Hutchinson, 1993). For example, if dominant alleles generally contribute to vigor and recessive alleles tend to be neutral, harmful or deleterious, then crossing 2 inbred parents that carry different dominant alleles will lead to an F_1 that is more vigorous than either parent.

B) The overdominance theory

Another possible explanation is the accumulation of heterozygous in the F_1 progeny. It was originally thought to be the favorable expression of heterozygosity. The hypothesis was proposed independently by Shull and East in 1908, assuming that the heterozygous combination of the alleles at a single locus is superior to either of the homozygous combination of the alleles at that locus (Johnson and Hutchinson,

1993, Xiao *et al*., 1995, and Crow, 1999). One of the most popular explanation has been that of favorable enzymatic production associated with heterozygous alleles. That is a hybrid enzyme system driving the plant mechanism is produced by heterozygous allelic form.

Heterosis plays a dominant role in accelerating the agricultural production and heterosis breeding opens up tremendous potential among the crops for quantitative trait improvement. The magnitude of heterosis provides basis for determining genetic diversity and serves as a guide to the choice of desirable parents (Swindell and Poehlman, 1976). The presence of heterosis in mungbean have been proved and demonstrated by many researchers (Shinde and Deshmukh, 1989). At present, heterosis breeding is being extended from cross-pollinated to self-pollinated crops like rice and wheat. However, there has been no attempt to produce hybrid varieties in legumes so far.

Inheritance of leaflet characteristics in mungbean

Veeraswamy and Kunjamma (1958) described a mutant with all leaves having four or five leaflets, instead of the normal three and found that mutant trait is governed by single dominant gene. Santos (1969) induced unifoliate and multifoliate leaf mutants which both behaved as monogenic recessives. Singh *et al.* (1981) described that a pentafoliate was monogenic reccessive. Chhabra (1990) observed that trifoliate (normal) trait was monogenically dominant over pentafoliate. AVRDC (1987) and Satyanarayana *et al.* (1989) also reported that multiple leaflet mutant also behaved as monogenic recessive to normal trifoliate leaves.

Singh and Saxena (1959) described a crinkled lanceolate leaf mutation. They showed that the trait is conditioned by a single major factor that they designated *N* and further reported that the gene is semidominant and lethal when it is in heterozygous condition. Since the symbol *N* was latert used by Frey (1980). The allele governing crinkled lanceolate leaf was proposed that this gene be redesignated as *La*.

Sripisut and Srinives (1986) reported that lobed and trifoliate leaflets were dominant to normal and multiple leaflets. Each trait was governed by a single locus of gene on different chromosomes.

Bhadra (1991) studied the inheritance of nine-foliate leaf in mungbean using F_1, F_2, BC_1, BC_2 and F_3 generations of a cross involving a normal trifoliate and a nine-foliate cultivars. The nine-foliate leaf was monogenically recessive to normal trifoliate leaf. The symbol *tf* and *Tf* were proposed respectively for the genes controlling these two characters.

The role of leaf in photosynthesis

Crop productivity and yield depend on many physiological processes and environmental factors, which photosynthesis is the most prominent one. Photosynthesis contributes about 90% of total dry matter accumulation. As a result, crop production aims to maximise photosynthesis. A plant must possess an efficient photosynthetic mechanism to be highly productive biologically.

Mungbean yield bears a close relationship with the duration and rate of photosynthesis which is dependent on light intensity (Kuo *et al*., 1978). Leaf photosynthesis can be influenced by many factors such as leaf age, leaf position, and mutual shading, as well as environmental factors such as light, temperature, nutrition, and water availability (Constable and Rawson, 1980; Lieth and Pasian, 1990). Leaf position and age also influence leaf area, gas exchange, leaf conductance, and saturated net photosynthetic rate (Constable and Rawson, 1980). Leaves are the main site of transpiration, which provides most of the energy necessary to draw water and minerals up from the roots of the plant. In addition, carbon dioxide for photosynthesis and oxygen for respiration are usually exchanged through the leaves. An erect leaf canopy could theoretically increase crop assimilation rate, especially under high-radiation environment (Duncan, 1971; Sakamoto and Matsuoka, 2004). Most of the highest yielding cultivars of maize, rice and wheat already have erect leaf canopies. It has been shown that leaf metabolism can adapt to different light

intensities according to the position in the canopy. The upper leaves in a canopy may show elevated photo-protective responses. Photosynthesis also varies from time to time during periods of a day depending on the dynamics of light intensity, air temperature and relative humidity.

Leaf area and dry matter accumulation in blackgram were studied by Biswas *et al.* (2001). They found that leaf area increased slowly during pre-flowering but rapid increase during post-flowering stage. The highest leaf area was recorded in pod filling stage and decreased sharply thereafter due to senescence of leaf. Similar trend of leaf area in blackgram also reported by Rahman *et al*. (1994). A positive correlation was found between photosynthesis and leaf area during flowering (r = 0.78) and pod filling (r = 0.97) stages. Similar results also reported by Mahon and Hobbs (1987). Blackgram showed rapid increase of dry matter during post-flowering than pre-flowering stage and the highest accumulation of dry matter was recorded at harvest. It showed significant correlation between leaf area and total dry matter accumulation at vegetative (r = 0.97), flowering (r = 0.96) and pod filling stages (r = 0.97). Total dry matter accumulation also strongly correlated with leaf photosynthesis both at flowering (r = 0.93) and pod filling (r = 0.91) stages of blackgram. Similar result in peas was also reported by Mahon (1982).

Genetic and molecular markers

Genetic marker is a gene with known location on chromosome and clear-cut phenotype used as a point of reference (King and Stansfield, 1985). Liu (1998) stated that a genetic marker has to be a polymorphic marker. However, the inverse is not true, i.e., a polymorphic marker may not be a genetic marker. A genetic marker may be operationally defined as a heritable polymorphic marker with clear genetic interpretation and repeatability. Genomic analysis using genetic markers should be based on well established genetic models. If the underlying genetics of a marker is not clear, then the analysis may be misleading. It is also important that the marker assay is repeatable at different times in the same or different laboratories. Different types of markers may identify different polymorphisms. The genetic interpretation of a marker

strongly depends on the sequence complexity of the genome and the kind of variation the marker identifies.

Three types of genetic markers have been used in genomic analysis: morphological markers (e.g. height, color, response to pathogens), protein based markers (e.g. isozymes), and DNA based markers (Liu, 1998). Phenotypic markers depend on expression of genes and are limited to those genes expressed at a particular time or under particular developmental or environmental conditions, whereas DNA-based markers provide an almost unlimited supply of markers that identify specific sequences across the genome (Mohan *et al*., 1997).

The advantages of DNA markers are: (a) single base changes in DNA can be identified, providing many potential marker sites across a genome, (b) they are independent of developmental stage, environment, or expression, (c) markers can be found in non-coding or repetitive sequences, and (d) most DNA marker sequences are selectively neutral (Jones, 2000). Thus, for example, because about 80 % of the wheat genome is non-coding DNA, only molecular marker can be used to identify polymorphisms and to map 'loci' in these regions of the genome. There are two major ways to utilize DNA markers: 1) as genetic markers for mapping and/or tagging traits of interest , and 2) as indicators of genetic diversity (Karp and Edwards, 1998).

Amplified Fragment Length Polymorphism (AFLP) marker technology

The amplified fragment length polymorphism (AFLP) is a marker used for genotyping individuals for a large number of loci using a minimal number of PCR reactions. The AFLP technique is based on detection of genomic restriction fragments by PCR amplification and can be used for DNA of any origin or complexity (Vos *et al*., 1995).

There are three key steps to the method (Vos and Kuiper, 1998).

1. DNA is cut with restriction enzymes and then linkers are ligated on. Typically, this involves a combination of two restriction enzymes: a 4 base cutter (MseI) and a 6 base cutter (EcoRI).

2. Pre-selective PCR is performed using primers which match the linkers. These primers have a two base overhang.

3. Selective PCR is performed using primers with three base overhangs. For any given pre-selective amplification, there are 16 possible selective primer combinations that can be used. PCR products are then analyzed by gel electrophoresis.

AFLP is based on PCR amplification of restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of few nucleotide bases (Vos *et al*., 1995). This technique generates a large number of restriction fragment bands facilitating the detection of polymorphisms. The number of DNA fragments which are amplified can be monitored by choosing the different base number and composition of nucleotides in adapters. Although not many maps have been developed so far using AFLP markers, this method is now widely used for developing polymorphic markers. Linkage map using AFLP has recently been developed in some crops as in barley (Becker *et al*., 1995) and genus *Vigna* (Tomooka *et al*., 2002). This approach is very useful in saturation mapping and for discrimination between varieties. Lin *et al*. (1996) compared three different DNA mapping techniques, viz. RFLP, RAPD and AFLP and found that AFLP is the most efficient technique in detecting polymorphism in soybean. High reproducibility, rapid generation, and high frequency of identifiable AFLP polymorphisms make AFLP DNA analysis an attractive technique for identifying polymorphisms and for determining linkages by analysing individuals from a segregating population. However, AFLPs are still expensive to generate as the bands are detected by silver staining, fluorescent dye or radioactivity.

AFLP fingerprints are increasingly used as a source for DNA markers since the molecular basis for AFLP polymorphisms occurs at the nucleotide level. Single nucleotide changes can be detected by AFLP when either restriction sites themselves or nucleotide adjacent to the restriction sites are affected, causing the AFLP primers to mispair at the 3' end and preventing amplification (the selective nucleotides do not exactly match the sequence next to the restriction site). In addition, deletion, insertion, and rearrangement affecting the presence or size of restriction fragments will lead to polymorphisms detected by AFLP. Most AFLP markers are mono-allelic markers, and thus the corresponding allele is not detected. A low frequency of bi-allelic markers is due to small insertion or deletion in the restriction fragments. The frequencies with which AFLP markers are identified are similar to what is found with RFLP and arbitrarily-primed PCR-based DNA marker techniques. The advantage of AFLP over these techniques lies in the high marker densities which can be obtained with AFLP with modest efforts. Most AFLP markers inherit in a Mendelian way, indicating that they are unique DNA fragments (Vos and Kuiper, 1998).

AFLP marker can be integrated with any type of genetic and physical DNA markers, which allows the integration of genetic and physical maps. This makes AFLP a powerful technology in genome research.

Mapping genes of interest

One of the most important applications of genetic maps is to locate specific genes of interest, such as those controlling traits of economic importance in plants and animals. These traits can be controlled by single genes or a number of genes. When more than one gene is involved in a quantitatively varying phenotype, the loci are commonly described as quantitative trait loci (QTL) and the mapping procedure is called QTL mapping.

Mapping and sequencing of plant genomes would help to elucidate gene function, gene regulation and expression. High resolution linkage maps are being developed in many crop plants. Molecular markers are used to identify and tag desired

genes. Linkage analysis is one of the basic and indispensable methods in genetics study. Linkage can define the genetic distances between polymorphic traits which may be recognized as difference in appearance of enzyme activity, restriction fragment length, or nucleotide sequence at an allelic locus. Linkage maps based on morphological and isozyme markers have been constructed for rice, maize, wheat, barley and many other cultivated plants including genus *Vigna* (Tomooka *et al*., 2002).

Polymorphism in the nucleotide sequence is usually sufficient to function as a molecular marker in mapping. This polymorphism is revealed by molecular techniques such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), microsatellite or simple sequence repeat polymorphism (SSRP), random amplified polymorphic DNA (RAPD), cleavable amplified polymorphic sequences (CAPS), and single-strand conformation polymorphism (SSCP). Genetic maps have been constructed in many crop plants using these markers on a single segregating population (Mohan *et al*., 1997).

Progress has been made in mapping and tagging many agricultural important genes with molecular markers which forms the foundation for marker-assisted selection (MAS) in crop plants. Molecular tags, a prerequisite for MAS, have been developed for many crop plants using different kinds of molecular markers. MAS offers a form of genotypic selection with some advantages over the traditional phenotypic markers that were previously available to plant breeders. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait. This, of course, would require a molecular marker to be tightly linked to the trait of interest. Besides, these markers are not environmentally regulated and are, therefore, unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth.

Applications of molecular markers in *Vigna* sp.

Genetic engineering and biotechnology hold great potential for plant breeding as it promises to expedite the time taken to produce crop varieties with desirable characters. With the use of molecular techniques it would now be possible to hasten the transfer of desirable genes among varieties and to introgress novel genes from related wild species. Polygenic characters which were previously very difficult to analyse using traditional plant breeding methods, would now be easily tagged using molecular markers.

Genome maps are valuable in providing insights into genome organization, inheritance and linkage of traits. Much of the emphasis on understanding genome organization is a tool in plant breeding to enable genes to transferred to elite breeding lines. Several important traits have been mapped on the mungbean genome. Fuji and Miyazaki (1987) and Fuji *et al*. (1989) found bruchid resistance in wild mungbean by using RFLP analysis and it was first mapped as a single major locus on linkage group VIII (Young *et al*., 1992). The nearest RFLP marker was 3.6 cM distance from this locus. Since this resistance gene from *V. radiata* var. *sublobata* (TC1966) also has an inhibitory activity against stink bug (*Riptortus clavatus* Thunberg) and yet associated with novel cyclopeptide alkaloids, further efforts were made to map this gene (Kaga and Ishimoto, 1998). The resulting genetic map reveled that the resistant dominant locus was located within 0.2 cM from the nearest RFLP markers. This map distance may enable the gene to be cloned within a genomic library for eventual introduction into susceptible mungbean lines or other crops.

Molecular markers associated with quantitative trait loci (QTL) have been reported in many crops and many important traits (El Attari *et al*., 1998; Maughan *et al*., 1996, and Saghai-Maroof *et al*., 1996). Several important traits such as resistance to bruchid beetle and powdery mildew have been mapped on the mungbean genome (Tomooka *et al*., 2002). After a linkage between a QTL and molecular markers has been determined, the QTL can be transferred into different genetic backgrounds by marker-assisted selection.

Young *et al*. (1993) identified 3 QTL of powdery mildew resistance on three different linkage groups that accounted for 58% of the total variation. In addition, Chaiteng *et al*. (2002) reported using a different powdery mildew line that the total of 96 RFLP probes failed to identify any QTLs associated with the resistance. Subsequently, 100 AFLP primer pair combinations were tested and 4 out of more than 5,000 polymorphic bands were found to be associated with the resistance. A major QTL was found on a new linkage group and accounted for 68% of the total variation.

Kaga *et al*. (2000) developed a genetic linkage map from 86 F2 plants derived from an interspecific cross between azuki bean (*Vigna angularis*, 2n=2x=22) and rice bean (*V. umbellata*, 2n=2x=22). It comprises one phenotypic, 114 RFLP, and 74 RAPD markers organized into 14 linkage groups, each contains at least 5 markers. The map covers a total distance of 1702 cM with an average distance of 9.7 cM between markers. The azuki-rice bean linkage map was compared with other available maps of *Vigna* species in subgenus *Ceratotropis*. Based on the lineage of the common loci, there were conserved blocks distributed in most of the linkage groups. This map may facilitate gene tagging, QTL mapping, and further useful gene transfer for azuki bean breeding.

Menancio-Hautea *et al*. (1992) constructed mungbean linkage map from RFLP markers using both homologous (mungbean) and heterologous (cowpea, soybean, and bean) clones and a mapping population derived from the intersubspecific hybrid between *V. radiata* ssp. *radiata* and *V. radiata* ssp. *sublobata*. The map now consists of 171 markers and several important traits have been assigned, including seed size and resistance to powdery mildew.

Lambrides *et al*. (2000) reported two genetic linkage maps of mungbean derived from the cross Berken x ACC 41. The F_2 map constructed from 67 individuals consisted of 110 markers (52 RFLPs and 56 RAPDs) that grouped into 12 linkage groups. The linked markers spanned a total map distance of 758.3 cM. The recombinant inbred population map, composed entirely of RAPD markers, consisted

of 115 markers in 12 linkage groups. The linked markers spanned a total map distance of 691.7 cM.

MATERIALS AND METHODS

1. Study on heterosis and heterobeltiosis

1.1. Plant materials

Three mungbean genotypes, viz. 5 small-multiple leaflet (S-5), 7 large-multiple leaflet (L-7), and normal-trifoliate (N) were crossed in four combinations, including reciprocals, during June to August 2002 at Kasetsart University - Kamphaeng Saen Campus (KU-KPS), Nakhon Pathom Province, Thailand. The S-5 parent is a new mutant line obtained from gamma-rays irradiation of F_2 seed from a cross between the cultivated 'Chai Nat 36' with wild mungbean 'TC 1966' (Srinives *et al*., 2000). The L-7 parent is a BC_9 progeny having the cultivar 'Kamphaeng Saen 1' as the recurrent parent and the large-multiple leaflet mutant (V5926) from the Asian Vegetable Research and Development Center (AVRDC), Taiwan as the donor (Kowsurat *et al*., 1999). The N parent, VC6468-11-1B, is an advanced breeding line carrying powdery mildew resistant genes. It derived from crossing between VC 6040A and VC 6209-1 at the Asian Regional Center of the Asian Vegetable Research and Development Center (AVRDC), located in KU-KPS. To minimize the environmental effect under growing condition, the parents and F_1's were sown with 2 plants per pot in 10 inch pots filled with mixed potted soil. Each genotype consisted of 20 plants. All the optimum recommended practices for mungbean growth was applied (Park, 1978). At 50 days after sowing, the leaf area was measured using leaf area meter model LI-3100 Licor, Inc., Lincoln, Nebraska, USA. At harvesting, ten plants were randomly measured for plant height (cm), no. of leaves per plant, leaf area per plant (cm^2), no. of pods per plant, pod length (cm), no. of seeds per pod, 100-seed weight (g), and yield per plant (g).

1.2. Significant testing of heterosis and heterobeltiosis

For each F_1 cross, percent heterosis (%H) and heterobeltiosis (%Hb) for a particular trait were calculated as followed.

$$\%H = \left(\bar{F}_1 - \overline{MP}\right) x\ 100 / \overline{MP}$$

$$\%Hb = \left(\bar{F}_1 - \bar{P}_i\right) x\ 100 / \bar{P}_i$$

Where $\bar{F}_1$ = mean observation of the F_1 progenies from the total of n_1 plants

$\overline{MP}$ = mean observation of both parents from $n_2 + n_3$ plants

$\bar{P}_i$ = mean observation of the i^{th} parent from n_2 plants for P_1, and n_3 plants for P_2

Significance of H and Hb were determined by a t-test as followed.

$$\text{t-test for H} = \frac{\bar{F}_1 - \overline{MP}}{S_H}$$

$$\text{t-test for Hb} = \frac{\bar{F}_1 - \bar{P}_i}{S_{Hb}}$$

Where S_H and S_{Hb} are the standard error of estimates of H and Hb which can be derived as followed:

$$H = \bar{F}_1 - \frac{\left(\bar{P}_1 + \bar{P}_2\right)}{2}$$

$$= \bar{F}_1 - \frac{\bar{P}_1}{2} - \frac{\bar{P}_2}{2}$$

Using the property of expectation (Steel and Torrie, 1980; Chapter 5, topic 5.10) then,

$$\text{Variance of H} = \text{Var}\left(\bar{F}_1 - \frac{\bar{P}_1}{2} - \frac{\bar{P}_2}{2}\right)$$

$$= V\bar{F}_1 + \frac{V\bar{P}_1}{4} + \frac{V\bar{P}_2}{4}$$

(assuming no covariation between generations)

$$= \frac{VF_1}{n_1} + \frac{VP_1}{4n_2} + \frac{VP_2}{4n_3}$$

$$= \frac{SSF_1}{n_1(n_1 - 1)} + \frac{SSP_1}{4n_2(n_2 - 1)} + \frac{SSP_2}{4n_3(n_3 - 1)}$$

Where $V\bar{F}_1, V\bar{P}_1,$ and $V\bar{P}_2$ are the variances of the mean of each generation; $VF_1, VP_1, VP_2, SSF_1, SSP_1$ and SSP_2 are variances and sums of squares of the specified generations, respectively.

Then, the standard error of estimate of H (or S_H) = $\sqrt{\text{variance of H}}$

In the same manner, variance of Hb can be obtained from

Variance of Hb $= \text{Var}(\bar{F}_1 - \bar{P}_i)$

$$= \frac{VF_1}{n_1} + \frac{VP_i}{n_i}$$

$$= \frac{SSF_1}{n_1(n_1 - 1)} + \frac{SSP_i}{n_i(n_i - 1)}$$

and $S_{Hb} = \sqrt{\text{variance of Hb}}$

The degree of freedom (*df*) for each test was obtained by summing up the *df* of each generation participating in the estimate. Thus the *df* for testing H is $(n_1-1)+(n_2-1)+(n_3-1)$, and the *df* for testing Hb is $(n_1-1)+(n_i-1)$, i = 2 or 3, depending on whether the high parent is P_1 or P_2.

2. Inheritance of multifoliate leaflets

2.1. Plant materials

A cross was made between two parental lines L-7 and S-5 (same genotypes which were used for heterosis study) during early rainy season 2002 at Kasetsart University, Kamphaeng Saen Campus. The S-5 was used as the paternal plant since it has purple hypocotyl which is a dominant character for identifying the true F_1 hybrid from crossing with the green hypocotyl L-7, used as the maternal plant. The F_1 seeds were planted and harvested separately and four F_1 plants with the highest number of F_2 seeds were grown in the field to form F_2 families. Field management of the trials followed the optimum recommended practices advocated by Park (1978). The F_2 plants were counted based on leaflet number classified into trifoliate, pentafoliate, and heptafoliate plants, and based on leaflet size classified into large and small leaflet.

2.2. Genetic analysis

Number of F_2 plants in each leaflet class was tested against the 3:1 Mendelian ratio following the Chi-square (χ^2) goodness-of-fit test as sugested by Mather (1951). Chi-square test was also performed against 9:3:3:1 ratio for independent both traits. Since all the F_1 plants derived from L-7 and S-5 are normal trifoliate leaf, the hypothesis is that number of leaflets is controlled by 2 loci which can be tentatively assigned as $N_1n_1N_2n_2$ segregating for leaflet number (3 and 5 vs 7), while for the leaflet size (large vs small) is assigned as Ss, with the genetic model as shown in Fig. 1. In this model 9/16 of the F_2 plants are normal trifoliate with the genotype $N_1\_N_2\_$, 3/16 are pentafoliate with the genotype $N_1\_n_2n_2$, 3/16 are heptafoliate ($n_1n_1N_2\_$), and 1/16 are heptafoliate ($n_1n_1n_2n_2$).

The formula for calculating Chi-square in this test was as follows:

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where O = observed number of multifoliate leaflets type

E = expected number of multifoliate leaflets type, and

i = number of classes

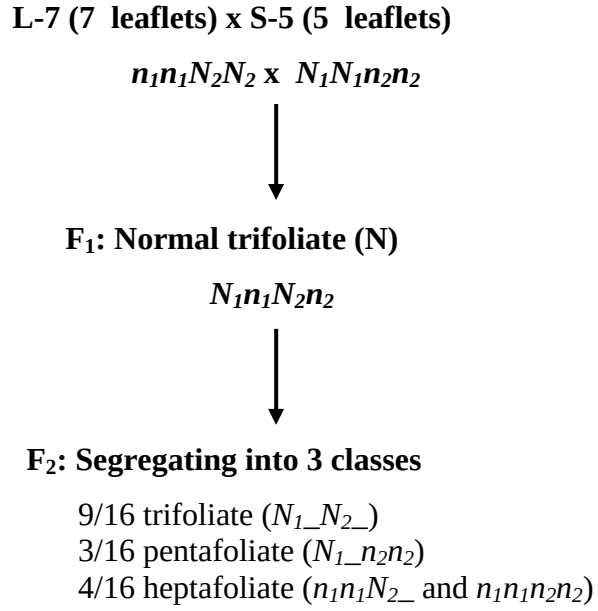

Figure 1 . The proposed 2-locus genetic model for number of leaflets type in L-7 x S-5 mungbean cross.

3. AFLP markers associated with leaflet and agronomic characters

3.1. Plant materials

Two parental lines, L-7 and S-5, and 4 isogenic lines from each of the 4 families as described in Figure 2 were grown in a crossing block at Kasetsart University, Kamphaeng Saen Campus (KU-KPS) during early rainy season 2005. Young expanded leaves from 3 plants each of the 16 mungbean lines and their parents were collected for DNA extraction.

3.2. DNA preparation

The plant samples were extracted using the protocol of the Center for Agricultural Biotechnology (CAB) laboratory, Kasetsart University, Kamphaeng Saen Campus. The procedures were modified from the CTAB method of Doyle and Doyle (1987) as follows:

1. Pre-chill the mortar and pestle in a –20^{o}C freezer before use, put 3 grams of leaves and pour liquid nitrogen into it, crush the leaf tissue and then grind to powder.

2. Add 500-700 μl of pre-warmed (65^{o}C) Plant Extraction Buffer to tube, mix thoroughly by vigorous shaking, and incubate in 65^{o}C water bath for 10-15 minutes, occasionally shake tubes during incubation.

3. Add 300 μl 5 M KAc to tubes, mix thoroughly and incubate on ice for 30-60 minutes.

4. Centrifuge the tube at 13,000 rpm at 4^{o}C for 30 minutes, collect supernatant into a new tube.

5. Add chloroform: isoamyl 24:1 (1 volume), mix by slowly inverting the tubes several times and allow the tube to rest undisturbed for 5 minutes, slowly shake with shaker for 5 minutes.

6. Spin down at 13,000 rpm for 10 minutes, collect supernatant into a new tube.

7. Add equal volume of absolute ethanol, mix and chill in refrigerator for 10 minutes, the DNA become aggregate.

8. Centrifuge for 5 minutes and pour off the supernatant.

9. Wash the DNA with 500-600 μl of 70 % cold ethanol, centrifuge for 5 minutes, pour of the supernatant. This step was done twice.

10. Dry the pellet DNA for 2 hours at 37^{o}C in incubator.

11. Add 50 μl TE into tube, incubate for several hours at 37^{o}C, store at -20^{o}C until use.

3.3. AFLP marker analysis

The AFLP analysis involved restriction digestion of genomic DNA, ligation of adapter sequences, pre-amplification, and selective amplification, as described by Vos *et al*. (1995) with modifications. The selective primer was screened to determine the high polymorphic combinations before performing AFLP analysis of the 16 isogenic lines.

Two hundred nanograms of genomic DNA from each mungbean line was digested and ligated simultaneously with 5 units of *Eco*RI (a rare 6-base cutter) and *Mse*I (a rare 4-base cutter) (Fermentaz Life Sciences), 3 μl of buffer A (1X final concentration), 5 ρmol of EcoRI and 50 ρmol of MseI adapters, and 1 unit of T4

DNA ligase. The reaction was completed to 30 µl using distilled water. The reaction was incubated over night at 37 °C.

Preamplification (PCR I) was performed in a total volume of 10 µl containing 1 µl of the 10 fold dilution ligated DNA fragments, 0.5 µl each of *Eco*RI and *Mse*I primers with one selective nucleotide (5 µM), 1 µl of 10X buffer, 0.6 µl of $MgCl_2$ (25 mM), 2 µl of dNTP (1 µM), and 0.2 µl of *Taq* DNA polymerase (Fermentas Life Sciences) (5U/ µl). The PCR procedure follows initial denaturation step at 94°C for 2 min, 20 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s and extension at 72 °C for 60 s, then incubated at 72 °C for 5 min as the final extension. The PCR I product was diluted 10 folds and used as template for the selective amplification (PCR II). The PCR II procedure began with denaturation step at 94°C for 2 min, 12 cycles of denaturation at 94°C for 30 s, annealing at6 5°C for 30 s (less 0.7°C per cycle after the first cycle), extension at 72 °C for 60s and 24 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72 °C for 60s, followed by the final extension at 72^{o}C for 2 min.

The PCR II products was added with 5 µl of sequencing dye (10 mM EDTA pH 8.0, 98% formamide, 0.01 % bromphenol blue and 0.01 % xylene cyanol). Three microliters of each sample was loaded on 4.5 % denaturing polyacrylamide gel compose of 80 ml of 4.5 % acrylamide-methylene bisacrylamide (19:1), 7 M urea, 500 µl of 10 % ammonium persulfate (APS), and 40 µl TEMED. Electrophoresis was performed at 45 – 50°C, with 60W power for 80 – 90 min using 1X TBE (100 mM tris, 100 mM boric acid, and 2 mM EDTA) as running buffer.

DNA fragments were detected by silver staining method as described by Promega Crop, USA as follows. The gel was fixed in 10 % acetic acid for 20 minutes after electrophoresis. Fixation was followed by washing using distilled water for 3 times for 2 minutes each wash. Staining was done for 30 minutes with silver stain consisted of silver nitrate (1 g/L) and 37 % formaldehyde. The stained gel was washed quickly (less than 10 seconds) and developed in solution of 30 % sodium carbonate, 10 mg/ml sodium thiosulfate, and 37 % formaldehyde. Developing was

stopped with fixer solution (same solution which was used for fixing) with shaking for 3 minutes. The gel was washed with distilled water for 5 minutes, then dried by keeping at room temperatute over night.

Different DNA fragments amplified with each primer were treated as discrete characters and numbered sequentially. Genotypes were scored for the presence (1) or absence (0) of each fragment.

3.4. Data analysis

Single factor analysis of ANOVA was carried out to identify the association between traits of interest (leaf and agronomic characters) and AFLP markers using Proc Anova (SAS Inst., 1999).

4. Effect of multifoliate leaflets on yield and agronomic characters

4.1. Plant materials : extraction of recombinant inbred lines

From F_2 and on, the normal trifoliate leaflet plants (with the hypothetical genotype $N_1\_N_2\_S\_$) were individually harvested each time to obtain the $N_1\_N_2\_S\_$ plants. The process was repeated until F_5 where 4 families each with 4 phenotypes (normal trifoliate, 7 large, 5 small and 7 small) were extracted (Fig. 2). Seeds of the four phenotypes in each family were increased until F_7 and considered isogenic lines with regard to leaflet number, but genetically uniform in the genotypic background (93.75 % homozygosity) within the family.

4.2. Field experiments

A series of 3 field experiments were conducted during September 2004 to March 2005. Two experiments were done at Kasetsart University, Kamphaeng Saen Campus (KU-KPS) in rainy season 2004 and dry season 2005 and at Chainat Field Crops Research Center (CNFCRC), Thailand in dry season 2005. The soil type

in both experimental fields is sandy clay loam. The experiment was arranged in a randomized complete block design (RCBD) with four replications. The treatments were four isogenic lines from each of the four families, their parents, and two check varieties widely grown in Thailand, viz. Kamphaeng Saen 1 (KPS1) and Chainat 36 (CN 36). A plot size of 10 m^2 (4 rows of 5m long) was planted to each line in a replication with a spacing of 50 cm between rows and 12.5 cm between plants within row with one plant per hill. The recommended practices for optimum mungbean growth and protocols for data collection were according to Park (1978). Days to flowering and maturity were recorded when 50% of plants showed open flowers and mature pods. At pod filling stage, number of leaflets per plant, leaf area per plant, and leaf area per leaflet (cm^2) were recorded using leaf area meter model LI-3100 (Licor, Inc., Lincoln, Nebraska, USA). At maturity, ten plants were randomly measured for plant height (cm), number of pods per plant, number of seeds per pod, 100-seed weight (g), and yield per plot (g). The yield was harvested from 2 middle rows, skipping 50 cm at each end of the row so that the harvested area in each plot is 4 m^2.

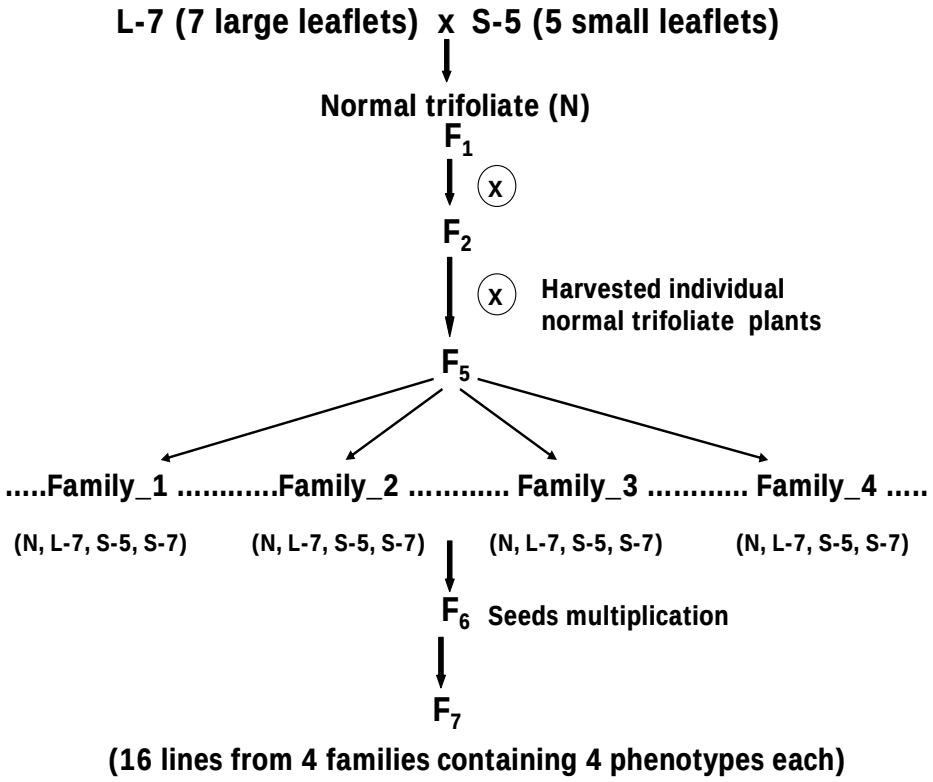

Figure 2. Extraction of 4 $F_{5:7}$ mungbean families, each with 4 leaflet types**.**

4.3. Statistical analysis

The data collected from the field trials were subjected to statistical analysis through the procedures described by Cody and Smith (1997) using SAS Program Ver.8 (SAS Inst., 1999). A combined analysis was performed across the 3 experiments (Gomez and Gomez, 1984). Group comparisons between genotypes were formed by partitioning the genotype sums of squares into appropriate components. General form of the combined analysis of variance across environments are presented in Table 1. Upon detecting the significance of the F-test for line difference, mean yield and yield components were compared among the lines using Duncan's Multiple Range Test (DMRT) at P=0.05.

Table 1. Combined analysis of variance of 20 mungbean lines in RCBD experiments with 4 replications over 3 environments.

SOV	df	MS
Environments	e-1 = 2	
Rep./Environments (Error a)	e (r-1) = 9	
Genotypes	v-1 = 19	
Between checks	c-1 = 3	
Checks vs. lines	1	
Between lines	l-1 = 15	
Genotype x environment	(v-1) (e-1) = 38	
Error (b)	171	
Total	(erv-1) = 239	

Where:
e = number of environments
r = number of replications
v = number of genotypes
c = number of check varieties and parental lines
l = number of lines tested

RESULTS

1. Study on Heterosis and Heterobeltiosis

All the characters observed from the cross S-5 x L-7 were similar to those from its reciprocal cross (L-7 x S-5) and the data from both sets could be combined to gain degrees of freedom for the t-test. Test of significance was separated at each cross.

Seed yield per plant in the normal-trifoliate VC6468 (N), small-multiple leaflet mutant (S-5), and large-multiple leaflet (L-7) an isogenic line of KPS1 were 12.47, 10.56, and 8.70 g/plant, respectively. The F_1 of S-5 x L-7 gave the highest seed yield (18.85 g) among the progenies (Table 2). All four crosses showed significant heterosis over the mid- and better parent for seed yield. Superiority over the mid-parent ranged from 52.2 to 95.7% (Table 3), while those over the better parent ranged from 31.8 to 78.5% (Table 4). The highest heterosis, both over mid- and better parents, was found in S-5 x L-7.

Even though N had the highest seed yield among the three parents, its hybrids had lower heterosis and heterobeltiosis values than those from the other parents. Crosses showing heterosis for seed yield also gave heterosis for pod length, number of seeds per pod, plant height, and leaf area per plant.

The highest number of pods per plant was found in the parent S-5 and in the F_1 of S-5 x N with 43 and 31 pods, respectively. The heterosis was not significant over either mid- or better parent, except only in the cross L-7 x N (28.2%). All crosses having S-5 as a parent showed negative heterobeltiosis for number of pods per plant (Table 4) indicating that S-5 had a very high number of pods.

Number of seeds per pod in the hybrids ranged from 11.5 to 12.2 (Table 2). S-5 had the lowest number of seeds (9.6) compared to L-7 and N (10.9 and 11.5,

respectively). All four crosses showed significant heterosis ranging from 8.9 to 13.2% (Table 3), but heterobeltiosis was not significant, ranging from 2.6 to 6.4 % (Table 4).

One hundred seed weight of S-5 was the lowest (2.57 g) compared to the parents L-7 and N (6.05 and 5.99 g, respectively). The hybrid L-7 x N showed the largest seed size and had significant heterosis and heterobeltiosis.

S-5 had the shortest pod and thus the hybrid S-5 x N expressed a negative heterobeltiosis of 7.7% (Table 4). The hybrid L-7 x N had the longest pods (10.0 cm). All cross combinations showed significant heterosis ranging from 8.4 to 24.7% (Table 3).

Plant height showed significant heterosis and heterobeltiosis in all cross combinations. The hybrid S-5 x L-7 and its reciprocal gave taller plants than the other crosses (Table 2). The range of significant heterosis and heterobeltiosis varied from 12.2 to 19.1% (Table 3), and from 4.3 to 12.8%, repectively (Table 4). The hybrid S-5 x N gave the highest value both in heterosis and heterobeltiosis.

The small-multiple leaflets (S-5) had the highest average number of leaves (66.0), as compared to L-7 and N which had 7.8 and 12.2 leaves, respectively. The F1 derived from S-5 showed negative values for both heterosis and heterobeltiosis. The F1 of L-7 x N showed significant heterosis (30%; Table 3), but not heterobeltiosis (6.6%; Table 4).

For leaf area per plant, all crosses showed significant heterosis and heterobeltiosis. Heterosis values ranged from 64.9 to 86%, while heterobeltiosis values ranged from 51.9 to 78.8%. The highest value was found in the cross S-5 x L-7 (Table 2).

Table 2. Yield, yield components, and agronomic characters of 3 mungbean lines and their F_1's grown at Kasetsart University, Kamphaeng Saen Campus, Thailand, Late Rainy Season, 2002.

Mungbean genotypes	Seed yield/ plant (g)	No. of pods/plant	No. of seeds/pod	100-seed weight (g)
	Value ± SE			
S-5	10.56 ± 0.45	43.0 ± 1.7	9.6 ± 0.3	2.57 ± 0.07
L-7	8.70 ± 0.51	13.3 ± 0.9	10.9 ± 0.4	6.05 ± 0.13
N	12.47 ± 0.98	17.9 ± 0.9	11.5 ± 0.5	5.99 ± 0.10
S-5 x L-7	18.85 ± 1.02	28.2 ± 1.7	11.6 ± 0.3	5.80 ± 0.26
L-7 x S-5	17.90 ± 0.8	27.1 ± 1.2	11.5 ± 0.3	5.77 ± 0.26
S-5 x N	17.55 ± 0.81	31.0 ± 1.2	11.8 ± 0.3	4.79 ± 0.27
L-7 x N	16.43 ± 1.2	20.0 ± 1.2	12.2 ± 0.4	6.70 ± 0.21

Mungbean Genotypes	Pod length (cm)	Plant height (cm)	No.leaves per plant	Leaf area/ Plant (cm^2)
	Value ± SE			
S-5	4.65 ± 0.13	47 ± 1.1	66.0 ± 5.9	1054 ± 127.0
L-7	8.75 ± 0.20	49 ± 0.4	7.8 ± 0.7	971 ± 169.1
N	9.70 ± 0.28	42 ± 0.4	12.2 ± 0.9	1153 ± 145.2
S-5 x L-7	8.20 ± 0.21	55 ± 0.8	16.8 ± 3.0	1884 ± 242.5
L-7 x S-5	8.25 ± 0.21	55 ± 0.8	16.4 ± 2.4	1800 ± 246.0
S-5 x N	8.95 ± 0.14	53 ± 1.2	15.6 ± 2.2	1824 ± 277.4
L-7 x N	10.00 ± 0.26	51 ± 0.6	13.0 ± 1.3	1751 ± 189.8

Tabel 3. Significant test of heterosis over mid parent (MP) in yield, yield components, and agronomic characters of four F_1 mungbean grown at Kasetsart University, Kamphaeng Saen Campus, Thailand, Late Rainy Season, 2002.

Cross combination	Seed yield /plant (g)		No. of pods / plant		No. of seeds /pod		100-seed weight (g)	
	value ± SE	% H	value ± SE	% H	value ± SE	% H	value ± SE	% H
S-5 x L-7 F_1	9.22 ± 1.07 **	95.7	0.05 ± 1.96 ns	0.2	1.35 ± 0.42 **	13.2	1.49 ± 0.27 **	34.6
L-7 x S-5 F_1	8.27 ± 0.87 **	85.9	-1.05 ± -0.04 ns	-0.1	1.25 ± 0.39 **	12.2	1.46 ± 0.27 **	33.9
S-5 x N F_1	6.04 ± 0.98 **	52.5	0.55 ± 1.54 ns	0.1	1.25 ± 0.39 **	11.9	0.51 ± 0.28 ns	11.9
L-7 x N F_1	5.84 ± 1.32 **	55.2	4.40 ± 1.34 **	28.2	1.00 ± 0.47 **	8.9	0.68 ± 0.23 *	11.3

Cross combination	Pod length (cm)		Plant height (cm)		No. of leaves /plant		Leaf area /plant (cm^2)	
	value ± SE	% H	value ± SE	% H	value ± SE	% H	value ± SE	% H
S-5 x L-7 F_1	1.50 ± 0.25 **	22.4	7.15 ± 0.96 **	14.9	-20.1 ± 3.75 **	-54.5	871.7 ±215.5 **	86.0
L-7 x S-5 F_1	1.55 ± 0.25 **	23.1	7.05 ± 0.99 **	14.7	-20.5 ± 3.83 **	-55.6	787.2 ±267.8 **	77.7
S-5 x N F_1	1.78 ± 0.21 **	24.7	8.50 ± 1.30 **	19.1	-23.5 ± 3.68 **	-60.1	720.9 ±293.6 **	65.3
L-7 x N F_1	0.78 ± 0.31 **	8.4	5.55 ± 0.71 **	12.2	3.0 ± 1.43 *	30.0	689.3 ±220.1 **	64.9

*significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; ns = non-significant

Tabel 4. Significant test of heterobeltiosis over better parent (BP) in yield, yield components, and agronomic characters of four F_1 mungbean grown at Kasetsart University, Kamphaeng Saen Campus, Thailand, Late Rainy Season, 2002.

Cross combination	Seed yield /plant (g)		No. of pods / plant		No. of seeds /pod		100-seed weight (g)	
	value ± SE	%Hb	value ± SE	% Hb	value ± SE	% Hb	value ± SE	% Hb
S-5 x L-7 F_1	8.29 ± 1.11 **	78.5	-14.8 ± 2.41 **	-34.4	0.7 ± 0.49 ns	6.4	-0.25 ± 0.28 ns	-4.13
L-7 x S-5 F_1	7.33 ± 0.92 **	69.4	-15.9 ± 2.09 **	-36.9	0.6 ± 0.46 ns	5.5	-0.28 ± 0.29 ns	-4.62
S-5 x N F_1	5.08 ± 1.27 **	40.7	-12.0 ± 2.08 **	-27.9	0.3 ± 0.56 ns	2.6	-1.20 ± 0.29 **	-20.03
L-7 x N F_1	3.96 ± 1.55 **	31.8	2.1 ± 1.49 ns	11.7	0.7 ± 0.62 ns	6.1	0.65 ± 0.25 *	10.74

Cross combination	Pod length (cm)		Plant height (cm)		No. of leaves /plant		Leaf area /plant (cm^2)	
	value ± SE	%Hb	value ± SE	% Hb	value ± SE	% Hb	value ± SE	% Hb
S-5 x L-7 F_1	-0.55 ± 0.29 ns	-6.3	6.2 ± 0.87 **	12.7	-49.2 ± 6.32 **	-74.6	830.3 ± 226.7 **	78.8
L-7 x S-5 F_1	-0.50 ± 0.29 ns	-5.7	6.1 ± 0.91 **	12.5	-49.6 ± 6.37 **	-75.2	745.9 ± 276.9 *	70.8
S-5 x N F_1	-0.75 ± 0.31 *	-7.7	6.0 ± 1.58 **	12.8	-50.4 ± 6.27 **	-76.4	671.3 ± 313.1 *	58.2
L-7 x N F_1	0.30 ± 0.38 ns	3.1	2.1 ± 0.76 *	4.3	0.8 ± 1.59 ns	6.6	598.4 ± 239.0 *	51.9

*significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; ns = non-significant

2. Inheritance of multifoliate leaflets

All F_1 plants from the cross L-7 x S-5 had normal trifoliate leaflets. The F_2 plants in each family segregated into 4 leaflet types, viz. large-trifoliate (N), large-heptafoliate (L-7), small-pentafoliate (S-5), and small-heptafoliate (S-7) as shown in Fig.3. Data classified based on leaflet size from each F_2 family were tested against a 3:1 ratio for the *s* locus (large vs small leaflet). The gene controlling leaflet size which the large one is dominant over the small one (Table 5). When the observations from 4 families were combined, the χ^2 value was so low that the observed number fitted well with the expected one. The low χ^2 value also caused high heterogeneity (P = .05 - .01) among the family. However, this is a normal phenomena when number of plants in each family are rather different.

The F_2 data also supported monohybrid hypothesis for leaflet number which tri- and pentafoliate are completely dominant to heptafoliate (Table 6). The combined data did not deviate significantly from 3:1 ratio, and heterogeneity among the families were not significant, revealing that the segregation of this trait agreed well with each other among all 4 F_2 families.

The linkage relationship between the genes controlling leaflet size and leaflet number was tested against the 9:3:3:1 ratio using number of plants observed in N, L-7, S-5, and S-7. The results presented in Table 7 showed that the the *s* and *n* loci were independent with no evidence of linkage. The segregation pattern in the combined data also followed the 9:3:3:1 ratio, with homogeneity among the families.

Since the F_1 are all trifoliate leaflet plants, the gene controlling leaflet number should have more than one locus. Assuming that the second locus is n_2, then the inheritance model for both loci is postulated as in Fig. 1, where there are 3 classes of leaflet number, viz. 3, 5, and 7 leaflets per leaf. Number of plants in different leaflet classes from each F_2 family were tested against 9:3:4 ratio for trifoliate ($N_1\_N_2\_$), pentafoliate ($N_1\_n_2n_2$), and heptafoliate ($n_1n_1N_2\_$ and $n_1n_1n_2n_2$) leaflets, respectively (Table 8). The results revealed that there are 2 loci of gene controlling number of

leaflets. The combined data did not deviate significantly from the 9:3:4 ratio. Heterogeneity among the families were not significant, revealing that the segregation of this trait agreed weel with each other among all 4 F_2 families. If this model is acceptable, the gene action should be such that n_1 and n_2 loci express interallelic interaction in which N_1 allele dictates trifoliate at the present of N_2 allele (*i.e.* genotype $N_1\_N_2\_$) but showed pentafoliate in $N_1\_n_2n_2$. Whereas n_1n_1 genotype expresses heptafoliate regardless the other genotypes (*i.e.* $n_1n_1N_2\_$ and $n_1n_1n_2n_2$ show large and small heptafoliate leaflets, respectively). The genotype $N_1\_n_2n_2S\_$ is not found, possibly because n_2 is highly linked with *s* and N_2 with *S* and thus n_2 and *s*, and N_2 and *S* were co-segregated in the progenies.

Table 5. Chi-square test for goodness-of-fit against a 3:1 ratio for leaflet size (large vs small) in 4 F_2 mungbean families from the cross between L-7 and S-5 parents.

Family	No. of plants*)		$\chi^2_{(1)}$	Prob
	S_	***ss***		
1	161	65	1.705	0.20 - 0.10
2	85	39	2.753	0.10 - 0.05
3	219	58	2.437	0.20 - 0.10
4	106	28	1.204	0.30 - 0.20
Total	571	190	0.000	< 0.99
Heterogeneity (3 df)			8.099	0.05 - 0.01

*)No. of plants with large leaflets (*S_*) was obtained from N and L-7; those with small leaflets (*ss*) were from S-5 and S-7.

Table 6. Chi-square test for goodness-of-fit against a 3:1 ratio for number of leaflets (tri- and pentafoliate vs heptafoliate) in 4 F_2 mungbean families from the cross between L-7 and S-5 parents.

Family	No. of plants*)		$\chi^2_{(1)}$	Prob
	$N_1\_$	n_1n_1		
1	159	67	2.602	0.20 - 0.10
2	91	33	0.172	0.70 - 0.50
3	213	64	0.531	0.50 - 0.30
4	102	32	0.090	0.80 - 0.70
Total	565	196	0.232	0.70 - 0.50
Heterogeneity (3 df)			3.162	0.30 - 0.20

*)No. of plants with 3 and 5 leaflets ($N_1\_$) were obtained from N and S-5; those with 7 leaflets (*nn*) were from L-7 and S-7.

Table 7. Chi square test for independence (9:3:3:1 ratio) between leaflet size and leaflet number in 4 F_2 mungbean families from the cross between L-7 and S-5 parents.

Family	No. of plants					$\chi^2_{(3)}$	Prob
	N	L-7	S-5	S-7	Total		
	$N_1\_S\_$	$n_1n_1S\_$	N_1_ss	n_1n_1ss			
1	117	44	42	23	226	6.448	0.10 - 0.05
2	64	21	27	12	124	3.627	0.50 - 0.30
3	172	47	41	17	277	4.460	0.30 - 0.20
4	82	24	20	8	134	1.695	0.80 - 0.70
Total	435	136	130	60	761	4.806	0.30 - 0.20
Heterogeneity (9 df)						11.424	0.30 - 0.20

Table 8. Chi-square test for goodness-of-fit against a 9:3:4 ratio for leaflet number in 4 F_2 mungbean families from the cross between L-7 and S-5 parents.

Family	No. of plants with no. leaflets				$\chi^2_{(2)}$	Prob
	3 ***(N_1_N_2_)***	**5** **(N_1_n_2n_2)**	**7** **($n_1n_1N_2$_ and $n_1n_1n_2n_2$)**	**Total**		
1	117	42	67	226	2.761	0.30 - 0.20
2	64	27	33	124	1.208	0.70 - 0.50
3	172	41	64	277	4.383	0.30 - 0.20
4	82	20	32	134	1.695	0.50 - 0.30
Total	435	130	196	761	1.414	0.50 - 0.30
Heterogeneity (6 df)					8.633	0.20 - 0.10

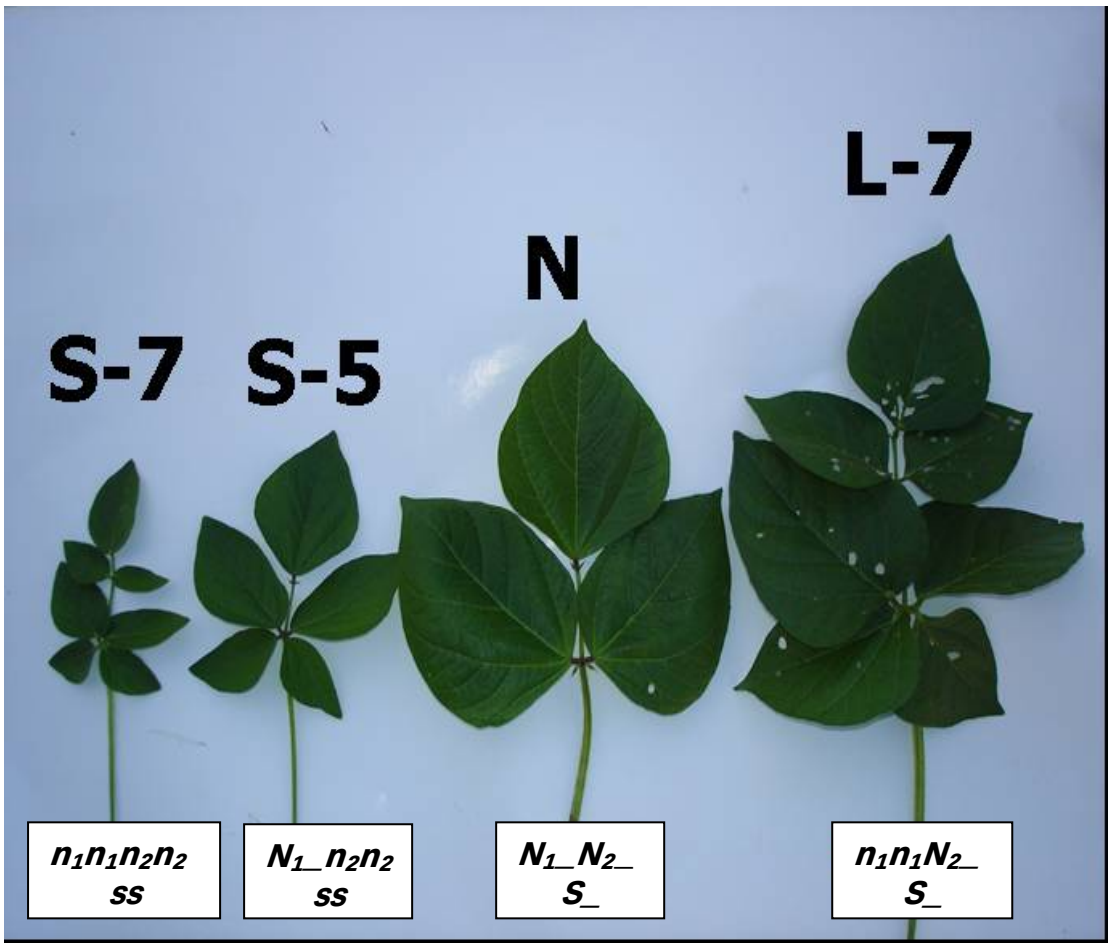

Figure 3. Leaflet types and proposed gene symbols of mungbean progenies derived from the cross between L-7 and S-5.

3. AFLP markers associated with leaflet and agronomic characters

A total of 180 primer combinations were evaluated for detection of polymorphism between L-7 and S-5 parental genotypes. Amplification was observed and 94 primer pairs showed polymorphism between them. From 94 primer combinations, 47 of them showed clear and sharp bands and thus used to amplify fragments of 16 isogenic lines. Twenty primer pairs were found polymorphic between parents and isogenic lines and produced 56 polymorphic DNA bands (Appendix Table 5). An example of segregation of polymorphic AFLP markers in 16 isogenic lines tested was shown in Fig. 4. The molecular weight (MW) of each polymorphic AFLP marker was estimated by comparing with HinfI marker (Fermentaz Life Sciences).

Results of single factor analysis of variance showed that 22 AFLP markers significantly associated with leaf and agronomic characters (Table 9 – Table 14). The size of detected fragments range from 82-713 bp, but most of them were between 151-249 bp. Among them, 12 markers (AAA_CAG2, AAA_CAG3, AAA_CTA1, AAA_CTT2, ACG_CAG4, ACG_CAG1, ACG_CAG2, ACT_ACG, ACT_AGC, CAG_ACG3, CT_AAT, GCC_ACA1) associated with leaflet size in term of large vs small, 3 markers (AAA_CTT3, ACG_CAC1, and GCC_ACT3) linked to leaflet number in term of 3 and 5 vs 7 leaflets, 2 markers (ACG_CAC1 and GCC_ACT3) associated with number of leaflet per leaf, 9 markers (AAA_CAG3, AAA_CTA1, AAA_CTT2, ACG_CAG4, ACG_CAG1, ACT_ACG, CT_AAT, GCC_ACA1, and GCC_ACT2) linked to leaf area per leaflet, 7 markers (AAA_CTA1, ACG_CAG4, ACG_CAG1, ACG_CAG2, ACT_ACG, CT_AAT, and GCC_ACT2) linked to leaf area per plant, 11 markers (AA_ACG3, AAA_CTA1, ACG_CAG4, ACG_CAG1, ACG_CaG2, ACT_ACG, CAG_ACG3, CT_AAT, GCC_ACA1, GCC_ACT1, and GCC_ACT2) linked to number of leaflet per plant, 12 markers (AAA_CTA1, AAA_CTC3, ACC_AGG2, ACG_CAG4, ACG_CAG1, ACG_CAG2, ACT_ACG, CAG_ACG3, CT_AAT, GCC_ACA1, GCC_ACT1, and GCC_ACT2) linked to seed yield, 9 markers (AAA_CTA1, AAA_CTC1, AAA_CTC3, ACC_AGG2, ACG_CAG4, ACG_CAG1, ACT_ACG, CT_AAT, and GCC_ACA) linked to 100-

seed weight, 7 markers (AAA_CTA1, ACG_CAG4, ACG_CAG1, ACT_ACG, CAG_ACG3, CT_AAT, and GCC_ACA1) linked to number of pods per plant, and 9 markers (AAA_CTA1, AAA_CTT2, ACG_CAG4, ACG_CAG1, ACG_CAG2, ACT_ACG, CAG_ACG3, CT_AAT, GCC_ACA1) linked to number of seeds per pod (Table 13).

There are 12 markers associated with leaflet size, 10 of them were contributed from P_1 alleles, the other 2 markers, viz. ACT_AGC and GCC_ACA1 were from P_2 alleles. For number of leaflets per leaf, 3 markers were contributed from P_2 alleles (Table 9).

There are 2 alleles contribute to higher number of leaflets per leaf and both come from P_1 (L-7) parent. Leaf area per leaflet was also contributed largely from P_1 alleles, except for marker number 54 (GCC_ACT2) was from P_2 (S-5) allele (Table 10).

Among 7 AFLP markers linked to leaf area per plant, 6 of them were from P_1 while only one was from P_2. For number of leaflets per plant, 3 out of 11 were P_1 alleles that help increasing the number (Table 11).

For seed yield, most of the alleles linked to the trait come from P_1, only 2 alleles were contributed from P_2. While all the alleles contributed to the traits of 100-seed weight, and number of seeds per pod were from P_1 (Table 12 and 13). However, the small pentafoliate parent (S-5) contributed to high number of pods per plant. This yield component, although very desirable, showed negative correlation with the other components and thus makes it difficult to select from this mungbean population.

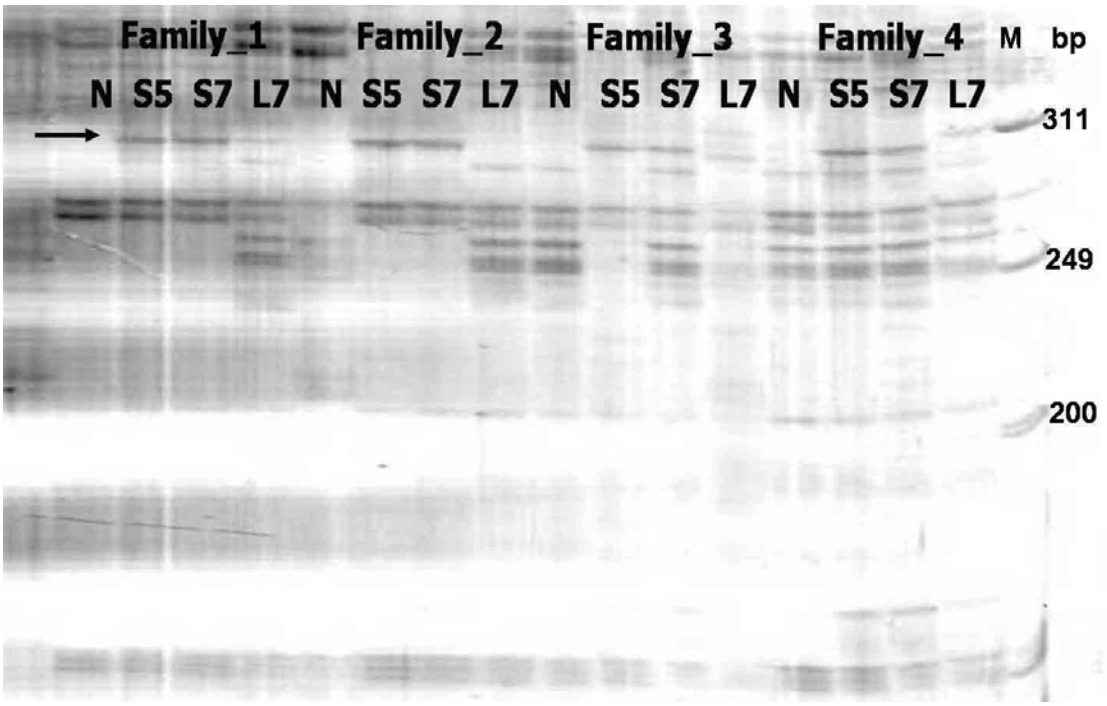

Figure 4. An example of AFLP profile for a specific primer combination on 16 mungbean isogenic lines. The arrow indicates a possible multifoliate leaflet marker. Each lane contains DNA restriction fragments amplified from multifoliate leaflet lines classified as present or absent based on polymorphism in the parental lines.

Table 9. AFLP markers showing association with leaflet size and leaflet number based on single factor analysis of variance.

No.	Marker no.	Marker	Marker size (bp)	Leaflet size (N, L-7 vs S-5, S-7)			Leaflet number (N, S-5 vs L-7, S-7)		
				Prob	Allele mean		Prob	Allele mean	
					P_1	P_2		P_1	P_2
1	8	AAA_CAG2	200-249	0.04	0.70	0.17	-	-	-
2	9	AAA_CAG3	151-200	0.04	0.70	0.17	-	-	-
3	12	AAA_CTA1	200-249	< 0.01	0.80	0.00	-	-	-
4	20	AAA_CTT2	200-249	< 0.01	0.88	0.13	-	-	-
5	21	AAA_CTT3	82-100	-	-	-	0.04	0.17	0.7
6	26	ACG_CAC1	200-249	-	-	-	0.01	0.22	0.86
7	29	ACG_CAG4	311-413	< 0.01	1.00	0.18	-	-	-
8	31	ACG_CAG1	151-200	< 0.01	1.00	0.10	-	-	-
9	34	ACG_CAG2	151-200	< 0.01	1.00	0.30	-	-	-
10	38	ACT_ACG	151-200	0.01	0.86	0.27	-	-	-
11	39	ACT_AGC	200	0.05	0.33	0.83	-	-	-
12	44	CAG_ACG3	100-118	0.03	0.73	0.17	-	-	-
13	48	CT_AAT	100-118	0.01	0.86	0.27	-	-	-
14	51	GCC_ACA1	151-200	< 0.01	0.30	1.00	-	-	-
15	55	GCC_ACT3	200-249	-	-	-	0.01	0.27	0.86

Table 10. AFLP markers showing association with number of leaflets per leaf (3, 5, and 7) and leaflet area based on single factor analysis of variance.

No	Marker no.	Marker	Marker size (bp)	No. leaflets per leaf (3,5,7)			Leaf area (cm^2)/ leaflet		
				Prob	Allele mean		Prob	Allele mean	
					P_1	P_2		P_1	P_2
1	9	AAA_CAG3	151-200	-	-	-	0.05	24.74	8.70
2	12	AAA_CTA1	200-249	-	-	-	0.01	26.74	5.37
3	20	AAA_CTT2	200-249	-	-	-	0.05	26.56	10.89
4	26	ACG_CAC1	200-249	0.02	6.33	4.43	-	-	-
5	29	ACG_CAG4	311-413	-	-	-	< 0.01	33.56	8.23
6	31	ACG_CAG1	151-200	-	-	-	0.01	28.35	9.86
7	38	ACT_ACG	151-200	-	-	-	0.03	28.19	11.65
8	48	CT_AAT	100-118	-	-	-	0.02	28.49	11.46
9	51	GCC_ACA1	151-200	-	-	-	0.05	29.36	13.74
10	54	GCC_ACT2	249	-	-	-	0.04	13.00	29.22
11	55	GCC_ACT3	200-249	0.02	6.27	4.43	-	-	-

Table 11. AFLP markers showing association with leaf area per plant and number of leaflets per plant based on single factor analysis of variance.

No	Marker no.	Marker	Marker size (bp)	Leaf area (cm^2)/ plant			No. leaflets per plant		
				Prob	Allele mean		Prob	Allele mean	
					P_1	P_2		P_1	P_2
1	4	AA_ACG3	200-249	-	-	-	0.05	102.3	60.1
2	12	AAA_CTA1	200-249	0.01	923	610	0.01	55.0	114.2
3	29	ACG_CAG4	311-413	< 0.00	999	608	0.01	37.5	115
4	31	ACG_CAG1	151-200	< 0.00	989	655	0.01	39.5	106.1
5	34	ACG_CaG2	151-200	0.01	1014	723	0.02	39.2	90.8
6	38	ACT_ACG	151-200	< 0.00	978	693	0.01	44.3	96.9
7	44	CAG_ACG3	100-118	-	-	-	0.02	57.8	108.1
8	48	CT_AAT	100-118	< 0.00	986	687	0.01	44.3	96.9
9	51	GCC_ACA1	151-200	-	-	-	0.01	37.1	91.6
10	53	GCC_ACT1	413	-	-	-	0.03	97.0	51.4
11	54	GCC_ACT2	249	0.03	727	968	0.05	91.3	46.7

Table 12. AFLP markers showing association with seed yield (kg/ha) and 100-seed weight (g) based on single factor analysis of variance.

No	Marker no.	Marker	Marker size (bp)	Seed yield (kg/ha)			100-seed weight (g)		
				Prob	Allele mean		Prob	Allele mean	
					P_1	P_2		P_1	P_2
1	12	AAA_CTA1	200-249	< 0.01	970	589	< 0.01	5.76	4.18
2	15	AAA_CTC1	553-713	-	-	-	0.02	5.52	4.12
3	17	AAA_CTC3	200-249	0.01	1018	679	0.01	5.9	4.6
4	24	ACC_AGG2	200-249	0.05	1031	735	0.03	6.04	4.78
5	29	ACG_CAG4	311-413	< 0.01	1048	626	< 0.01	6.08	4.33
6	31	ACG_CAG1	151-200	< 0.01	1038	676	< 0.01	5.99	4.57
7	34	ACG_CAG2	151-200	0.04	1043	758	-	-	-
8	38	ACT_ACG	151-200	< 0.01	1065	692	< 0.01	6.19	4.57
9	44	CAG_ACG3	100-118	0.03	937	648	-	-	-
10	48	CT_AAT	100-118	< 0.01	1073	687	< 0.01	6.16	4.59
11	50	GCC_ACA	151-200	-	-	-	0.05	5.6	4.58
12	51	GCC_ACA1	151-200	0.05	1035	761	-	-	-
13	53	GCC_ACT1	413	0.04	708	978	-	-	-
14	54	GCC_ACT2	249	0.04	738	1013	-	-	-

Table 13. AFLP markers showing association with number of pods per plant and number of seeds per pod based on single factor analysis of variance.

No	Marker no.	Marker	Marker size (bp)	No. pods/ plant			No. seeds/ pod		
				Prob	Allele mean		Prob	Allele mean	
					P_1	P_2		P_1	P_2
1	12	AAA_CTA1	200-249	<0.01	17.2	22.9	<0.01	11.47	9.05
2	20	AAA_CTT2	200-249	-	-	-	<0.01	11.60	9.52
3	29	ACG_CAG4	311-413	<0.01	15.8	22.6	<0.01	11.97	9.69
4	31	ACG_CAG1	151-200	<0.01	15.9	21.9	<0.01	12.05	9.40
5	34	ACG_CAG2	151-200	-	-	-	<0.01	12.09	9.99
6	38	ACT_ACG	151-200	<0.01	16.2	21.1	0.03	11.56	9.96
7	44	CAG_ACG3	100-118	0.04	17.5	21.8	0.04	11.23	9.63
8	48	CT_AAT	100-118	0.02	16.5	20.9	0.03	11.56	9.95
9	51	GCC_ACA1	151-200	0.04	16.0	20.4	<0.01	12.16	9.97

Table 14. Total of AFLP markers showing association with yield and agronomic characters based on single factor analysis of variance.

No	Marker no.	Marker	Marker size (bp)	Leaflet size (N_1N_1 vs n_1n_1)	Leaflet no. (N_2N_2 vs n_2n_2)	No. leaflets /leaf	Leaf-area/ leaflet	Leaf-area/ plant	No. leaflets /plant	Seed yield	100-seed weight	No. pods/ plant	No. seeds/ pod
				Probability									
1	4	AA_ACG3	200-249	-	-	-	-	-	0.05	-	-	-	-
2	8	AAA_CAG2	200-249	0.04	-	-	-	-	-	-	-	-	-
3	9	AAA_CAG3	151-200	0.04	-	-	0.05	-	-	-	-	-	-
4	12	AAA_CTA1	200-249	< 0.01	-	-	0.01	0.01	0.01	< 0.01	< 0.01	< 0.01	< 0.01
5	15	AAA_CTC1	553-713	-	-	-	-	-	-	-	0.02	-	-
6	17	AAA_CTC3	200-249	-	-	-	-	-	-	0.01	0.01	-	-
7	20	AAA_CTT2	200-249	< 0.01		-	0.05	-	-	-	-	-	< 0.01
8	21	AAA_CTT3	82-100	-	0.04	-	-	-	-	-	-	-	-
9	24	ACC_AGG2	200-249	-	-	-	-	-	-	0.05	0.03	-	-
10	26	ACG_CAC1	200-249	-	0.01	0.02	-	-	-	-	-	-	-
11	29	ACG_CAG4	311-413	< 0.01	-	-	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
12	31	ACG_CAG1	151-200	< 0.01	-	-	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
13	34	ACG_CAG2	151-200	< 0.01	-	-	-	0.01	0.02	0.04	-	-	< 0.01
14	38	ACT_ACG	151-200	0.01	-	-	0.03	< 0.01	0.01	< 0.01	< 0.01	< 0.01	0.03
15	39	ACT_AGC	200	0.05	-	-	-	-	-	-	-	-	-
16	44	CAG_ACG3	100-118	0.03	-	-	-	-	0.02	0.03	-	0.04	0.04
17	48	CT_AAT	100-118	0.01	-	-	0.02	< 0.01	0.01	< 0.01	< 0.01	0.02	0.03
18	50	GCC_ACA	151-200	-	-	-	-	-	-	-	0.05	-	-
19	51	GCC_ACA1	151-200	< 0.01	-	-	0.05	-	0.01	0.05	-	0.04	< 0.01
20	53	GCC_ACT1	413	-	-	-	-	-	0.03	0.04	-	-	-
21	54	GCC_ACT2	249	-	-	-	0.04	0.03	0.05	0.04	-	-	-
22	55	GCC_ACT3	200-249	-	0.01	0.02	-	-	-	-	-	-	-

4. Effect of multifoliate leaflets on yield and agronomic characters

Analysis of variance for yield and its components were combined across 3 locations, giving the mean data as shown in Fig. 5 to Fig. 7. Number of total leaflets per plant was found different among multifoliate lines tested which was highest in S-7 (132.0 leaflets). The average number of leaflets per plant was 100.7 in S-5, 49.1 in L-7, and 24.2 in N. The parents and progenies carrying the same size and number of leaflets, (i.e. S-5 parent and lines; L-7 parent and lines) showed similar number of leaflets per plant. However, in term of leaf area per plant, normal trifoliate gave similar value to that of L-7 parent and line, but higher than that of S-5 and S-7. Among the tested lines, N had the highest leaf area per plant (1058.8 cm^2), while S-7 gave the smallest value of 576.2 cm^2 (Fig. 5). Although the number of leaflets per plant of S-7 was the highest, its leaf area per plant was the smallest due to smaller leaflet size throughout the plant. This character also caused shorter plants with smaller seed and fewer number of seeds per pod, thus produced lower yield

The 100-seed weight of N and L-7 lines was higher than that of S-5 and S-7 (Fig. 6). The L-7 parent gave the largest 100-seed weight with the average of 7.02 g. Although the plants were slightly shorter and number of pods per plant were less than those of the normal genotype, the advantage in seed size resulted in comparatively high yield in L-7.

For number of seeds per pod, N and L-7 lines had similar value, viz. 11.9 and 12.2, while S-5 and S-7 set shorter pods with 9.4 and 8.9 seeds per pod, respectively. The number of seeds per pod in the lines were also comparable to the parents of the same leaflet type (Fig. 6).

Distribution of data in each family (Fig. 8 to Fig. 14) showed that N and L-7 tended to have higher values than those of S-5 and S-7 in all agronomic characters in this study, except for number of leaflets and number of pods per plant. Whereas days to flowering and maturity were the same among all leaflet types. Across all 3 tests, the normal mungbean gave similar yield to L-7, while S-5 was similar to S-7. The L-7

parent, N and L-7 gave higher yield, while S-5 parent gave similar yield to those small multifoliate S-5 and S-7 lines (Fig. 8).

Leaves are the primary sites of photosynthesis with varying in number, shape and size. They are efficient interceptors of light because of their flat shape with the chloroplats on the surface. The N and L-7 had higher leaf area than the other multifoliate types, thus can absorb light in more quantity during photosynthesis. The resulting grain yield is controlled by many factors within and outside the plant which may be measured in the form of yield components. Correlations between mungbean yield and its components in this study are shown in Table 14. Seed yield correlated well with leaf area per leaflet, leaf area per plant, number of seeds per pod and 100-seed weight. The multiple leaflet mungbeans tended to give shorter but more pods per plant. Thus the number of pods was negatively correlated with seed yield in this study. Although number of pods per plant was positively correlated with total number of leaflets per plant, both traits were negatively correlated with seed yield. This gave no yield advantage even though S-5 and S-7 set more profuse pods. The multiple leaflet lines set smaller seed which is a major contribution to low yielding. In term of plant height, the normal leaflet lines were taller than the others.

Since mungbeans are a relatively high priced seed (about twice the cost of soybeans), it is not cost effective to feed good quality seed to livestock. However, splitted and cracked seed, and other materials left after cleaning mungbeans may be fed to cattle. Mungbeans can also be used as a green manure crop and as forage for livestock. In response to specific requirements such as developing forage or grazing types of mungbean, 4 F_7 mungbean lines selected from 7 large leaflets were analyzed for their nutritional quality and presented in Table 15. The whole plant of these lines gave high protein percentage of up to 15 % with acceptable detergent fiber and lignin. This open another use of multiple leaflet mungbean as an alternative animal feeds.

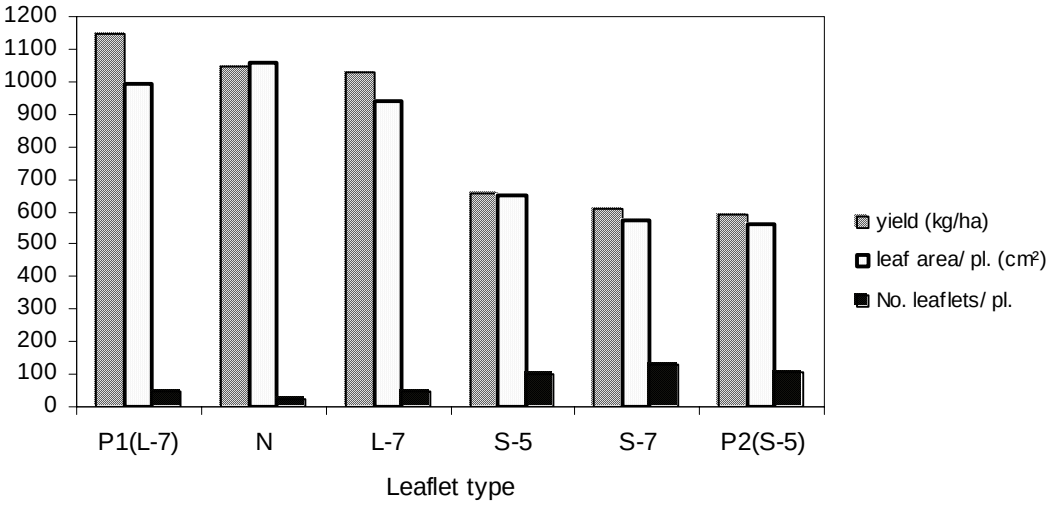

Figure 5. Effect of multifoliate leaflet types on yield, leaf area per plant, and number of leaflets per plant of mungbean lines derived from the cross between L-7 and S-5 parents.

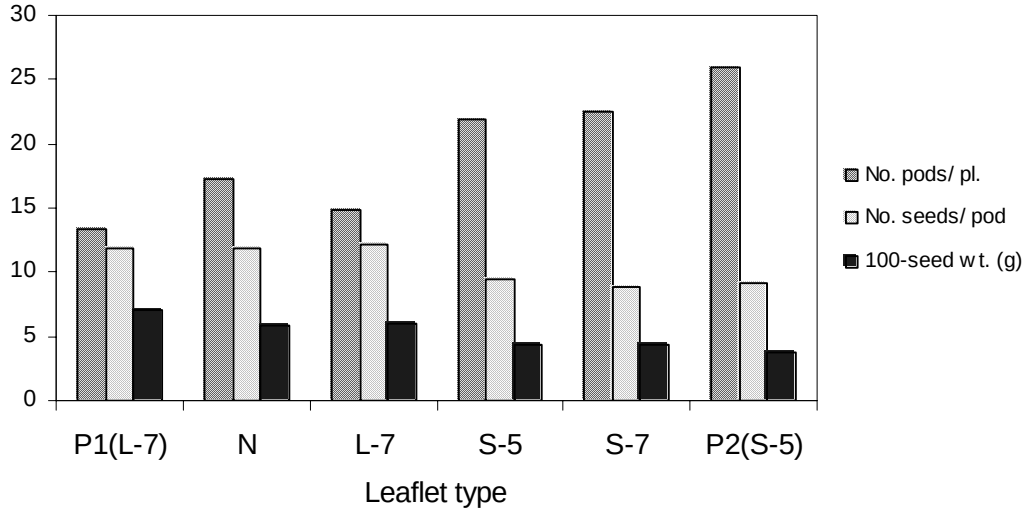

Figure 6. Effect of multifoliate leaflet types on number of pods per plant, number of seeds per pod, and 100-seed weight of mungbean lines derived from the cross between L-7 and S-5 parents.

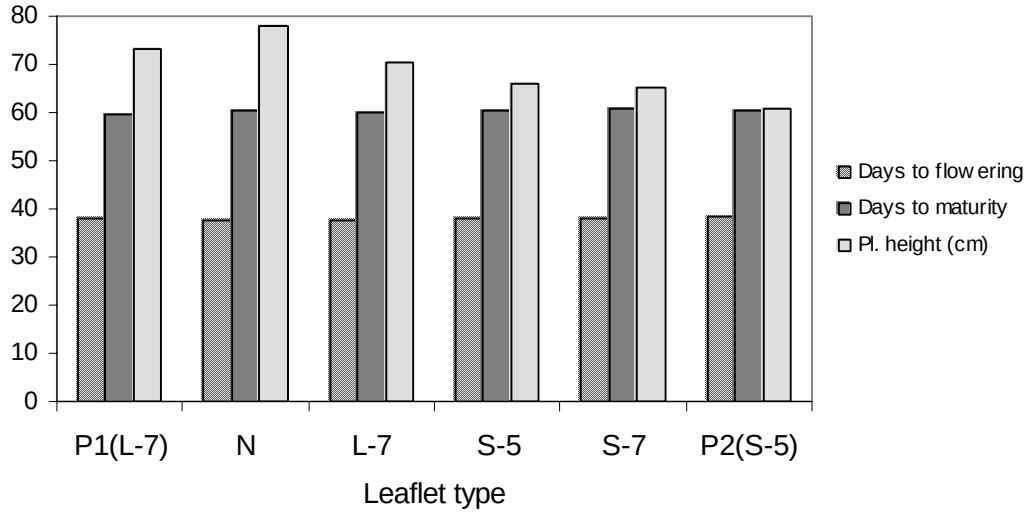

Figure 7. Effect of multifoliate leaflet types on days to flowering, days to maturity, and plant height of mungbean lines derived from the cross between L-7 and S-5 parents.

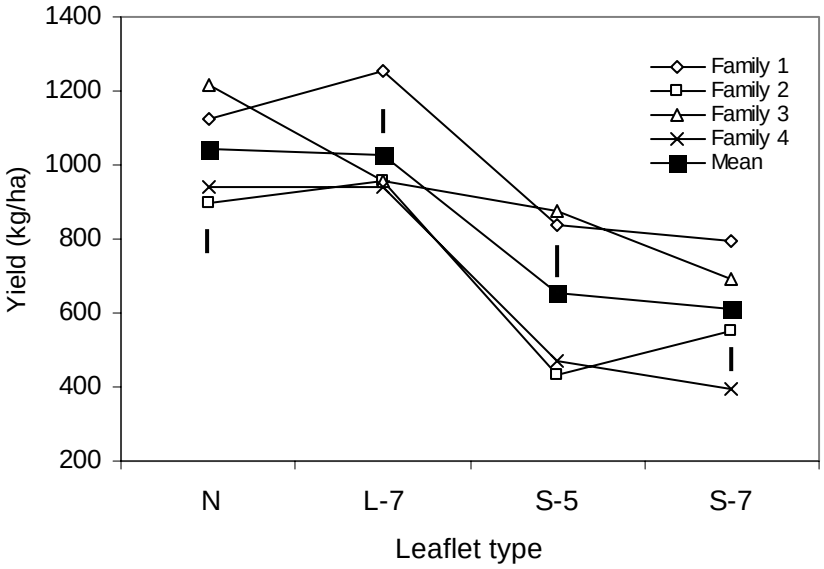

Figure 8. Effect of multifoliate leaflet types on yield in 4 mungbean families each with 4 isogenic lines. S.E. of mean at each multifoliate leaflet type is represented by a vertical bar.

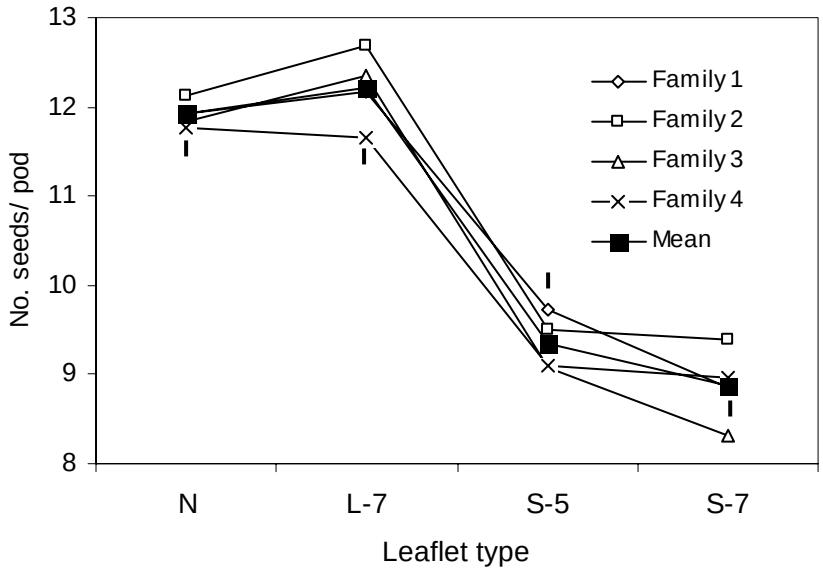

Figure 9. Effect of multifoliate leaflet types on number of seeds per pod in 4 mungbean families each with 4 isogenic lines. S.E. of mean at each multifoliate leaflet type is represented by a vertical bar.

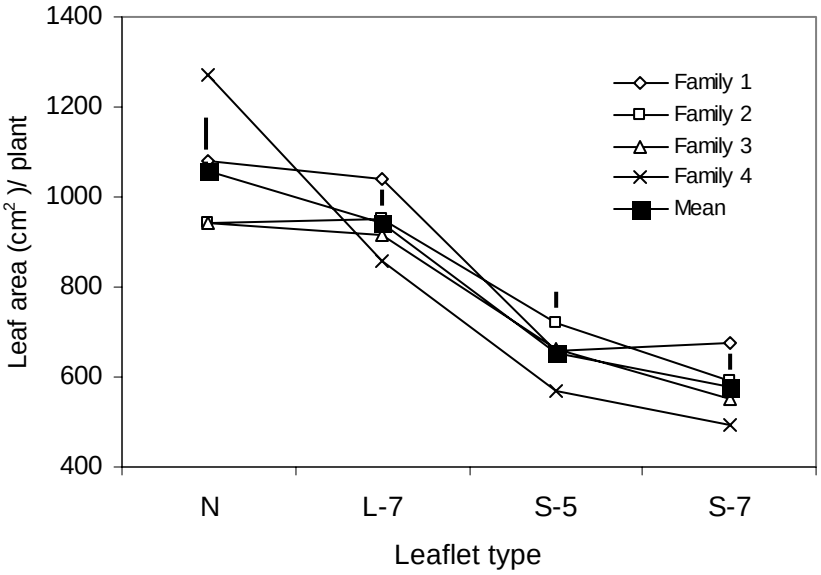

Figure 10. Effect of multifoliate leaflet types on leaf area per plant in 4 mungbean families each with 4 isogenic lines. S.E. of mean at each multifoliate leaflet type is represented by a vertical bar.

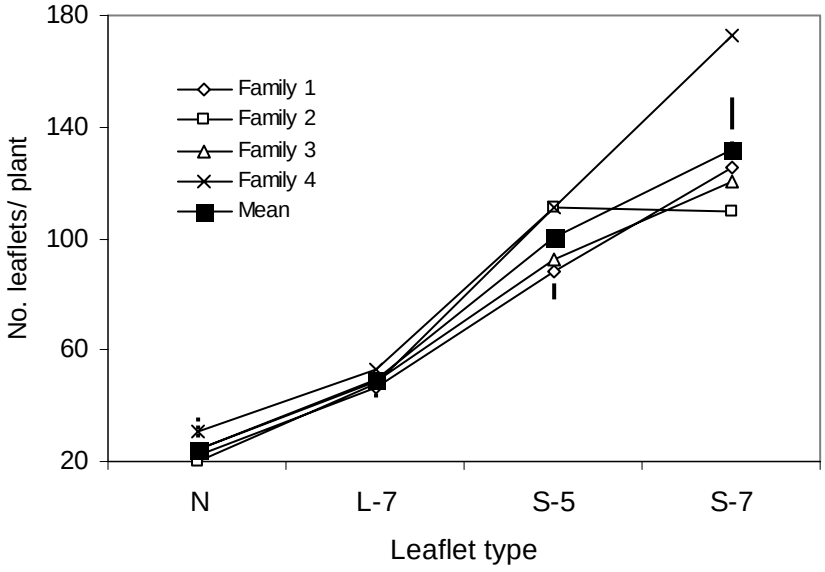

Figure 11. Effect of multifoliate leaflet types on number of leaflets per plant in 4 mungbean families each with 4 isogenic lines. S.E. of mean at each multifoliate leaflet type is represented by a vertical bar.

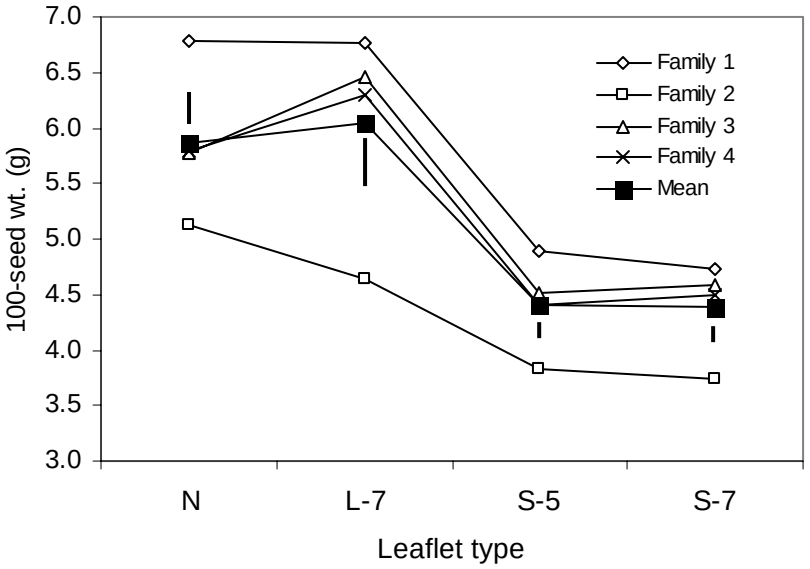

Figure 12. Effect of multifoliate leaflet types on 100-seed weight in 4 mungbean families each with 4 isogenic lines. S.E. of mean at each multifoliate leaflet type is represented by a vertical bar.

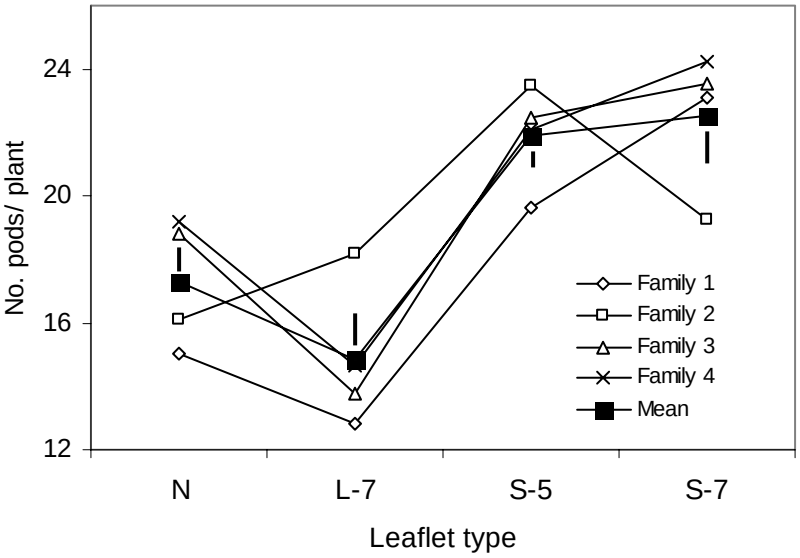

Figure 13. Effect of multifoliate leaflet types on number of pods per plant in 4 mungbean families each with 4 isogenic lines. S.E. of mean at each multifoliate leaflet type is represented by a vertical bar.

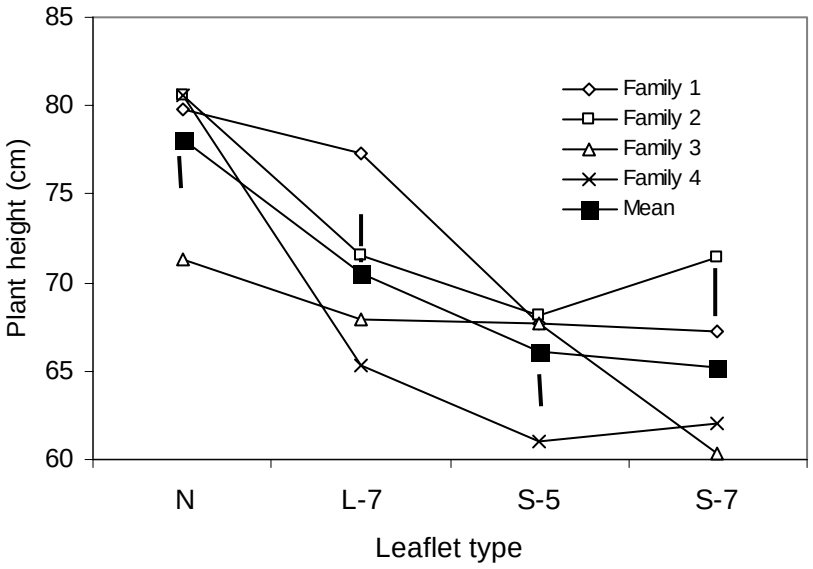

Figure 14. Effect of multifoliate leaflet types on plant height in 4 mungbean families each with 4 isogenic lines. S.E. of mean at each multifoliate leaflet type is represented by a vertical bar.

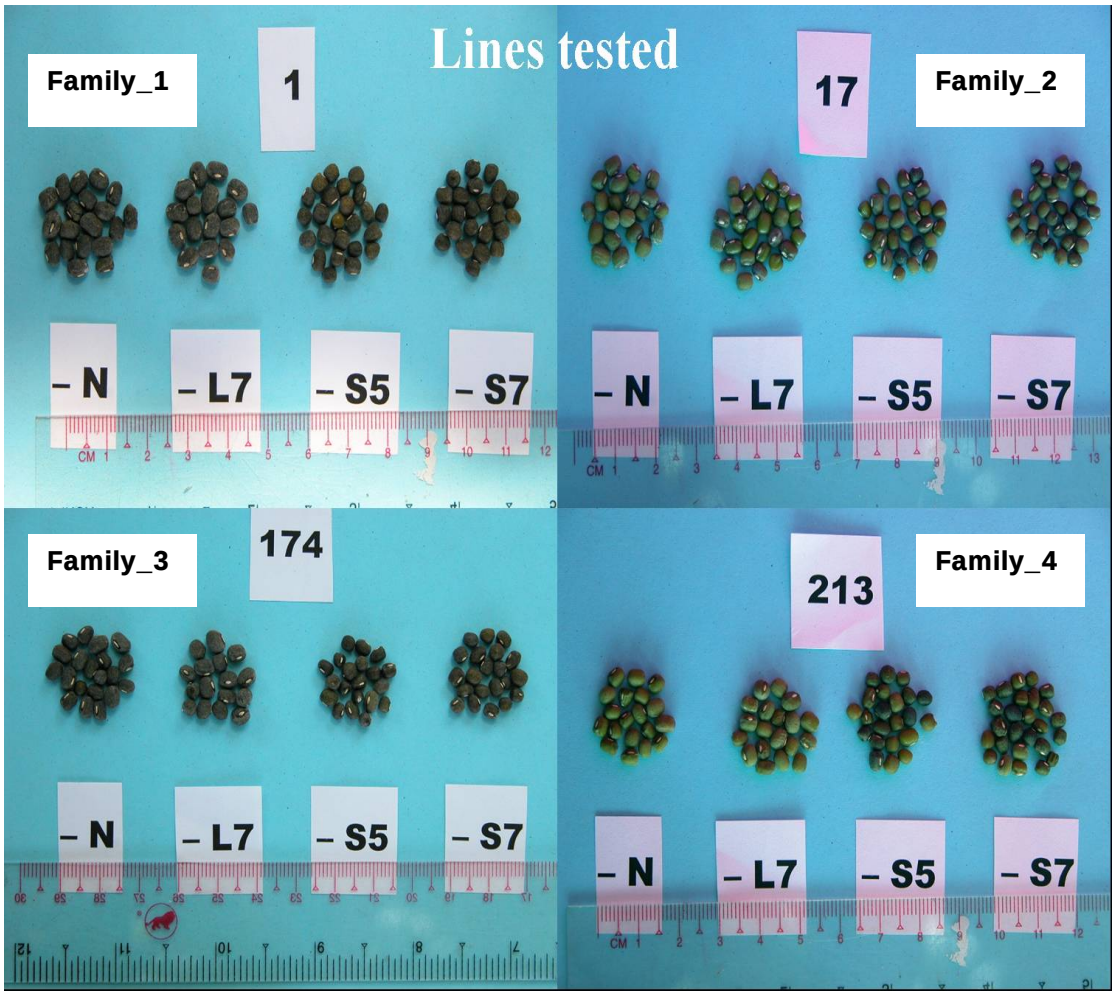

Figure 15. Seed characteristics of 16 isogenic mungbean lines.

Table 15. Correlation between yield and yield components of 16 mungbean lines tested.

Trait	Seed yield	No. leaflets/ leaf	Leaf area/ leaflet	Leaf area/ plant	No. leaflets/ plant	Plant height	No. pods/ plant	No. seeds/ pod
No. leaflets/ leaf	-0.285	-						
Leaf area/ leaflet	0.681 **	-0.736 **	-					
Leaf area/ plant.	0.762 **	-0.483	0.853**	-				
No. leaflets/ plant	-0.832 **	0.547 **	-0.873 **	-0.888 **	-			
Plant height	0.610 *	-0.575 *	0.842 **	0.836 **	-0.765 **	-		
No. pods/ plant	-0.740 **	0.079	-0.601 *	-0.702 **	0.794 **	-0.596 *	-	
No. seeds/ pod	0.752 **	-0.279	0.771 **	0.871 **	-0.894 **	0.694 **	-0.859 **	-
100-seed wt.	0.814 **	-0.192	0.635 **	0.734 **	-0.706 **	0.474	-0.816 **	0.731 **

*significant at $P \leq 0.05$; ** significant at $P \leq 0.01$

Table 16. Nutritional composition of selected mungbean recombinant inbred lines with 7 leaflets. The samples were analyzed by Animal Nutrition Laboratory, Department of Animal Science, Kasetsart University, Kamphaeng Saen Campus, 2005.

Chemical composition *)	RIL no.4				RIL no.55				RIL no.68				RIL no.105			
	Part of plant				Part of plant				Part of plant				Part of plant			
	Stem + petiole	Leaves	Pods + flower	Whole plant	Stem + petiole	Leaves	Pods + flower	Whole plant	Stem + petiole	Leaves	Pods + flower	Whole plant	Stem + petiole	Leaves	Pods + flower	Whole plant
FW/ plant (g)	40	28	9	77	48	21	11	50	36	33	35	104	55	46	42	143
DM/ plant (g)	11.93	9.76	4.42	26.11	19.01	6.66	4.28	29.95	8.69	9.33	11.18	29.2	15.25	13.56	7.56	36.37
DM (%)	29.83	34.87	49.15	33.9	39.6	31.7	38.94	37.4	24.15	28.27	31.93	28	27.72	29.48	18	25.4
NDF (%)	55.02	36.18	36.68	44.87	52.16	34.41	36.24	45.94	59.55	32.29	32.64	40.54	57.36	36.66	30.99	44.16
ADF (%)	45.05	18.94	21.89	31.37	43.46	15.08	19.31	33.7	51.53	18.88	20.03	29.04	47.95	17.05	21.96	31.01
ADL (%)	8.09	3.49	3.81	5.64	7.86	3.67	3.82	6.35	8.67	5.81	3.23	5.67	8.32	4.01	3.03	5.61
Moisture %)												75.15				72.47
Protein (%)												13.53				16.58
Condensed Tannin (%)												1.09				1.20

*) FW:fresh weight ; DM:dry mass; NDF: Neutral Detergent Fiber; ADF:Acid Detergent Fiber; ADL:Acid Detergent Lignin

DISCUSSION

In self-pollinated plant species, it is rather easy to produce hybrid seed if male sterile lines are available and can be used as the female parent. Cross and Schulz (1997) discussed a development in chemical induction of male sterility. There are at least four classes of chemical agents, viz. plant-growth regulators and substances that disrupt floral development, metabolic inhibitors, inhibitors of microspore development, and inhibitors of pollen fertility. Since the hybrid seeds must be harvested from the female parent only, the magnitude of heterosis should be sufficiently high to compensate with the cost of producing open-pollinated seeds in self-pollinating crops. Our significant test show that heterosis obtained from two diverse mungbeans was sizable and worth exploring further.

Hybrid rice breeding has been very successful in China since the 1970s. With the development of photo-thermo-sensitive genic male sterile (P/TGMS) or environment-sensitive genic male sterile (EGMS) lines, a two-line breeding system has been developed as a simplified alternative to the traditional three-line breeding that requires a male-sterile line, a sterility maintainer line, and a fertility restorer (Yuan, 1992). The two-line breeding system is much simplified since an EGMS line can serve as a sterile line under one environmental condition and can propagate itself under different environments. The ability to maintain sterility makes EGMS lines practicable as a female to cross with other lines. In recent years, a number of two-line hybrids have been commercialized in China, and several other Asian countries have established hybrid breeding programs using EGMS lines (Lu *et al.*, 1994; Li and Yuan, 2000).

With the success in the use of hybrid rice varieties, the possibility of using hybrid mungbean should be explored. Chopra (1994) reported a high degree of heterosis for seed yield and its components in almost all grain legumes, which are essentially self-pollinated. A high degree of heterosis was reported in the F_1s of various grain legumes, such as pigeon pea, pea, and lentil (Singh *et al.*, 1975). In mungbean, Khattak *et al*. (2000) found heterosis over the mid-parent for pod clusters

on the main stem in the cross VC 3902A x ML-5. Chen *et al.* (2003) reported that a Korean mungbean variety (K7) gave F1 progenies with significant heterobeltiosis for seed yield in many crosses. In these studies, the magnitudes of heterosis were dependent upon the genotype of the parents. The high heterosis identified in this study and by Chen *et al*. (2003) are encouraging. However, a large-scale production of hybrid seed is possible only when a male sterility system is available, coupled with the availability of insect pollinator. Generally, legume pollen is heavier than that of cereals and thus could not be effectively transferred by wind. These are interesting topics for mungbean breeders to investigate in the future. A male sterile line, if available, should open up a large dimension of yield improvement through the use of hybrid seed in mungbean.

In number of leaflets per leaf, the F_2 population segregated into a 9:3:4 ratio consistently in all 4 F_2 families. A gene action with epistatic expression was proposed for alleles controlling leaflet number. $N_1\_$ gave trifoliate leaf upon the presence of $N_2\_$ genotype, but gave pentafoliate leaflet at the presence of n_2n_2. Whereas n_1n_1 expressed heptafoliate regardless the presence of $N_2\_$ or n_2n_2. The previous study reported by Sripisut and Srinives (1986) showed that lobed and trifoliate leaflets were dominant over normal and multiple leaflets. Each trait was governed by a single locus of gene on different chromosomes. Chhabra (1990) observed that trifoliate (normal) trait was monogenically dominant over pentafoliate in mungbean. Thus it is clear that the small heptafoliate (with the proposed genetic symbol $n_1n_1n_2n_2$) mutant allele in this study is not the same as those previously reported. The F_2 population showed segregation in leaflet size and leaflet number into 9:3:3:1 ratio, indicating that these 2 characters were each controlled by a separate locus of genes.

Three markers, AAA_CTT3, ACG_CAC1, and GCC_ACT3, showed association with genes controlling leaflet number, 2 of them also linked to number of leaflets per leaf. Marker AAA_CTT3 was from P_2 allele, while ACG_CAC1 and GCC_ACT3 were from P_1 alleles. There are 3 markers associated with N_1, n_1, N_2, and n_2 alleles which can be confirmed with phenotypic data (Appendix Table 5). The marker AAA_CTT3 was most likely linked to N_1 allele, while markers ACG_CAC1

and GCC_ACT3 linked to n_1. These 3 markers did not correspond to other traits and it was shown evidently that number of leaflets per leaf had no correlation with seed yield, yield components, and leaf area per plant (Table 14). This finding supported that the genes controlling leaflet size and leaflet number located at different chromosomes or probably in the same chromosome but far in distance.

There were 5 AFLP markers, viz. AAA_CTA1, ACG_CAG4, ACG_CAG1, ACT_ACG, and CT_AAT showing association with leaflet size, leaf area per leaflet, leaf area per plant, number of leaflets per plant, seed yield, 100-seed weight, and number of pods per plant. Phenotypic correlation among these characters indicated that the markers linked to the genes controlling these characters. The association between these markers and the genes controlling seed yield had negative effect to number of leaflets per plant and number of pods per plant. Unfortunately that the genes conditioning higher number of leaflets per plant and number of pods per plant were adversely correlated with yield potential. Although a set of AFLP markers was identified to linked with leaflet and agronomic characters, more investigation on their map distance need to be explored.

In this study, the trifoliate N and L-7 lines were higher in leaf area and yield. These results supported the earlier finding of Kowsurat *et al*. (1999). Eventhough S-5 and S-7 had numerous leaflets distributing evently in the canopy, each leaf is comparatively much smaller in area than those of N and L-7, thus consequently absorbs less sun light. The number of leaflets per plant was found negatively correlated with leaf area (Table 15).

Although the small leaflet mungbean set more pods per plant, it also has smaller seed and less number of seeds per pod. Thus it may be used as a source to increase pod number to improve seed yield. However, the plant breeder must break the negative linkage with seed size and seeds per pod in order to utilize this trait.

The yield varied in different leaflet types with the same trend in all families as well as for leaf area, number of seeds per pod, and seed weight (Fig. 8, 9, 10 and 12),

while yield potential was less in small multifoliate leaflets (S-5 and S-7). This indicated that the leaflet types significantly influenced seed yield, although the genetic background is up to 94 % homozygous among lines within the same family. Thus the difference between lines within each family is clearly affected by the qualitative genes controlling the leaflet types.

Days to flowering and maturity showed no difference among multifoliate lines. Tickoo *et al*. (1996) explained that in mungbean, like in other grain legumes, flowering comes in different flushes. In rainy season, the flowering continues until harvesting and plants tend to become indeterminate, thus a competitive phase for photosynthate starts between developing seeds or pods and the vegetative plant parts. Vigorous growth before flowering is needed to encourage assimilate production in order to be translocated into the seed after flowering (Tickoo *et al*., 1996 and Kuo *et al*., 1978). The continuation of vegetative growth would utilize a certain amount of the source, thereby diverting it from the sink.

Isogenic lines used in this study showed less genetic variation as confirmed by less polymorphism of the AFLP markers, suggesting that the genetic background among the isogenic lines are similar, except for the leaflet trait. Effect of leaflet types in mungbean was clear on yield and yield components. However, the multiple leaflet type in this study did not give yield advantage over the normal trifoliate lines. This experiment can serve as a model to discover the association between a major mutant character and yield which is a promising approach to increase yield and yield components in mungbean.

CONCLUSION

The yield superiority of the F_1 hybrid over the mid- and better parents is evident in mungbean. A simple t-statistical test was developed to help support evidence of heterosis and heterobeltiosis. The test is sufficiently sensitive to be able to conclude that mid-parent and better parent heterosis occurred in varying degrees for seed yield and its components. The highest mid- and better parent heterosis for seed yield were 95.7% and 78.5%, respectively, as shown in the cross S-5 x L-7. Commercialization of hybrid cultivars in mungbean can be justified by the significant heterosis in yield. Future research should be directed to commercial hybrid seed production.

Crossing between 7 large leaflet (L-7) and 5 small leaflet (S-5) mungbean mutants resulted in the normal-trifoliate (N) F_1. The F_2 can be classified into number of leaflets per leaf with tri- ($N_1\_N_2\_$), penta- ($N_1\_n_2n_2$), and heptafoliate ($n_1n_1N_2\_$ and $n_1n_1n_2n_2$) at the dihybrid ratio of 9:3:4. The finding is thus evident that leaflet number character was controlled by n_1 and n_2 loci of genes. However, all 3 AFLP markers associated with leaflet number in this study corresponded to n_1 locus only. The n_2 locus might be closely linked to the *s* locus such that there was no progenies with large pentafoliate leaflet (hypothetically carrying $N_1\_n_2n_2S\_$ genotype).

Four F_5 families were derived from the cross. Each family has 4 lines of each leaf type but 93.75 % uniform in the other genetic background. The results from yield testing revealed that normal and L-7 multifoliate lines gave higher leaf area and yield than the other multifoliate lines tested over three environments. Although S-5 and S-7 had numerous leaflets but comparatively much smaller in leaf area. Yet, the plants are shorter with fewer number of seeds, thus produced lower yield.

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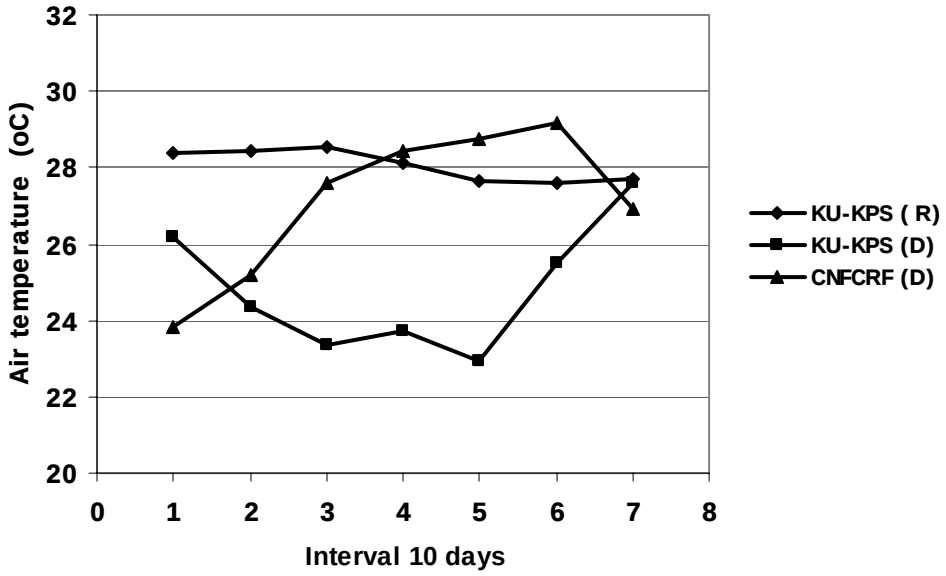
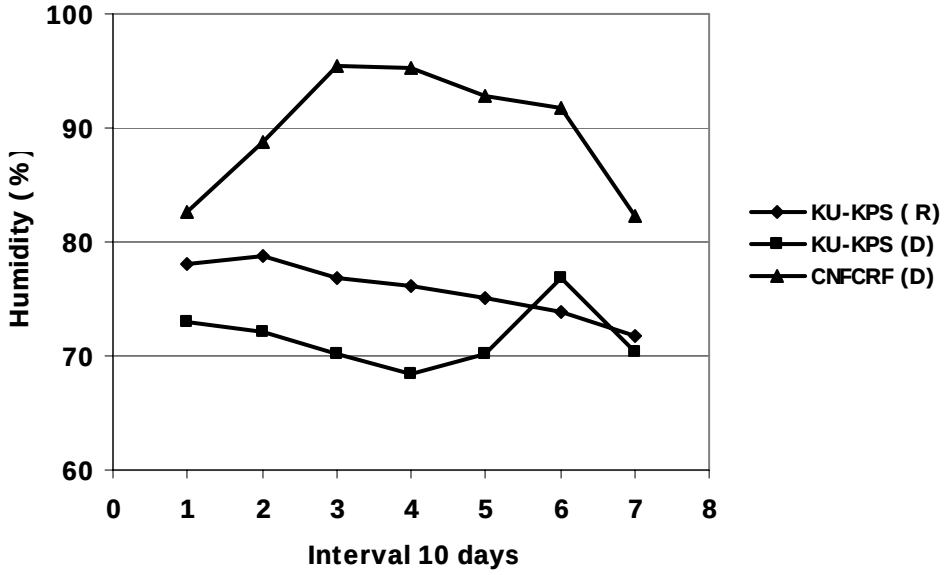
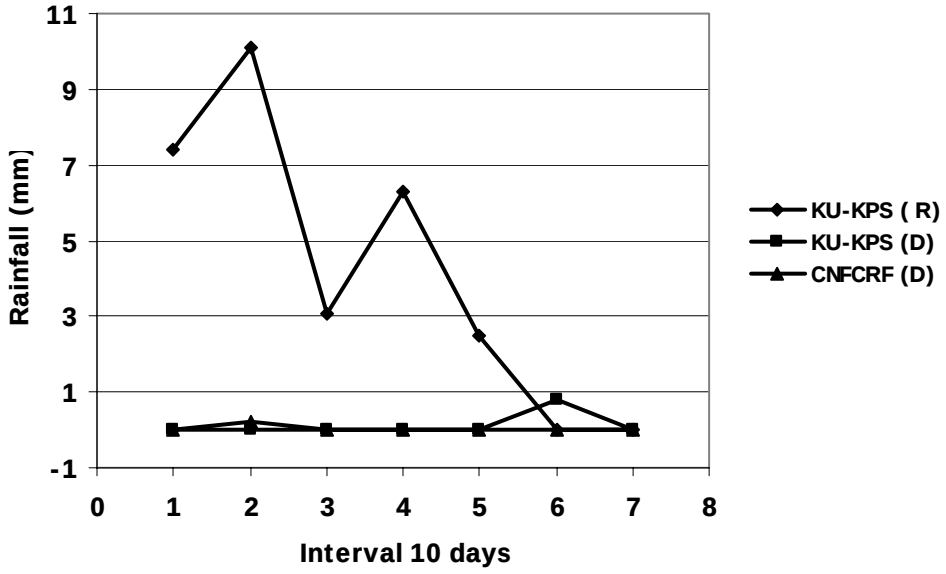
APPENDIX



Appendix Figure 1 Air temperature during crop growing period at Kasetsart University, Kamphaeng Saen Campus (KU-KPS) in rainy (R) and dry (D) season, and at Chainat Field Crops Research Center (CNFCRC) in dry season (D), 2004/2005.



Appendix Figure 2 Air humidity during crop growing period at Kasetsart University, Kamphaeng Saen Campus (KU-KPS) in rainy (R) and dry (D) season, and at Chainat Field Crops Research Center (CNFCRC) in dry season (D), 2004/2005.



Appendix Figure 3 Rainfall during crop growing period at Kasetsart University, Kamphaeng Saen Campus (KU-KPS) in rainy (R) and dry (D) season, and at Chainat Field Crops Research Center (CNFCRC) in dry season (D), 2004/2005.

Appendix Tabel 1. Combined analysis of variance of yield (kg/ha), leaf area per plant (cm^2), and number of leaflets per plant of 20 mungbean genotypes tested over 3 environments.

Source of Variation	DF	Mean square		
		Yield	Leaf area	No. leaflets per plant
Environment	2	4987491.5 **	1250940 *	14015.9 **
Rep./envs. (Error a)	9	339930.8	216531.6	544.5
Genotype	19	1018185.6 **	636838.4 **	24637.9 **
Between Check	3	1415756.9 **	796173.5 **	20138.7 **
Check vs Lines	1	2445210.9 **	653994.5 **	30016.7 **
Between Lines	15	843536.3 **	603827.6 **	25179.2 **
Genotype x Env.	38	196044.0 **	53946.88 *	784.2 **
Error (b)	171	45523.0	35826.77	330.7
Total	239			

*significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; ns = non-significant

Appendix Tabel 2. Combined analysis of variance of number of pods per plant, number of seeds per pod, and 100-seed weight (g) of 20 mungbean genotypes tested over 3 environments.

Source of Variation	DF	Mean square		
		No. pods Per plant	No. seeds per pod	100-seed weight
Environment	2	1252.95 **	1.4 ns	141.73 **
Rep./envs. (Error a)	9	65.6	0.9	0.1
Genotype	19	217.95 **	29.2 **	17.67 **
Between Check	3	452.63 **	29.4 **	34.80 **
Check vs Lines	1	203.78 **	24.3 **	50.47 **
Between Lines	15	171.95 **	29.5 **	12.06 **
Genotype x Env.	38	68.50 **	1.2 **	0.18 **
Error (b)	171	28.38	0.5	0.04
Total	239			

*significant at P ≤ 0.05; ** significant at P ≤ 0.01; ns = non-significant

Appendix Tabel 3 Combined analysis of variance of days to flowering, days to maturity, and plant height (cm) of 20 mungbean genotypes tested over 3 environments.

Source of Variation	DF	Mean square		
		Days to flowering	Days to maturity	Plant height
Environment	2	450.50 **	3986.18 **	7834.95 **
Rep./envs. (Error a)	9	17.4	20.9	216.1
Genotype	19	6.39 **	7.64 **	547.52 **
Between Check	3	2.08 ns	9.30 **	756.69 **
Check vs Lines	1	4.40 ns	7.18 ns	176.65 *
Between Lines	15	7.38 **	7.33 **	530.41 **
Genotype x Env.	38	2.14 ns	3.10 *	62.72 **
Error (b)	171	1.93	2.09	38.45
Total	239			

*significant at P ≤ 0.05; ** significant at P ≤ 0.01; ns = non-significant

Appendix Table 4 Mean yield, yield components, and some agronomic characters of 20 mungbean genotypes tested over 3 environments.

No.	Genotype	Yield (kg/ha)	Days to flowering	Days to maturity	No. of leaflets /pl.	Leaf area /pl. (cm^2)	Plant ht. (cm)	No. of pods /pl.	No. of seeds /pod	100-seed wt. (g)
1	P1(LM)	1144 b	37.9 a-f	58.9 e	44.7 ef	993 bcd	73.4 bcd	13.3 fg	11.8 bc	7.02 b
2	P2(SM)	589 fg	38.5 a-d	60.5 bcd	107.6 c	560 fg	61.0 h	25.9 a	9.1 de	3.78 l
3	KPS1	1365 a	37.8 b-g	59.8 cde	20.1 g	1070 bc	74.3 bc	15.4 efg	12.3 abc	7.13 b
4	CN36	1246 ab	38.6 a-d	60.9 a-d	21.8 g	1126 ab	79.9 a	12.7 g	12.4 ab	7.36 a
5	1-N	1126 bc	38.9 ab	62.1 a	22.1 g	1081 bc	79.8 a	15.0 efg	11.9 bc	6.78 c
6	1-L7	1252 ab	38.3 a-d	60.6 bcd	46.9 e	1041 bc	77.3 ab	12.8 g	12.2 abc	6.77 c
7	1-S5	838 de	38.6 a-d	60.5 bcd	88.2 d	658 fg	67.7 def	19.6 b-e	9.7 d	4.89 h
8	1-S7	795 de	38.3 a-e	61.1 abc	125.8 b	674 f	67.2 efg	23.1 a-d	8.9 ef	4.74 hi
9	17-N	900 d	37.3 d-g	59.6 de	19.9 g	943 cd	80.6 a	16.1 efg	12.1 abc	5.13 g
10	17-L7	956 cd	37.5 c-g	59.5 de	47.6 e	949 cd	71.6 cde	18.2 def	12.7 a	4.65 ij
11	17-S5	431 gh	38.8 abc	60.9 a-d	111.0 bc	718 ef	68.1 def	23.4 abc	9.5 de	3.82 l
12	17-S7	553 fgh	39.2 a	61.6 ab	109.5 c	590 fg	71.4 cde	19.2 b-e	9.4 de	3.73 l
13	174-N	1216 ab	36.9 efg	59.7 de	24.3 g	940 cd	71.3 cde	18.8 cde	11.8 bc	5.78 f
14	174-L7	959 cd	37.9 a-f	60.3 b-e	48.8 e	915 cd	68.0 def	13.8 fg	12.4 abc	6.46 d
15	174-S5	875 de	37.7 b-g	60.3 b-e	92.6 d	660 fg	67.7 def	22.5 a-d	9.1 de	4.51 jk
16	174-S7	694 ef	37.5 c-g	60.3 bcd	120.2 bc	550 fg	60.3 h	23.5 abc	8.3 f	4.58 ij
17	213-N	938 cd	38.1 a-f	61.2 ab	30.5 fg	1270 a	80.5 a	19.2 cde	11.8 bc	5.80 f
18	213-L7	942 cd	36.8 fg	59.5 de	53.1 e	860 ed	65.3 fgh	14.7 efg	11.7 c	6.29 e
19	213-S5	471 gh	36.5 g	59.6 de	111.0 bc	569 fg	61.1 h	22.1 a-d	9.1 de	4.41 k
20	213-S7	392 h	37.4 c-g	60.7 bcd	172.7 a	491 g	62.1 gh	24.2 ab	9.0 e	4.49 jk
	cv (%)	24.1	3.7	2.4	25.6	22.7	8.8	28.5	6.8	3.6
	mean	884	37.9	60.4	70.9	832.9	70.4	18.7	10.8	5.41

Mean followed by the same letter in each column are not significantly different using Duncan's Multiple Range Test (P=0.05).

Appendix Table 5. Binary data of AFLP markers in 16 lines of multiple leaflet mungbean and their parents.

N0	Marker	P1 (L-7)	P2 (S-5)	Family_1				Family_2				Family_3				Family_4			
				N	S5	S7	L7	N	S5	S7	L7	N	S5	S7	L7	N	S5	S7	L7
1	AA_AAT	0	1	0	1	.	1	1	0	0	1	1	0	1	1	1	1	1	1
2	AA_ACG1	0	1	0	0	0	1	1	1	0	1	1	0	1	1	0	0	1	1
3	AA_ACG2	0	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	0
4	AA_ACG3	0	1	1	1	0	1	1	1	1	1	1	0	0	0	1	0	0	1
5	AA_ACG4	0	1	0	0	0	1	1	0	1	1	1	0	1	0	1	1	1	1
6	AA_ACG5	0	1	1	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1
7	AAA_CAG1	0	1	0	0	0	1	1	0	0	1	1	.	1	.	0	0	1	1
8	AAA_CAG2	1	0	0	0	0	1	1	0	0	1	1	.	1	.	1	1	1	1
9	AAA_CAG3	1	0	1	0	0	0	1	0	0	1	1	.	1	.	1	1	1	1
10	AAA_CAG4	1	0	1	0	1	0	1	1	0	1	1	.	1	.	0	1	1	0
11	AAA_CAG5	0	1	1	0	1	0	1	0	0	0	1	.	1	.	1	0	1	1
12	AAA_CTA1	0	1	0	0	1	0	0	1	1	0	0	.	1	.	0	1	0	0
13	AAA_CTA2	0	1	0	0	1	1	0	1	0	0	1	.	0	.	1	1	1	1
14	AAA_CTA3	1	0	0	0	0	0	1	1	1	1	1	.	1	.	0	0	1	1
15	AAA_CTC1	1	0	1	1	1	1	0	0	0	1	1	.	1	.	1	1	1	1

Appendix Table 5. (cont'd.)

N0	Marker	P1 (L-7)	P2 (S-5)	Family_1				Family_2				Family_3				Family_4			
				N	S5	S7	L7	N	S5	S7	L7	N	S5	S7	L7	N	S5	S7	L7
16	AAA_CTC2	1	0	0	0	0	1	1	0	0	1	1	.	1	.	0	1	1	1
17	AAA_CTC3	1	0	0	1	1	1	0	0	0	0	1	.	0	.	1	0	0	1
18	AAA_CTC4	0	1	1	1	1	1	0	0	0	0	1	.	1	.	0	0	0	0
19	AAA_CTT1	0	1	0	0	0	1	1	0	0	1	1	.	1	.	1	1	1	1
20	AAA_CTT2	0	1	0	1	1	0	1	1	1	0	0	.	1	.	0	1	0	0
21	AAA_CTT3	0	1	1	1	1	1	0	1	0	0	1	.	0	.	1	1	0	1
22	AAA_CTT4	1	0	0	0	0	0	1	1	1	1	1	.	1	.	0	0	0	0
23	ACC_AGG1	0	1	0	0	0	1	1	0	0	0	1	.	1	.	1	1	1	1
24	ACC_AGG2	0	1	0	0	0	0	1	1	1	1	1	.	1	.	1	1	1	1
25	ACC_ATG	1	0	0	0	0	1	1	0	0	0	1	.	1	0	1	1	1	1
26	ACG_CAC1	1	0	0	0	0	1	1	0	1	1	0	.	1	.	0	1	1	1
27	ACG_CAC2	1	0	0	0	0	1	1	1	1	1	0	.	1	.	0	1	1	0
28	ACG_CAC3	1	0	0	0	0	0	1	1	1	1	0	.	0	.	0	1	1	0
29	ACG_CAG4	0	1	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0
30	ACG_CAG3	1	0	0	0	0	1	1	0	0	1	1	0	1	0	1	1	1	1

Appendix Table 5. (cont'd.)

N0	Marker	P1 (L-7)	P2 (S-5)	Family_1				Family_2				Family_3				Family_4			
				N	S5	S7	L7	N	S5	S7	L7	N	S5	S7	L7	N	S5	S7	L7
31	ACG_CAG1	0	1	1	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0
32	ACG_CAG6	0	1	0	0	0	1	1	0	0	1	1	0	1	1	1	1	1	1
33	ACG_CAG5	0	1	1	1	1	1	0	1	1	0	1	1	0	1	0	0	0	0
34	ACG_CAG2	0	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1	1	1
35	ACG_CTT1	0	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1	1
36	ACG_CTT2	1	0	1	0	0	0	0	0	1	1	0	1	0	1	1	1	0	1
37	ACG_CTT3	1	0	0	0	0	1	1	0	0	1	1	0	1	0	0	0	1	1
38	ACT_ACG	1	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1
39	ACT_AGC	1	0	0	1	1	1	1	1	1	0	0	1	1	0	1	1	1	0
40	ACT_AGG1	1	0	1	0	0	0	1	1	1	1	0	0	1	1	0	0	1	0
41	ACT_AGG2	1	0	0	1	1	1	1	1	1	1	0	0	0	1	0	0	1	0
42	CAG_ACG1	0	1	0	0	1	1	1	1	0	1	1	.	1	0	1	1	1	1
43	CAG_ACG2	1	0	0	1	1	1	1	1	1	1	1	.	1	0	1	1	1	1
44	CAG_ACG3	0	1	1	0	1	0	0	1	1	0	0	.	0	0	0	0	1	0
45	CAG_ACT1	1	0	0	0	0	1	1	1	0	1	1	.	1	1	1	1	1	1

Appendix Table 5. (cont'd.)

N0	Marker	P1 (L-7)	P2 (S-5)	Family_1				Family_2				Family_3				Family_4			
				N	S5	S7	L7	N	S5	S7	L7	N	S5	S7	L7	N	S5	S7	L7
46	CAG_ACT2	0	1	0	0	0	1	1	0	0	1	1	.	1	1	1	1	1	1
47	CAG_ACT3	0	1	0	0	0	0	1	1	0	0	0	.	0	0	1	0	0	1
48	CT_AAT	0	1	0	1	1	0	1	1	1	1	0	0	1	0	0	1	1	1
49	GCA_ACA	0	1	1	0	0	1	0	1	1	0	1	.	1	0	1	1	1	1
50	GCC_ACA	1	0	1	1	1	1	0	0	0	0	1	0	1	0	1	1	1	1
51	GCC_ACA1	0	1	1	1	1	1	0	1	1	0	0	1	1	0	1	1	1	1
52	GCC_ACA2	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0
53	GCC_ACT1	1	0	0	0	1	0	0	1	1	1	0	.	1	0	1	1	1	0
54	GCC_ACT2	1	0	0	1	1	0	1	1	1	1	0	.	1	0	0	1	1	1
55	GCC_ACT3	1	0	0	0	1	1	0	1	1	1	0	0	1	0	1	1	1	1
56	GCC_ACT4	1	0	0	0	1	1	1	1	0	0	1	0	1	1	1	1	1	1