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# Genetics, Agronomic, and Molecular Study of Leaflet Mutants in Mungbean (*Vigna radiata* (L.) Wilczek)

**Rudy Soehendi[1], Sontichai Chanprame[2], Theerayut Toojinda[3], Sumana Ngampongsai[4] and Peerasak Srinives[2*]**

[1] *Indonesian Legumes and Tuber Crops Research Institute (ILETRI), Malang 65101, Indonesia*
[2] *Department of Agronomy, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*
[3] *Rice Gene Discovery Unit, National Center for Genetic Engineering and Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*
[4] *Chai Nat Field Crops Research Center, Chai Nat 17000, Thailand*

## Abstract

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 could alter seed yield. Two multiple leaflet mutants were obtained from gamma-ray irradiation and used to study the mode of inheritance related to leaflet types and to evaluate their agronomic features. The cross between large-heptafoliate leaflet with small-pentafoliate leaflet mutants produce all $F_1$ plants with normal trifoliate leaflets. The $F_2$ plants segregated in leaflet size and leaflet number into a 9:3:3:1 ratio of large-trifoliate: large-heptafoliate: small-pentafoliate: small-heptafoliate plants, suggesting that independent loci control leaflet size and leaflet number. Regarding leaflet number, the $F_2$ population can be classified into normal-trifoliate, small-pentafoliate, large-heptafoliate, and small-heptafoliate at the dihybrid ratio of 9:3:3:1. The gene symbols $N_1,n_1$ and $N_2,n_2$ are proposed to represent leaflet number. Since no plant was found with large-pentafoliate leaflets, we hypothesize that the $N_2$ allele expresses pleiotropic effect on both leaflet number and leaflet size. Another possibility is that an additional locus with *S* and *s* alleles controls leaflet size and *S* is tightly linked with $N_2$. The effect of multifoliate leaflet on yield and yield components was evaluated in four mungbean families each with four leaflet isolines under three environments. Averaging across the families and environments, the normal-trifoliate and large-heptafoliate lines gave higher yield than small pentafoliate and heptafoliate ones. These two large leaflet lines also had higher leaf area per plant than the other multifoliate lines. Therefore, the mungbean lines with a greater leaf area, which were likely to intercept more sunlight, gave greater yield. Three AFLP markers that were found to be linked to number of leaflets per leaf, corresponded to the $N_1$ allele of the small-pentafoliate parent.

Key words: *Vigna radiata*, mungbean, multifoliate, inheritance, AFLP marker, trait correlation

## Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is a widely-grown, short-duration grain legume crop grown 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 from 500 to 600 kg/ha. One reason is due to the use of traditional cultivars and low management inputs used by most farmers.

Mungbean plants generally have a relatively close canopy compared 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 may alter seed yield. A new mungbean variety 'Samgang' was released in Korea with lobed leaflets that can intercept more sunlight 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

* **To whom correspondence should be addressed**
Peerasak Srinives
E-mail: agrpss@yahoo.com
Tel: +66-34281267

low compared to 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. Sandhu and Saxena (2003) reported high variability found in 34 mungbean mutants for yield per plant and nutritional quality traits such as the content of protein, methionine, tryptophan, sulfur, phenol, and total sugars.

Multiple leaflet mutants express the potential to alter mungbean yield and thus are worth a more detailed investigation on inheritance of the trait, effect of the trait on yield and yield components, and gene tagging using molecular markers. The use of certain mutants can help to break the yield limit encountered in the available mungbean germplasm. Dwivedi and Singh (1985) reported that narrow leaf character in mungbean appears to be governed by two recessive genes symbolized by $nl_1$ and $nl_2$, 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 environmental 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, a crop for which molecular marker technology is at the beginning stage. AFLP markers were chosen in this study because of their excellent reproducibility which is essential if screening protocols are to be established (Jones *et al.* 1998; Matthes *et al.* 1998). AFLP can screen a high number of loci for polymorphism and simultaneously detect a greater number of DNA markers than any other polymerase chain reaction-based detection system (Vos *et al.*1995). A linkage map has recently been developed in some crops including the genus *Vigna* (Tomooka *et al.* 2002; Somta *et al.* 2006).

The objectives of this experiment were: 1) to study the inheritance of multifoliate leaflet mutants in mungbean; 2) to study the effect of multifoliate leaflet character on yield and its components; and 3) to identify AFLP markers associated with the multifoliate leaflet character.

## Materials and Methods

### Plant materials

A cross was made between two parental lines, one with large-heptafoliate leaflets (L-7) and the other with small-pentafoliate leaflets (S-5) during the early rainy season of 2002 at Kasetsart University, Kamphaeng Saen Campus, Thailand. The L-7 parent is a $BC_9$ progeny having the most popular Thai cultivar 'Kamphaeng Saen 1' as the recurrent parent and the large-heptafoliate leaflet mutant (V5926) from the World Vegetable Center (AVRDC), Taiwan, as the donor parent (Kowsurat et al. 1999). The S-5 parent is a 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 leaflet size of this mutant is only about 1/5 of the normal (large) leaflet and thus they can be distinctly differentiated (Fig. 1). 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 sown and harvested individually 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 number of $F_2$ plants was recorded according to leaflet number classified into three, five, and seven leaflets, and leaflet size into large and small leaflets.

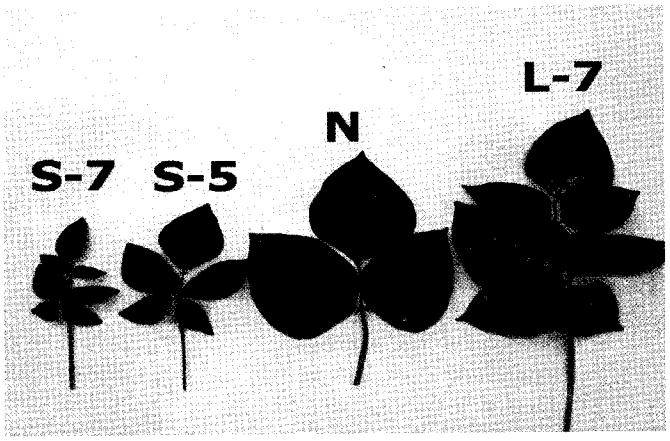


**Fig. 1.** Leaflet of $F_2$ mungbean progenies derived from the cross between L-7 and S-5.

### Genetic data analysis

The number of $F_2$ plants in each leaflet type was tested againt the 3:1 Mendelian ratio using the Chi-square ($x^2$) goodness-of-fit test suggested by Mather (1951). Since all the $F_1$ plants have normal trifoliate leaf, we hypothesize that the leaflet number is controlled by two loci of genes which can be assigned as $N_1$ and $N_2$, respectively. Thus, the trifoliate leaf type should carry a dominant gene in each locus, i.e. $N_1\_N_2\_$. The segregation was tested against a 9:3:3:1 ratio. The heterogeneity among the four $F_2$ families was also tested accordingly.

### Extraction of recombinant inbred lines

From $F_2$ onward, the normal trifoliate leaflet plants (with the hypothetical genotype $N_1\_N_2\_$) were individually harvested each time to obtain the $N_1\_N_2\_$ plants. The process was repeated until $F_5$ when four families each with four phenotypes (normal-trifoliate, large-heptafoliate, small-pentafoliate, and small-heptafoliate) were extracted (Fig. 2). Seed 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.

### Field experiments

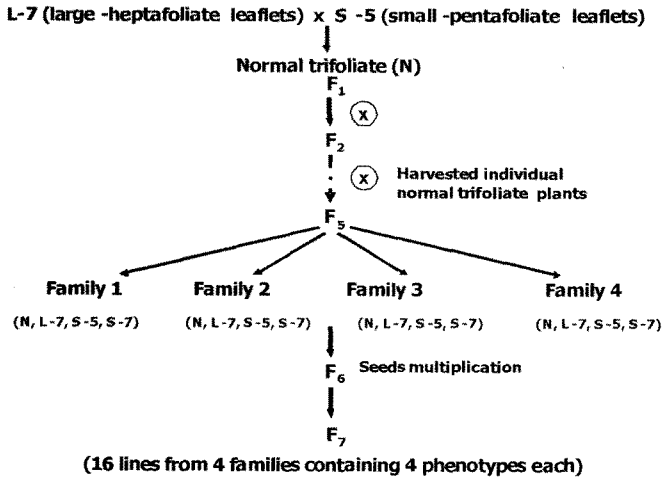


**Fig. 2.** Extraction of 4 $F_{5:7}$ mungbean families, each with four leaflet types.

Three field experiments were conducted during September 2004 to March 2005. Two experiments were done at Kasetsart University, Kamphaeng Saen Campus (KU-KPS) in the rainy and dry seasons, and at Chainat Field Crops Research Center (CNFCRC), Thailand in the dry season. The soil type in the experimental fields is sandy clay loam. The experiment was arranged in a randomized complete block design (RCBD) with four replications. The 18 treatments were four isogenic lines from each of the four families and their parents. A plot size of 10 $m^2$ (four rows of 5 m-long) was planted for each line in a replication with a spacing of 50 cm between rows and 12.5 cm between plants within rows, with one plant per hill. The recommended practices for optimum mungbean growth and protocols of data collection were according to Park (1978). Days to flowering and maturity were recorded when 50% of the plants showed open flowers and mature pods, respectively. At the pod-filling stage, the number of leaflets per plant and leaf area per plant ($cm^2$) were determined 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 collected from two middle rows, skipping 50 cm at each end of the row so that the harvested area in each plot was 4 $m^2$.

### 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 (Gomez and Gomez, 1984) was performed across the three experiments. 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 = 05.

### AFLP marker analysis

Young expanded leaves from three plants in each of the 16 mungbean lines and their parents were collected for DNA extraction using the modified CTAB method of Doyle and Doyle (1987). Two hundred nanograms of genomic DNA from each line was digested and ligated simultaneously in a total volume of 30 µl at 37 °C overnight. The genomic DNA was digested with 10U *Eco*RI and 10U *Mse*I (Fermentaz Life Sciences, Hanover, Maryland, USA), while ligation required adapters of 5 mol of *Eco*RI and 50 mol of *Mse*I.

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 EcoRI and MseI 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 mM) and 0.2 µl of Taq DNA polymerase (Fermentas Life Sciences, Hanover, Maryland, USA) (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-fold and used as the template for 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 at 65 °C for 30 s (less 0.7 °C per cycle after the first cycle), extension at 72 °C for 60 s, denaturation for 24 cycles at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 60 s, followed by the final extension at 72 °C for 2 min.

The PCR II products were loaded on 4.5% denaturing polyacrylamide gel with 1x TBE at 60 W for 80 min. DNA fragments were detected by silver staining method as described by Promega Corp., Madison, Wisconsin, USA. 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. Single factor analysis of variance was carried out to identify the association between leaflet types and AFLP markers using Proc ANOVA (SAS Inst. 1999).

## Results

### Genetic analysis of $F_2$ populations

All the $F_1$ from L-7 x S-5 were trifoliate leaflet plants, suggesting that there were at least two loci controlling the leaflet number. Assuming that the L-7 and S-5 carry the genotype $n_1n_1N_2N_2$ and $N_1N_1n_2n_2$, respectively, the $F_1$ should have the genotype $N_1n_1N_2n_2$. There was no large-pentafoliate plant (L-5) found among several thousand plants segregating from $F_2$ to $F_7$ and in the yield trials. Instead, there were only four classes of leaflet number and size, viz. large-trifoliate, small-pentafoliate, large-heptafoliate, and small-heptafoliate. The number of plants in different leaflet classes from each $F_2$ family were tested against a 9:3:3:1 ratio of the respective phenotypes $N_1\_N_2\_$, $N_1\_n_2n_2$, $n_1n_1N_2\_$, and $n_1n_1n_2n_2$, respectively (Table 1). The $x^2$-test supported the hypothesis that there were two loci controlling the number of leaflets. The combined data did not deviate significantly from the 9:3:3:1 ratio. Heterogeneity among the families was not significant, revealing that the segregation of this trait among the $F_2$ families agreed well with each other. With this model of gene

**Table 1.** Chi square test for independence (9:3:3:1 ratio) between the $n_1$ and $n_2$ alleles controlling leaflet number in four $F_2$ mungbean families from the cross between L-7 and S-5 parents.

| Family | No. of plants | | | | | $X^2_{(3)}$ | Prob |
|---|---|---|---|---|---|---|---|
| | N $N_1\_N_2\_$ | L-7 $n_1n_1N_2\_$ | S-5 $N_1\_n_2n_2$ | S-7 $n_1n_1n_2n_2$ | Total | | |
| 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 |

action, interaction in $N_2$ allele should have a pleiotropic effect on leaflet size, so that the plants with $N_2\_$ and $n_2n_2$ phenotypes always have large and small leaflets regardless of leaflet number.The $x^2$-test for the goodness-of-fit of 3 large:1 small leaflet plants as supposedly controlled by the $n_2$ locus was given in Table 2. The results supported that the $n_2$ locus also conditioned leaflet size in all of the tested families. The $N_1$ allele dictated normal-trifoliate at the present of $N_2$ but showed pentafoliate in $N_1\_n_2n_2$. Whereas $n_1n_1$ genotypes expressed heptafoliate regardless of genotypes in $n_2$ locus (Table 1). Another less likely possibility is that the $n_2$ locus is tightly linked with the third locus (say *s*) controlling the leaflet size. With the latter hypothesis, the *S* allele attaches to the $N_2$ allele so tightly that they always cosegregate so that the respective genotypes for the large-trifoliate, small-pentafoliate, large-heptafoliate, and small-heptafoliate should be $N_1\_N_2\_S\_$, $N_1\_n_2n_2ss$, $n_1n_1N_2\_S\_$, and $n_1n_1n_2n_2ss$, respectively. The theoretical genotype $N_1\_n_2n_2S\_$ (supposedly controlling large-pentafoliate leaflet) was not found in this study.

**Table 2.** Chi-square test for goodness-of-fit against a 3:1 ratio for leaflet size (large vs small) as supposedly controlled by the $n_2$ locus in four $F_2$ mungbean families from the cross between L-7 and S-5 parents.

| Family | No. of plants | | $X^2_{(1)}$ | Prob |
|---|---|---|---|---|
| | $N_2\_$ | $n_2n_2$ | | |
| 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 |

[a] No. of plants with large leaflets ($N_2\_$) was obtained from N and L-7; those with small leaflets ($n_2n_2$) were from S-5 and S-7.

## Effect of multifoliate leaflets on yield and yield components

Analysis of variance for yield and its components were combined across three locations, giving the mean data as shown in Fig. 3. Total number of leaflets per plant differed 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

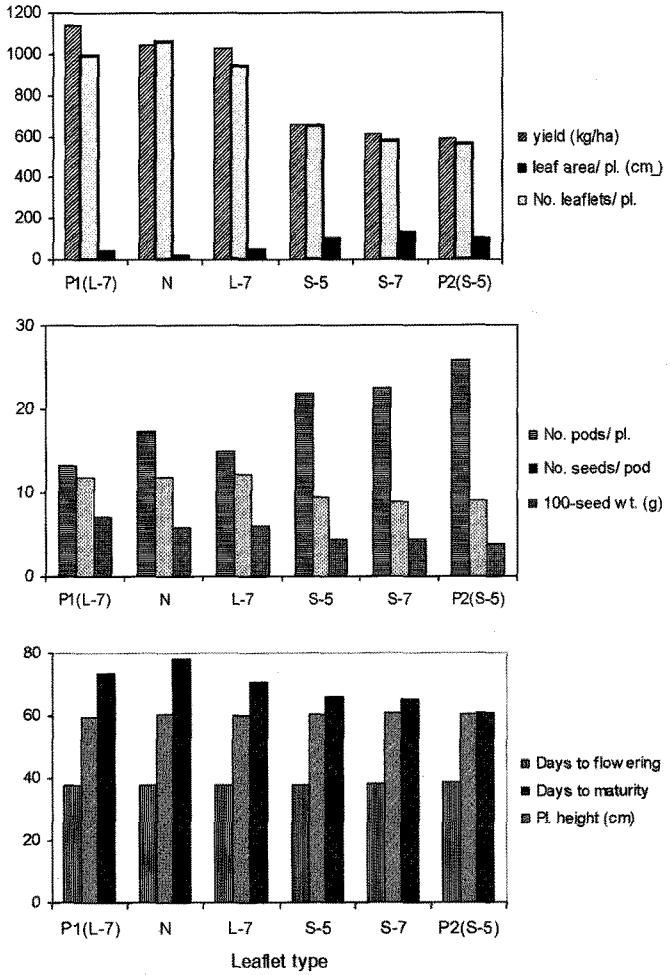


**Fig. 3.** Effect of multifoliate leaflet types on yield, yield components, and some agronomic characters of mungbean lines derived from the cross between L-7 and S-5 parents.

size and number of leaflets, (i.e. S-5 parent and lines; L-7 parent and lines) showed similar total number of leaflets per plant. However, in terms of leaf area per plant, normal trifoliate gave similar value to that of L-7 parent and line, but was higher than that of S-5 and S-7. Among multifoliate lines tested, N had the highest value of 1058.8 $cm^2$, while S-7 gave the smallest value of 576.2 $cm^2$. Even though the number of leaflets per plant of S-7 was the highest, its leaf area per plant was the lowest due to smaller leaflet size throughout the plant. This character also caused shorter plants with smaller and fewer number of seeds, 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. 3). The L-7 parent gave the largest 100-seed weight with an average of 7.02 g. Although the plants were slightly shorter and the number of pods per plant was less than those of the normal genotype; the advantage in seed size resulted in a comparatively high yield in L-7.

For number of seeds per pod, N and L-7 lines had similar values, 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 was also comparable to the parents of the same leaflet type (Fig. 3).

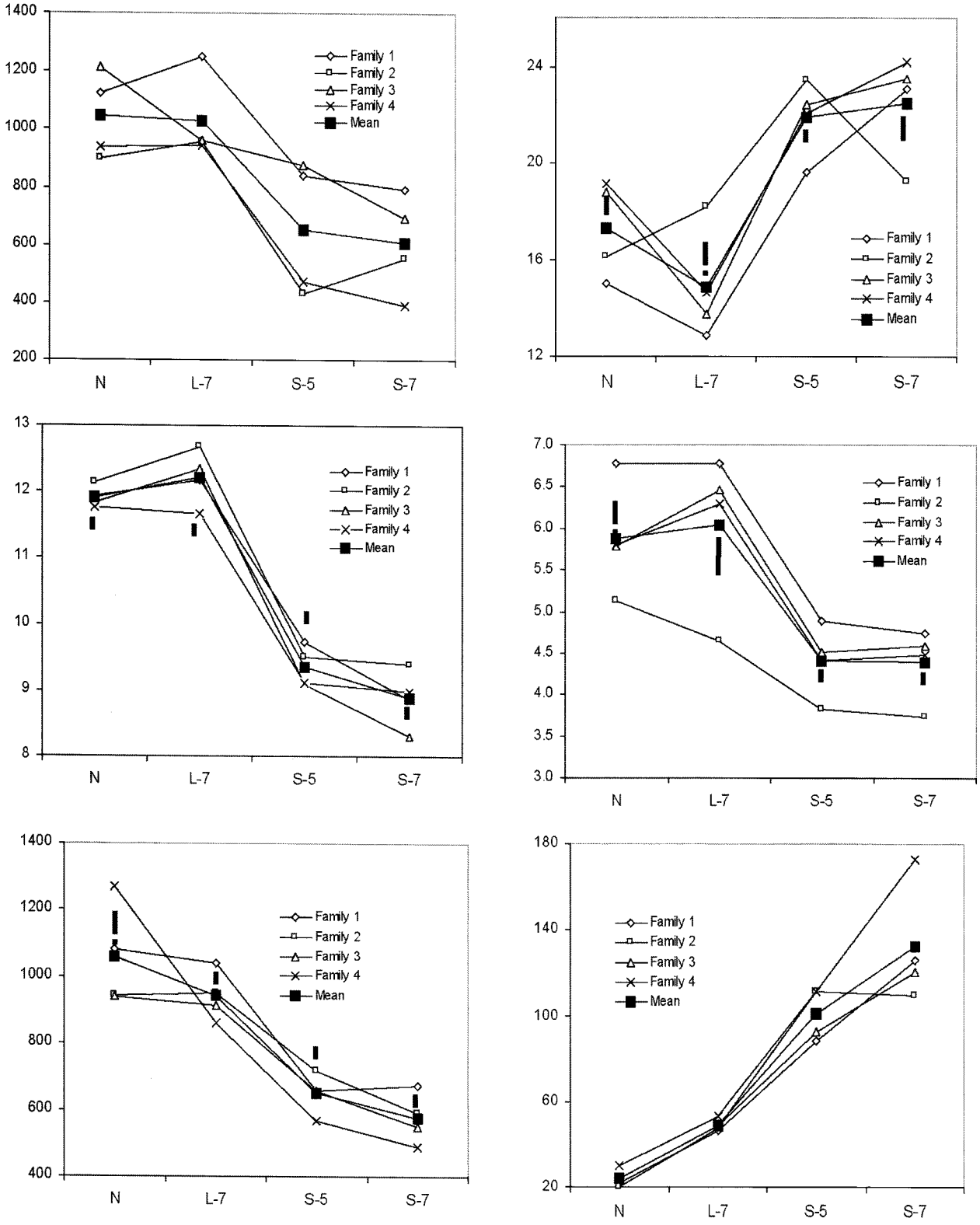


**Fig. 4.** Effect of multifoliate leaflet types on yield, yield components, and some agronomic characters in four isogenic lines each of four mungbean families. Standard error of mean of each leaflet type is represented by a vertical bar.

Distribution of data in each family (Fig. 4) 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. However, days to flowering and maturity were the same among all leaflet types. Across all three field trials, the normal mungbean gave similar yield to L-7,

**Table 3.** 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 = .05; ** significant at P = .01

while S-5 was similar to S-7. The L-7 parent, N and L-7 lines gave higher yield, while the S-5 parent gave similar yield to the small multifoliate S-5 and S-7 lines. The trend of yield potential was similar in each family where N and L-7 gave more yield compared to those of S-5 and S-7.

Correlations between mungbean yield and its components in this study are shown in Table 3. Seed yield correlated well with 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. Although number of pods per plant was positively correlated with total number of leaflets per plant, while 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 terms of plant height, the normal leaflet lines were taller than the others (data not shown).

### Identification of AFLP markers associated with multifoliate leaflet

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. Of 94 primer combinations, 47 showed clear and sharp bands and thus were used to amplify fragments of 16 isogenic lines. Twenty primer pairs can distinguish between parents and isogenic lines and produced 56 polymorphic DNA bands.

The results of single factor analysis of variance showed that 12 and three AFLP markers were significantly associated with leaflet size and leaflet number, respectively (Table 4). Of the 12 markers associated with leaflet size, 10 were contributed from $P_1$ (L-7) alleles, and the other two markers, viz. ACT_AGC and GCC_ACA1 were from $P_2$ alleles. For number of leaflets per leaf, all three markers were contributed from $P_2$ (S-5) alleles.

**Table 4.** AFLP markers showing association with leaflet size and leaflet number based on single factor analysis of variance.

| 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) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Allele mean | | Prob | Allele mean | | Prob |
| | | | $P_1$ | $P_2$ | | $P_1$ | $P_2$ | |
| 1 | AAA_CAG2 | 200-249 | 0.70 | 0.17 | 0.04 | - | - | - |
| 2 | AAA_CAG3 | 151-200 | 0.70 | 0.17 | 0.04 | - | - | - |
| 3 | AAA_CTA1 | 200-249 | 0.80 | 0.00 | < 0.01 | - | - | - |
| 4 | AAA_CTT2 | 200-249 | 0.88 | 0.13 | < 0.01 | - | - | - |
| 5 | AAA_CTT3 | 82-100 | - | - | - | 0.17 | 0.7 | 0.04 |
| 6 | ACG_CAC1 | 200-249 | - | - | - | 0.22 | 0.86 | 0.01 |
| 7 | ACG_CAG4 | 311-413 | 1.00 | 0.18 | < 0.01 | - | - | - |
| 8 | ACG_CAG1 | 151-200 | 1.00 | 0.10 | < 0.01 | - | - | - |
| 9 | ACG_CAG2 | 151-200 | 1.00 | 0.30 | < 0.01 | - | - | - |
| 10 | ACT_ACG | 151-200 | 0.86 | 0.27 | 0.01 | - | - | - |
| 11 | ACT_AGC | 200 | 0.33 | 0.83 | 0.05 | - | - | - |
| 12 | CAG_ACG3 | 100-118 | 0.73 | 0.17 | 0.03 | - | - | - |
| 13 | CT_AAT | 100-118 | 0.86 | 0.27 | 0.01 | - | - | - |
| 14 | GCC_ACA1 | 151-200 | 0.30 | 1.00 | < 0.01 | - | - | - |
| 15 | GCC_ACT3 | 200-249 | - | - | - | 0.27 | 0.86 | 0.01 |

## Discussion

Since the $F_2$ population segregated into a 9:3:3:1 ratio in leaflet size and number, a gene action with epistatic expression is proposed for alleles controlling leaflet number. With this particular gene action, $N_1\_$ gave trifoliate leaf upon the presence of $N_2\_$ genotype, but gave pentafoliate leaflets in the presence of $n_2n_2$, whereas $n_1n_1$ expressed heptafoliate regardless of 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 mutant (with the proposed genetic symbol $n_1n_1n_2n_2$) in this study is not the same as those previously reported. The $F_2$ population showed segregation in leaflet size and leaflet number at a 9:3:3:1 ratio, indicating that these two characters were each controlled by a separate gene locus.

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) in mungbean. Results of the present study and earlier reports showed that different leaflet types correlated with crop yield. Even though S-5 and S-7 had numerous leaflets distributing evenly in the canopy, each leaf was comparatively much smaller in area than those of N and L-7, thus they absorbed less sunlight. The number of leaflets was negatively correlated with leaf area as shown in Table 3.

Although the small leaflet mungbean had more pods per plant, it also had smaller and less seeds per pod and thus it could serve 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, leaf area, number of seeds per pod, and seed weight varied in different leaflet types with the same trend in all families (Fig. 4), while yield potential was lower in small multifoliate leaflets (S-5 and S-7). This indicated that the leaflet types significantly influenced seed yield, although the genetic background was up to 94% homozygous among lines within the same family. Thus the difference between lines within each fam-

ily was affected by the qualitative genes controlling the leaflet types.

Leaves are the primary sites of photosynthesis varying in number, shape and size. They are efficient interceptors of light because of their flat shape with the chloroplast on the surface. The N and L-7 have higher leaf areas than the other multifoliate types, thus they can absorb a higher quantity of light 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.

Three markers, AAA_CTT3, ACG_CAC1, and GCC_ACT3 showed association with genes controlling leaflet number. Marker AAA_CTT3 was from $P_2$ (S-5) allele, while ACG_CAC1 and GCC_ACT3 were from $P_1$ (L-7) alleles. These three markers were confirmed with phenotypic data for their association with $N_1$, $n_1$, $N_2$, and $n_2$ alleles; the marker AAA_CTT3 was most likely linked to $N_1$ allele, while markers ACG_CAC1 and GCC_ACT3 were linked to $n_1$. These three markers did not correspond to leaflet size and yet the number of leaflets per leaf had no correlation with leaflet area per plant (Table 3). This finding supports the notion that the genes controlling leaflet size and leaflet number are located at different chromosomes or probably in the same chromosome but far spaced. Although a set of AFLP markers was identified to link with leaflet number and leaflet size, more investigation on map distance is needed. To do so, a segregating mapping population such as $F_2$ or RILs is produced. Then the associated markers from Table 4 are applied to the DNA extracted from each individual/line. The recombination frequency between the markers and multiple leaflets can be determined and converted into a map distance.

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

## Conclusion

Crossing between seven large leaflet (L-7) and five small leaflet (S-5) mungbean mutants resulted in the normal-trifoliate (N) $F_1$. The $F_2$ can be phenotypically classified into number of leaflets per leaf and leaflet size with large-trifoliate ($N_1\_N_2\_$), small-pentafoliate ($N_1\_n_2n_2$), large-heptafoliate ($n_1n_1N_2\_$), and small-heptafoliate ($n_1n_1n_2n_2$) at the dihybrid ratio of 9:3:3:1. The finding is thus evident that leaflet number character was controlled by $n_1$ and $n_2$ loci of genes. However, all three AFLP markers associated with leaflet number in this study probably corresponded to $n_1$ locus only. The $n_2$ locus might be closely linked to the s locus such that there were no progenies with large pentafoliate leaflets (hypothetically carrying $N_1\_n_2n_2S\_$ genotype). Another hypothesis is that the $n_2$ locus can have a pleiotropic effect upon the leaflet size such that the $N_2$ allele controls large leaflet size as well.

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

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