# GENETIC STUDIES IN MUNGBEAN

# (*VIGNA RADIATA* (L.) WILCZEK)


By

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# CHAPTER I

## INTRODUCTION

The main task of a plant breeder in a world faced with food shortages is to increase grain yield potential to help insure an abundant and continuous food supply to meet the growing human requirements. Genetics has played a major role in contributing to improved yields in many major crops. A better understanding of the mechanisms of inheritance for characters affecting yield and their interaction is essential in planning an efficient breeding program. This knowledge will improve ability to select superior progeny and ultimately lead to increased yield potential.

The second chapter of this thesis concerns the detection of reciprocal differences for grain yield, days to flower, plant weight, vegetative weight, and number of pods per plant in several crosses in mungbean. Reciprocal differences have long been neglected or assumed to be of little importance in breeding programs. However, several studies have shown reciprocal differences for agronomic characters. Should reciprocal crosses be studied for important characters, the magnitude of genetic variabilty arising from reciprocal differences may need to be examined.

In the third chapter, a new crossing technique for mungbeans is discussed. This crossing technique is faster and more efficient than those previously reported. Using this technique mungbean breeders will be able to produce large segregating populations more efficiently than before, and thus increase the potential of selecting superior genotypes.

Chapters II and III will be presented in a form acceptable to the Crop Science Society of America. The same format is currently adopted in many professional journals. Chapter IV is a general summary of the two studies.

# CHAPTER II

## RECIPROCAL DIFFERENCES FOR SEVERAL AGRONOMIC CHARACTERS IN MUNGBEANS (VIGNA RADIATA (L.) WILCZEK).


### ABSTRACT

This study deals with reciprocal differences observed in the $F_1$ generation, when the advanced yield line M-1-77-OT-4 was crossed to several tester lines in mungbeans (*Vigna radiata* (L.) Wilczek). Highly significant reciprocal differences were observed for the following characters: days to flower, grain yield, plant weight, vegetative weight, and number of pods per plant in both individual $F_1$ crosses and the combined $F_1$ data. Highly significant negative correlations were found between days to flower and grain yield, plant weight, vegetative weight, and the number of pods per plant. Cytoplasmic inheritance for those characters exhibiting reciprocal differences could not be substantiated in this study.


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Additional index words: Yield, Reciprocal differences, yield components, cytoplasmic inheritance.

Many present breeding programs select their parental genotypes on the basis of their general and/or specific combining abilities. One of the major assumptions made in the formulas for these selection procedures is the lack of reciprocal differences within a cross. Many breeders do not separate reciprocals because they assume no reciprocal differences.

Reciprocal differences have been found in a number of major crops (3,4,5,6,7,12,14). Many papers have dealt with maternal effects on $F_1$ seeds (3,5,7,12). These papers usually discuss oil and protein quantity and quality. Several individuals, however, have observed significant reciprocal differences for agronomic characters in later generations (4,12,14). Wilcox and Simpson (14) found significant reciprocal differences in the $F_1$ generation for maturity date, plant weight, seed weight, harvest index, oil content, and protein content in soybeans (Glycine max (L.) Merrill). They concluded that cytoplasmic effects were not at work because the $F_1$ with the higher value almost always contained the cytoplasm of the parent with the lower value. Also when the reciprocal differences were averaged over the crosses, there was neither a significant reciprocal difference nor a trend toward one for any of the characters.

Crane and Nyquist (4) noted that in corn (Zea mays L.) when two lines (H42) and (B37) were crossed to several lines with exotic germplasm, significant reciprocal differences for yield were observed with the exotic cytoplasm being superior. They decided that although cytoplasmic expression for yield was significant, the magnitude of difference was lower than would be effective to use in a maize breeding program.

Kuo (8) stated that mungbean yield is directly determined during the post anthesis period. Mungbeans produce very little vegetation before flowering and thus store very little photosynthate to contribute to seed development. He found that flowering stimulated the growth of vegetation. He believed that the photosynthate produced during this time was the carbohydrate used by the plant to produce seed. He also felt that one of the greatest limitations to yield increases in mungbeans was the length of time required for optimum leaf area index (LAI) to be reached.

The AVRDC (2) found in their studies that carbon assimilation early in the mungbean's life cycle (pre anthesis) had no direct effect on seed development. They found that all carbohydrate in the seed was fixed during the post anthesis period. They also found that reductions in leaf area during the post anthesis period reduced grain yield. This suggested that, in the source-sink photosynthate mechanism, the source of photosynthate may be a limiting factor in increasing grain yield in mungbeans

Therefore they concluded that an increase in total photosynthate production during the reproduction period could increase grain yield.

Mckenzia (9), Ramanujam (10), and Singh and Singh (13) all found a strong positive correlation between the number of pods per plant and grain yield. Ramanujam believed selection for earlier flowering plants would increase pod set and thus increase yield. Both Singh and Mckenzia determined that the number of pods per plant was the yield component which had the most direct effect on grain yield in mungbeans of any of the yield components.

The objective of the present study was to evaluate the reciprocal differences observed in the $F_1$ generation for days to flower, grain yield, plant weight, vegetative weight, and number of pods per plant in crosses containing the advanced yield line M-1-77-OT-4 in mungbean.

# MATERIALS and METHODS

The study consisted of the line M-1-77-OT-4 crossed to four tester lines (MG-50-10A(Y), 52-22, 3-1, and 48-14). These lines are coded as follows: line 1=MG-50-10A(Y), line 2=52-22, line 3=3-1, line 4=48-14, line 7=M-1-77-OT-4. Line 7 came from an off-type individual plant selection from the cultivar 'Kiloga'. Kiloga was developed from selections made out of the population Purdue 3. Line 7 was chosen for this study because of its high yield potential. Line 1 is a cultivar from the Philippines. The origins of lines 2, 3 , and 4 are not clear. Lines 1, 2, 3, and 4 were chosen for this study because of their relatively large seed size (>8.00 grams per 100 seeds).

The field design for this study was a randomized complete block design with four blocks. Plants of the five parents and eight $F_1$ populations were planted on July eleventh 1983 at 37.5 cm intervals in rows 75 cm apart at Perkins, Oklahoma. The soil type was a thermic Udic Argiustoll, Teller loam 0-1% slope. Contrasting genetic markers were utilized to insure that $F_1$ plants were not self pollinated parentals.

The following characters were evaluated on an individual plant basis: days to flower (days from planting until the first flower fully opened), grain yield (g of air dried seed), plant weight (g of the aerial portion of plant at physiological maturity after air drying), vegetative weight (g of plant weight less grain yield), 100 seed weight (g of 100 random seeds), seeds per pod (average number of seeds from twenty pods taken at random), harvest index (grain yield divided by plant weight), and pods per plant (calculated by dividing the grain yield per plant by the number of seeds per pod times the weight per seed)

A protected LSD test was used to test for reciprocal differences in the combined $F_1$ data. P values were then calculated for all pairs of $F_1$'s using a t-test.

Correlations between all characters were made by testing the individual $F_1$ reciprocal groups within crosses, the individual $F_1$ crosses ignoring reciprocal groups, the reciprocal groups ignoring the individual crosses, the individual parent lines, and the combined parent lines.

Parental lines were tested for differences similar to testing for reciprocal differences in the $F_1$ crosses.

RESULTS

In the cross 7X1 highly significant reciprocal differences were observed for days to flower, grain yield, plant weight, vegetative weight, and number of pods per plant (Table 1 ) When Line 7 was used as the maternal parent the $F_1$ plants in this cross flowered later, produced less grain yield, had lower plant weights and vegetative weights, and produced fewer pods per plant than when line 1 was the maternal parent.

Results from the cross 7X2 showed that only the number of pods per plant exhibited a significant reciprocal difference (Table 1.) Line 7 as the maternal parent produced $F_1$ plants with fewer pods per plant than when Line 2 was the maternal parent. Reciprocal difference for grain yield was significant at P=.10, with Line 7 as the maternal parent producing $F_1$ plants lower in grain yield than when Line 2 was the maternal parent. Means for the other characters of the cross 7X2 were not significantly different, however, they followed the same trend as the cross 7X1.

The cross 7X3 showed significant reciprocal differences for grain yield, plant weight, vegetative weight, and number of pods per plant. Line 7 as the maternal parent produced $F_1$ plants lower in grain yield, plant weight, vegetative weight, and with fewer pods per plant than when Line 3 was the maternal parent (Table 1.).Days to flower for the cross

7X3 did not show a significant reciprocal difference.

In the cross 7X4, days to flower and the number of pods per plant showed highly significant reciprocal differences, with Line 7 as the maternal parent producing $F_1$ plants which flowered later and produced fewer pods per plant (Table 1.). A significant reciprocal difference for seeds per pod was also exhibited for this cross in which Line 7 as the maternal parent produced $F_1$ plants which averaged 10.34 seeds per pod, while Line 4 as the maternal parent produced $F_1$ plants which averaged 8.99 seeds per pod. Grain yield in the cross 7X4 exhibited a significant reciprocal difference at P=.08, with Line 7 as the maternal parent producing $F_1$ plants with lower grain yield than when Line 4 was the maternal parent. In the cross 7X4, reciprocal differences for vegetative weight, and plant weight were not significant.

When all crosses were combined highly significant reciprocal differences were declared for days to flower, grain yield, plant weight, vegetative weight, and the number of pods per plant (Table 2.). Line 7 as the maternal parent produced $F_1$ plants which flowered later, had lower grain yields, plant weights, and vegetative weights, and produced fewer pods per plant than when Line 7 was the paternal parent. Because the cross 7X4 was the only cross to show significant reciprocal differences for the number of seeds per pod, the reciprocal difference for the number of seeds per pod in the combined data was not found to be significant.

Correlations between reciprocals within a cross did not significantly differ from one another, therefore reciprocals were ignored and the data from each cross was combined (Table 3.). In the cross 7X1, highly significant negative correlations were found between days to flower and the following characters: grain yield (-.70), plant weight (-.68), and vegetative weight (-.64). At P=.10 the correlation between days to flower and the number of pods per plant was also significant (-.60). The cross 7X2 showed similar correlations to those found in the cross 7X1. Days to flower was highly significantly correlated to grain yield (-.81), plant weight (-.80), vegetative weight (-.78), and the number of pods per plant (-.97). The cross 7X3 showed significant correlations between days to flower and grain yield, plant weight, and vegetative weight, however they were lower than those seen in the previous two crosses. The correlations with days to flower were: grain yield (-.40), plant weight (-.43), and vegetative weight (-.45). The correlation between days to flower and the number of pods per plant was not significant in this cross. In the cross 7X4 highly significant correlations with days to flower were: grain yield (-.58), plant weight (-.62), and vegetative weight (-.58). The correlation between days to flower and the number of pods per plant was not significant for this cross.

When the parental lines were tested for differences for days to flower it was found that Lines 7 and 2 were the earliest flowering and not different from each other. Lines 1 and 4 were the next earliest, and Line 3 was the latest flowering line though it was not different from Line 4 (Table 4.). When differences for grain yield, plant weight, and vegetative weight were tested in the parental lines, the rankings were all similar. Lines 4 and 3 had the highest yields and were significantly different from Line 1. However,lines 4 and 3 were not different statistically from Lines 2 and 7. Nor were Lines 2 and 7 different from Line 1. When differences for the number of pods per plant were tested between the parental lines, Line 7 had the highest number of pods per plant though it was not significantly different from Lines 4, 3, and 2. Line 7 did however have significantly more pods per plant than Line 1. Line 1 did not differ from lines 4, 3, and 2 statistically.

When correlations were tested on the individual parental lines, Line 1 showed highly significant correlations between days to flower and grain yield (-.87), plant weight (-.87), vegetative weight (-.84), and the number of pods per plant (-.88) (Table 5.). Line 4 showed similar highly significant correlations between days to flower and the other characters. They were: grain yield (-.63), plant weight (-.66), vegetative weight (-.66), and the number of pods per plant (-.69). Line 7 showed highly significant correlations between days to flower and grain yield (-.79) and days to flower and the number of pods per plant (-.85). Line 7 also showed a significant correlation between days to flower and plant weight at P=.10. Line 7 did not show a significant correlation between days to flower and vegetative weight. Lines 2 and 3 did not showed significant correlations between days to flower and the other characters (Table 5.).

## DISCUSSION

This study found significant reciprocal differences in the $F_1$ plant generation for the following characters: days to flower, grain yield, plant weight, vegetative weight, and number of pods per plant, from the combined data of four crosses with line 7 (M-1-77-OT-4) as one parent.

Previous studies (2,8, and 10) all determined that days to flower had a strong influence on grain yield in mungbean. It has also been reported that flowering stimulates vegetative growth in mungbeans. Our correlations likewise determined that days to flower strongly influences grain yield, the number of pods per plant, plant weight, and vegetative weight. Results from our study indicate that in the $F_1$ generation earlier flowering may promote the production of more vegetation early in the growing season. Because mungbeans are an indeterminant crop this early increase in vegetation increases the total amount of photosynthate produced during the reproductive season. The increased photosynthate produced during the post anthesis period increases the number of pods produced per plant which directly increases yield. Therefore the reciprocal differences seen in the $F_1$ crosses for grain yield, plant weight, vegetative weight, and number of pods per plant appear to be dependent upon days to flower for their expression. The data also suggests that the source of photosynthate was the limiting factor in increasing yield in the late flowering plants in this study.

Only those crosses made between lines which showed significant correlations between flower date and the other characters (Lines 1 and 4) showed significant reciprocal differences for days to flower when crossed to Line 7. Since Lines 1, 4, and 7 showed significant correlations between days to flower and the other characters, this suggests that these lines are more sensitive to changes in days to flower.

Those lines which did not show significant correlations between days to flower and the other characters (Lines 2 and 3) are believed to be less sensitive to changes in days to flower. When these days to flower insensitive lines were crossed to Line 7 the $F_1$ progenies from these crosses did not exhibit significant reciprocal differences for days to flower.

This suggests that if Line 7 does contain a character in the cytoplasm which modifies days to flower, it must be incorporated into a progeny which exhibits a sensitivity to changes in days to flower for the reciprocal difference for days to flower to be expressed in the progeny.

The results from the correlations between days to flower and the other characters in the individual crosses were inconclusive. In the crosses 7X1 and 7X4, lines sensitive to days to flower were crossed to Line 7, and the $F_1$ progenies were sensitive to days to flower. Also, in the cross 7X3, Line 3 was considered insensitive to days to flower and when it was crossed to Line 7 the $F_1$ progeny showed a low sensitivity to days to flower roughly midpoint between the two parental levels. In these three crosses the correlations between days to flower and the other characters appeared as expected, with crosses between lines sensitive to changes in days to flower producing progeny sensitive to days to flower, and a cross between a line sensitive to changes in days to flower and a line not sensitive to changes in days to flower producing progeny with intermediate sensitivity to days to flower. In the cross 7X2, however, Line 2 was considered insensitive to changes in days to flower and yet when it was crossed to Line 7 the $F_1$ progeny was still sensitive to changes in days to flower. Therefore, progeny which did exhibit reciprocal differences for days to flower also were sensitive to changes in days to flower, but progeny which did not exhibit reciprocal differences for days to flower also were not insensitive to changes in flower date.

Simple cytoplasmic inheritance for flower date can be ruled out because line 7 as a variety flowered significantly earlier than three of the lines with which it was crossed. However, as the maternal parent in crosses to these same lines line 7 caused the $F_1$ plants to flower later. Although line 7 flowered earlier than some of the other lines in this study, its grain yield, plant weight, and vegetative weight were only intermediate compared to the larger, higher yielding, later flowering lines.The parental lines as a whole showed a reversal of the $F_1$ plants because later flowering lines were the higher yielding lines. Although these results appear contradictory, they are identical to the results found by Wilcox and Simpson (14) in their soybean study. The lines in this study may have differed in their genetic potential for characters affecting yield which were not examined. The genetic and environmental interactions present in this study appear to be too complex to resolve from a single year's data. Future studies will be necessary to test whether the reciprocal differences observed in this study can be maintained over different environments as well as different genotypes. Further work is also needed to substantiate the theory that genetic differences within the cytoplasms are causing the reciprocal differences seen in this study and to what extent flower date affects the reciprocal differences for the other characters.

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## LIST OF TABLES

TABLE 1. MEANS AND P VALUES FOR THE RECIPROCAL GROUPS WITHIN CROSSES 7X1, 7X2, 7X3, AND 7X4 FOR DAYS TO FLOWER, GRAIN YIELD, PLANT WEIGHT, VEGETATIVE WEIGHT, AND NUMBER OF PODS PER PLANT. FIRST LINE IN PEDIGREE IS FEMALE PARENT.

| ENTRY LINES | DAYS TO FLOWER | GRAIN YIELD g | PLANT WEIGHT g | VEG. WEIGHT g | NO. PODS PER PLANT |
|---|---|---|---|---|---|
| 7*1 | 24.19 | 22.10 | 51.58 | 29.48 | 35.08 |
| P VALUE | .004 | .003 | .004 | .007 | .002 |
| 1*7 | 20.03 | 43.74 | 108.58 | 64.85 | 65.82 |
| 7*2 | 23.47 | 24.67 | 61.78 | 37.11 | 34.39 |
| P VALUE | .127 | .100 | .220 | .330 | .037 |
| 2*7 | 21.33 | 36.31 | 85.80 | 49.49 | 53.94 |
| 7*3 | 21.79 | 33.42 | 79.68 | 46.27 | 58.41 |
| P VALUE | .246 | .042 | .024 | .021 | .036 |
| 3*7 | 20.17 | 47.90 | 124.25 | 76.35 | 81.84 |
| 7*4 | 24.42 | 34.01 | 87.39 | 53.38 | 51.97 |
| P VALUE | .009 | .077 | .153 | .228 | .001 |
| 4*7 | 20.70 | 46.56 | 115.39 | 68.83 | 81.54 |

$P \leq .05$=significant, $P \leq .01$=highly significant.

TABLE 2. MEANS AND P VALUES FOR THE RECIPROCAL GROUPS WITHIN THE COMBINED CROSSES TO LINE 7 FOR DAYS TO FLOWER, GRAIN YIELD, PLANT WEIGHT, VEGETATIVE WEIGHT, AND THE NUMBER OF PODS PER PLANT. FIRST LINE IN PEDIGREE IS FEMALE PARENT.

| ENTRY LINES | DAYS TO FLOWER | GRAIN YIELD g | PLANT WEIGHT g | VEGETATIVE WEIGHT g | NO. PODS PER PLANT |
|---|---|---|---|---|---|
| 7 AS FEMALE | 23.47 | 28.55 | 70.11 | 41.56 | 44.96 |
| P VALUE | .0001 | .0001 | .0002 | .0004 | .0001 |
| 7 AS MALE | 20.56 | 43.63 | 108.51 | 64.88 | 70.79 |

$P \leq .01$=highly significant.

TABLE 3. CORRELATIONS FOR THE INDIVIDUAL CROSSES BETWEEN DAYS TO FLOWER AND GRAIN YIELD, PLANT WEIGHT, VEGETATIVE WEIGHT, AND PODS PER PLANT.

| ENTRIES | GRAIN YIELD | PLANT WEIGHT | VEG. WEIGHT | PODS PER PLANT |
|---|---|---|---|---|
| 7X1 | -.700** | -.678** | -.636** | -.603+ |
| 7X2 | -.806** | -.800** | -.782** | -.971** |
| 7X3 | -.401* | -.427* | -.448* | -.465 |
| 7X4 | -.587** | -.623** | -.579** | -.429 |

+, *, ** = SIGNIFICANT AT .10, .05, AND.01 LEVELS OF PROBABILITY,RESPECTIVELY

TABLE 4. MEANS AND STATISTICAL DIFFERENCES FOR THE LINES 1,2,3,4, AND 7. FOR DAYS TO FLOWER, GRAIN YIELD, VEGETATIVE WEIGHT,AND NUMBER OF PODS PER PLANT.

| ENTRY LINES | DAYS TO FLOWER | GRAIN YIELD g | PLANT WEIGHT g | VEG. WEIGHT g | NO. PODS PER PLANT |
|---|---|---|---|---|---|
| 4 | 22.96 BC* | 42.74 A | 118.70 A | 75.96 A | 61.74 AB |
| 3 | 25.29 C | 42.15 A | 118.72 A | 76.57 A | 57.12 AB |
| 7 | 19.49 A | 38.00 AB | 96.97 AB | 58.97 AB | 69.78 A |
| 2 | 18.09 A | 34.20 AB | 95.71 AB | 61.51 AB | 47.18 AB |
| 1 | 21.13 B | 26.49 B | 66.89 B | 40.40 B | 32.60 C |

*DUNCANS MULTIPLE RANGE USED TO EXPRESS STATISTICAL DIFFERENCES WITHIN COLUMNS

TABLE 5 CORRELATIONS FOR THE PARENTAL LINES BETWEEN DAYS TO FLOWER AND GRAIN YIELD, PLANT WEIGHT, VEGETATIVE WEIGHT, AND PODS PER PLANT.

| ENTRY LINES | GRAIN YIELD | PLANT WEIGHT | VEG. WEIGHT | PODS PER PLANT |
|---|---|---|---|---|
| LINE 1 | -.869** | -.871** | -.841** | -.875** |
| LINE 2 | -.328 | -.377 | -.394 | -.347 |
| LINE 3 | -.239 | -.217 | -.198 | -.303 |
| LINE 4 | -.626** | -.662** | -.622** | -.697** |
| LINE 7 | -.794** | -.660+ | -.572 | -.849** |

+, *, ** = SIGNIFICANT AT .10, .05, AND .01 PROBABILITY LEVELS, RESPECTIVELY

CHAPTER III

# A NEW CROSSING TECHNIQUE IN MUNGBEANS

## ABSTRACT

Improved crossing techniques are needed to exploit the genetic potential of mungbeans (*Vigna radiata*(L.) Wilczek). In our technique only the tip of the bud is opened to expose the stigma and style. After the anthers are gently removed with forceps, the emasculated bud is then ready to be crossed. When pollination is completed, cellophane tape is placed over the opening to seal the bud and the process is complete. An experienced technician can complete an emasculation and pollination in one minute. The success rate using this technique averaged 60% with an average of six seeds per pod per successful cross. This technique differs from those presently being used in other legume crops because most other crossing techniques open the entire dorsal edge of the bud in order to make an emasculation and pollination (3,5,6,7). Our technique appears to reduce moisture and temperature fluctuations within the bud and reduces bud abortion.

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Additional index words: Artificial pollination.

Mungbean (Vigna radiata(L.) Wilczek) is a self-fertilizing legume used as a protein grain crop for human consumption throughout much of Asia, the Middle East, and India. Mungbeans are a preferred protein supplement in many developing areas because of their high protein (24%-28%) and ease of digestibility. In the United States, mungbeans are commonly eaten as bean sprouts in salads or oriental dishes, where they are an excellent source of vitamin C.

Mungbeans grow well in semiarid regions where many other legumes must be irrigated. A tolerance to hot, dry climates increases the potential of mungbeans as a useful seed crop in the semi-arid climates of the Southwest United States. Mungbeans are presently grown to a limited extent in the United States with the largest production in Oklahoma(40,000 hectares annually).

Currently used crossing techniques in mungbeans are not as efficient as desired. Boling et al(4) reported a success rate of 20%, however, in our preliminary attempts using the Boling method over 300 pollinations were attempted with only 23 pods forming. This resulted in a success rate of less than than 10%. One difficulty with the Boling method is that it requires the standards of the bud to be completely separated along the entire dorsal. This separation allows a rapid change in the humidity and temperature within the micro environment of the bud. We believe that this dramatic loss of moisture and fluctuation in temperature may excessively stress the reproductive organs in the bud and cause abortion. The objective of this study was to develop a more efficient crossing technique for mungbeans.

## MATERIALS AND METHODS

In 1983, eleven mungbean genotypes were planted in a greenhouse at the OSU-USDA Research Facility in Stillwater, Oklahoma. The eleven parent lines included two released cultivars 'Berken' and 'Oklahoma 12' and nine advanced breeding lines.

Twenty six-inch pots were planted per genotype with seedlings thinned to two plants per pot. The soil was a mixture of peatmoss, sand, and perlite in a 3:3:1 ratio, respectively. Nitrogen, phosphorous, and potassium were applied to assure adequate fertility. Lime was also mixed into the soil to achieve a pH of (6.6 to 7.2). Temperature within the greenhouse was kept at 25 C during the day and 20 C at night No special lighting was used as mungbeans are considered a day neutral crop (1). Equipment used to make crosses included fine tipped forceps, cellophane tape, and tags.

## RESULTS AND DISCUSSION

When flowers appeared, the technique described below was used in crossing. To select a female, a large bud one day from opening should be chosen. These buds should be approximately one centimeter in length and light green in color compared to the younger dark green buds. To emasculate the female, grasp the bud between the thumb and forefinger. Using forceps, grip the right side of the standard approximately two-thirds the distance from the base along the ventral edge of the bud. Gently tear the standard upward toward the dorsal edge of the bud and remove the loosened piece. The wing, if in the way, can be removed similarly in order to reveal the keel. Using the point of the forceps, carefully slit open one side of the keel. Then by grasping the loosened flap with forceps, remove the loosened tip of the keel. The stigma and some of the stamens should now be exposed. Carefully tease out the rest of the stamens by gently pulling on the filaments already exposed. When all ten stamens are exposed, remove them by pinching off the filaments below the anthers. Precision is important so the stigma or stylar tissue is not damaged during the emasculation. The stigma is now ready to be pollinated.

To select a pollen source, choose a flower which has just opened and has fresh, dry, light yellow pollen covering it's stigma. By applying pressure along the dorsal edge of the flower near its base the pollen ladened stigma will protrude from the keel and can be removed with forceps. The stigma can now be used as a pollen source to pollinate the female parent. Be careful to use only fresh dry pollen to ensure gamete viability.

To pollinate the female parent, gently brush the pollen ladened stigma of the male parent against the stigma of the female parent, checking to see that pollen has been transferred to the female parent's stigma. When pollen can be seen on the stigma of the female, tag the pollinated flower and seal the opening in the standard with cellophane tape (2). The cellophane tape helps control the loss of moisture in the stigma by resealing the opening.

If the pollination is successful, the flower will dehisce in two to three days and reveal a small pod. Emasculations can done either in the evening after 1700h or in the morning before 1000h. All our pollinations were done between 1000h and 1300h, however late afternoon pollinations should also be possible if pollen can be kept dry.

Over a six week period, 1800 pollinations were attempted. Of these, 1153 successfully produced seed-bearing pods. These pods averaged six seeds per pod to produce a total of 6523 $F_1$ seeds. When these seeds were grown in the field 0% - 20% were judged using marker genes to be self pollinated parentals depending on the female parent. A total of approximately 6200 $F_1$ seeds were thus actually produced, giving a success rate of 60%.

Our crossing technique was more effective then the Boling technique because it required a smaller opening be made in the standards and this opening was resealed after pollination was completed. We feel this helped to maintain the temperature and moisture levels within the bud and thus reduced bud abortion.

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# CHAPTER IV

## SUMMARY

This study consisted of two separate experiments which are referred to as experiments 1, and 2.

In experiment 1, a replicated nursery consisting of four $F_1$ crosses including reciprocals and their five parents was conducted at the Agronomy Research Station, Perkins, Oklahoma. This experiment was done to determine reciprocal differences in the $F_1$ plant generation for grain yield, plant weight, vegetative weight, number of pods per plant, and days to flower in four crosses containing the advanced yield line M-1-77-OT-4. The experiment was carried out during the 1983 growing season. It contained four replications. Individual plants were studied for the following characters: grain yield, plant weight, vegetative weight, number of pods per plant, number of seeds per pod, 100 seed weight, plant height, and days to flower.

Statistical analyses indicated that significant or highly significant reciprocal differences were observed for one or more of the following characters in all four crosses, grain yield, plant weight, vegetative weight, number of pods per plant, days to flower, and number of seeds per pod. A significant reciprocal difference for the number of seeds per pod was found however in only one cross (M-1-77-OT-4 X 48-14), such that Line M-1-77-OT-4 as the female parent produced progeny with 1.35 more seeds per pod than when line 48-14 was the female parent. No significant reciprocal differences were found in any cross for 100 seed weight or plant height. When the data from all the crosses were combined, overall reciprocal differences for grain yield, plant weight, vegetative weight, days to flower, and number of pods per plant were highly significant. With the Line M-1-77-OT-4 as the female parent producing progeny which flowered later, produced lower grain yields, less vegetative weight, less plant weight, and fewer pods per plant than when it was the male parent. Additional studies will be necesary to determine the inheritance of these reciprocal differences as well as their possible interaction with one another.

In experiment 2, a more efficient crossing technique in mungbeans was developed. Previously reported techniques induced a high percentage of bud abortions. This was due to stresses placed on the bud during emasculation. The new technique reduces the stress put on the bud by only making a small opening in the standards, and then resealing that opening with cellophane tape after pollination. In our study the new technique's success rate was between 50%-60%, as compared with the Boling method which produced a success rate of less than a 10%. The new technique proved very effective and will be used in future crossing blocks.

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