

Mutant Mungbean Lines from Radiation and Chemical Induction

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ABSTRACT

Gamma rays of 500 Gy and the chemical mutagen, 1% ethylmethane sulphonate (EMS) were administered to induce mutation in mungbean KPS 1 and CN 36 varieties for improvement of disease resistance to cercospora leaf spot and powdery mildew as well as producing high yields. The individual plant and plant-row selections were made in M2 and M3 and in M3 and M4 of KPS 1 and CN 36 respectively. The selected characters observed were the low level of powdery mildew, pods protruding up above the canopy, early maturity, and purple-red petioles. Preliminary yield test of 27 selected lines comparing to original varieties, KPS 1 and CN 36, revealed potentiality of some lines that might be developed into good varieties. These were, M5-8 with good pod setting, M5-19 with pods protruding up above the canopy, M5-21 and M5-22 with good plant type and high pod setting, and M5-24 with early flowering. All five mutant lines possessed moderate resistance to powdery mildew as did KPS 1, while CN 36 moderate susceptibility. The controls as well as the selected lines were also moderately resistant to cercospora leaf spot. For further investigation, artificial inoculation of the pathogens in severe infection would be made in yield test so that only most resistant lines could be chosen.

Key words : mungbean, radiation, chemical mutagen, mutant

INTRODUCTION

The application of mutagens, radiation or chemical, to induce mutation is one way to cause genetic variation and can be used in plant breeding for development of good varieties. The results of successful radiation and chemical uses in mungbean in biological, genetic and physiological changes, mutation frequency changes, as well as varietal development were reported (Chow and Loo, 1988;

Lamseejan *et al.*, 1983, 1988; Asencion *et al.*, 1994; Chotechuen and Lamseejan, 1994). The main objective of the research study was to apply mutation technique in mungbean breeding in order to obtain varieties which are more resistant to cercospora leaf spot and powdery mildew than the existing ones. In addition, the other good agronomic characters which might be selected from this population, were also brought into consideration.

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MATERIALS AND METHODS

1. Radiation treatment

Seeds of two mungbean varieties, KPS 1 and CN 36, each was divided into 3 parts. One part was irradiated with gamma rays from Co-60, one part from Cs-137 (with the dose of 500 Gy) and the other as the control.

2. Chemical treatment

Seeds of KPS 1 and CN 36 were separately put into net-cloth bags soaked in running water for 15 hours at room temperature. After that they were soaked in 1% EMS solution for another 4 hours, washed in running water for 4 hours, fan-dried for 18 hours, and followed by immediate planting. The control groups were similarly treated except that they were soaked in water instead of EMS for the same duration.

3. Procedures after treatment

3.1 Germination percentages and growth development studies of M1 generation were conducted in the laboratory and greenhouse, respectively.

3.2 Planting of M1 to M4 was conducted from dry season 1995 to rainy season 1997 at Chai Nat Field Crops Research Center and Dong Gane Luang Experimental Field, Wat Sing District, Chai Nat Province.

M1 generation - Planting was made with 5 × 50 cm spacing. Each row was 5 m-long. No selection was undertaken in M1 generation. At maturity, one pod from each M1 plants was harvested in order to obtain M2-seeds.

M2 generation - Similar planting procedure to M1 generation was applied. Plant selection of required characteristics was made and the selected plants were tagged. Harvesting was executed in 2 methods. In the first method, each

tagged plant was separately harvested and seeds obtained were categorized as M3-single. The second method was conducted in similar manner to M1 generation and the acquired seeds were the M3-bulk. Characters of M2 plants as plant height, number of pods/plant, number of seeds/pod, seed weight/plant and seed size were recorded.

M3 generation – Planting of M3-single seeds was made in plant to row, while M3-bulk in similar procedure to M1 and M2 generations. Selection in M3-single was conducted by selecting individual plants or rows with required characters. As for M3-bulk, the same selection method to M2 generation was applied. The separated harvesting of selected single plant produced M4-single seeds, whereas row selection of every pod of good plants in selected rows produced M4-bulk. The other characters were also recorded in similar manner to M2 generation.

M4 generation – Planting, selection, harvesting and recording various characters of M4-single and M4-bulk were conducted in similar ways to M3 generation, and M5-single and M5-bulk seeds were obtained. M5 seeds from the same varieties and treatments were then combined together in order to form new lines.

4. Preliminary Yield Trial

The trial was conducted during dry and rainy seasons of the year 1997 at Chai Nat Field Crops Research Center and Dong Gane Luang Experimental Field, Chai Nat, respectively. RCB with 3 replicates was employed. The tested mungbean lines were divided into 2 groups. One group were the lines selected from M3 generation while the other from M2. CN 36 and KPS 1 were used as the controls in each group. The seeds were sown on 5 m-long row, 2 rows/line, after that the seedlings were thinned to 10 seedlings/meter.

RESULTS AND DISCUSSION

1. The effect of mutagens on germination, growth, and physiological damages in M1 generation.

The results showed both radiation and chemical not to affect germination percentage of the 2 mungbean varieties while affecting seedling growth. CN 36 was found to be more sensitive to gamma-rays than KPS 1 with not much difference regarding to sources of gamma radiation. The results agreed with those reported by Malik (1996) that radiation had no effect on germination of every tested mungbean variety, even though the dose used was as high as 80 krad. Still seedling growth was noted to be affected by gamma-rays and the effect increased with the increasing doses. CN 36 was more sensitive than KPS 1, this might be caused by the difference in genetic background of varieties in radiation response. (Singh and Pandya, 1977; Sharma and Haque, 1983)

In chemical treatment, 1% EMS was found to assert no effect to germination but to seedling growth with similar response of the 2 varieties to EMS.

Both radiation and chemical were likely to affect seedling growth, yet the damage decreased with the growth of M1 plants. The finding was similar to the report of Chow and Loo (1988).

The other characters encountered in M1 generation were physiological damages, mainly chlorophyll mutation of various patterns. This caused leaves to show different color levels, mostly on the first single leaf or trifoliate leaves at early stages. However, leaves at later stages would appear more normal.

Radiation and chemical cause chlorophyll mutation to most plants and the severity increases with the increasing dose. Asencion *et al.* (1994), whose test employed mutagen with mungbean, reported the results of sectorial chlorophyll

deficiency as well. Chlorophyll mutation may relate to gene or chromosome changes or just relate to physiological mechanism of the plants. Frequency of chlorophyll mutation in M1 generation may be better used as an indicator to the effects of mutagens on gene mutation than plant growth or survival (Blixt *et al.*, 1965).

In addition to chlorophyll mutation, the other abnormal characters such as leaf shape, compound leaves with different number of leaflets including less podding due to pollen sterility were noticed. Malik (1996) also reported the reduction of pollen fertility with the increasing radiation doses. At 30-40 krad, 50% reduction of pollen fertility compared to the controls was found and the average harvesting age of M1 was observed to be longer than those of the controls.

2. Selection in M2-M4 generations

In M2 generation, many types of chlorophyll mutation were encountered in seedlings, for example, the first pair of single leaf appeared albina, xantha, chlorina, viridis, dark green. More xantha was found than albina. After 7 days of emergence, plants with xantha and albina leaves died, while those with viridis and dark green leaves survived. Malik (1996) noticed genotypes controlling such chlorophyll mutations to be usually heterozygous with the segregation of monogenic recessive. Treatment of mungbean by radiation or chemicals always resulted in chlorophyll mutations, as reported by Bahl and Gupta (1983), Malik (1996) and Asencion *et al.* (1994). Apart from these, the seedlings with 3-4 single leaves, variation in pollen sterility, days to first flowering and maturity were also observed.

Planting M2 in dry season of 1996 found severe infection by powdery mildew, hence resistance to this disease was then selected. The low infected plants from powdery mildew with good agronomic characters such as pods protruding

up above the canopy, large pods, high pod setting, synchronous maturity and purple-red petioles were selected.

M3 sown in dry season of 1996 was severely infected by powdery mildew disease. The infection started at seedling stage and its severity enhanced with the increasing ages of mungbean. Lightly infected plants were chosen as non infected plants were not found. Of all M3 rows, the row from M2 plant number ten (selected from CN 36 irradiated by 500 Gy of gamma rays from Cs-137 with purple-red petioles) showed mutant colors on hypocotyl and leaf petiole which differed from non-irradiated CN 36. That was, at seedling stage, the hypocotyl color of such seedling was purple-red and the color of stem and leaf petiole remained so even at later growth stage, while those of CN 36 normal green. Besides, it showed more resistance to powdery mildew than the other rows. Ten plants were separately harvested for M4 planting. In addition, the less-infected in M3 rows from the 52th and 60th M2 plants were selected. M3 plants were found to show sterility as well as M2.

In M4 planting, the development of mungbean was rated in good condition without severe disease infection or insect infestation. Only cercospora leaf spot appeared but not affecting yield, since the infection was small and found after podding. The selection was made on agronomic characters. Ten M4 plants derived from the 10th plant of M2, when grown in plant to row, the segregation of hypocotyl and leaf petiole colors of plants in each row was observed. In some rows, all plants were either purple-red or green while in the others both purple-red and green were found. Purple-red hypocotyl and leaf petioles were the mutant colors from green. According to the preliminary observation, it was speculated to be back mutation, that was from recessive green plants to dominant purple-red ones. This character is controlled by a single gene. Hypocotyl and seed

coat colors can be used as genetic markers in genetic study and cultivar identification. Swain (1965) reported that the color of seed coat and hypocotyl depended on the concentration of anthocyanin and chlorophyll. Whereas Ishikura *et al.* (1981) found 2 kinds of anthocyanin, A-I (delphinidin-3-glucoside) and A-IV (cyanidin-3-glucoside), in blackgram (*Vigna mungo*) with purple-red hypocotyl. According to the study of Pandey *et al.* (1989), *Vigna* had 4 kinds of anthocyanin, with 2 kinds, A-I and A-IV, similarly reported by Ishikura *et al.* (1981), while the other two, A-II and A-III, still unidentified chemical names. Anthocyanins in seed coat and hypocotyl are independent from each other. The report of Pandey *et al.* (1989) also stated that mungbean seedlings of purple-red had 3 kinds of anthocyanin namely A-I, A-II and A-III whereas green seedlings were free of anthocyanin. Since mutation in hypocotyl color occurred in this mutant line, the separate harvest of purple-red and green plants were made for further study such characters.

3. Preliminary yield trial of selected mungbean lines

The trials were conducted in dry and rainy seasons of 1997. Mungbean lines were divided into 2 groups, one group selected from M2 generation while the other from M3. The experiment in early rainy season 1997 consisting of 5 lines of group 1, namely M4-1, M5-4, M5-10, M5-11 and KPS 1 did not grow well due to poor soil at the growing site. Since the productions of these 5 lines were quite low, they were omitted from data analysis. Therefore, only 9 lines including the control were analyzed.

Table 1 and 2 present yields and agronomic characters in plant height, days to 50% flowering, pod length, number of pods per plant, number of seeds per pod, 100 seed weight, and yield.

It was found that among the tested mungbean

Table 1 Yield and agronomic data of mungbean mutant lines in group I, dry season 1997.

Mutant line/variety	Pedigree	Plant height (cm)	Days to 50% flowering	Pod length (cm)	No. pods/plant	No. seeds/pod	100 seed wt. (g)	Yield (kg/rai)
M4-1	CN 36, EMS	37.60	33.67 cd	8.37	7.43	9.03	6.43	69
M5-1	CN 36, Cs-137	36.53	34.67 bc	9.07	9.03	10.27	6.97	128
M5-2	CN 36, Cs-137	34.27	35.00 bc	8.83	6.73	9.77	6.33	57
M5-3	KPS 1, Co-60	34.60	35.33 bc	8.53	7.57	9.47	6.97	94
M5-4	KPS 1, Co-60	34.73	32.33 d	9.20	8.87	10.10	6.67	101
M5-5	KPS 1, Co-60	35.20	34.33 bc	9.17	8.50	10.23	6.73	113
M5-6	KPS 1, Co-60	34.13	34.67 bc	8.90	8.57	9.73	6.70	110
M5-7	KPS 1, Co-60	33.53	34.67 bc	9.13	8.50	10.07	6.83	98
M5-8	KPS 1, Co-60	35.87	34.67 bc	9.20	10.13	10.20	6.97	133
M5-10	KPS 1, Co-60	35.33	37.33 a	9.60	8.57	10.93	6.90	66
M5-11	KPS 1, Cs-137	37.40	35.67 ab	8.97	9.57	9.93	6.57	85
M5-13	CN 36, Cs-137	36.87	34.33 bc	9.17	8.10	10.50	6.80	108
CN36	check	37.40	36.00 ab	8.97	9.33	10.03	6.83	103
KPS1	check	32.73	35.67 ab	9.00	7.47	9.73	6.43	85
F-test		<1 ns	4.30 **	1.96 ns	1.32 ns	1.05 ns	1.45 ns	<1 ns
C.V. (%)		10.2	2.8	4.1	16.6	7.7	4.6	42.0

ns = non significance

Table 2 Yield and agronomic data of mungbean mutant lines in group II, dry season 1997.

Mutant line/variety	Pedigree	Plant height (cm)	Days to 50% flowering	Pod length (cm)	No. pods/plant	No. seeds/pod	100 seed wt. (g)	Yield (kg/rai)
M4-2	CN 36, EMS	33.40	35.67 a	9.10 bcd	8.17	10.57	6.87	79 bcd
M5-15	CN 36, Cs-137	37.93	35.33 ab	9.70 ab	7.17	11.00	6.80	119 abc
M5-16	CN 36, Cs-137	34.87	35.33 ab	9.10 bcd	6.40	11.07	6.70	84 a-d
M5-17	KPS 1, Co-60	34.07	34.33 ab	8.77 cd	7.03	10.17	6.83	106 abc
M5-18	KPS 1, Co-60	34.20	34.67 ab	9.17 bcd	6.57	10.73	6.63	114 abc
M5-19	KPS 1, Co-60	33.80	35.00 ab	9.47 ab	6.80	11.10	6.83	91 a-d
M5-20	KPS 1, Co-60	39.40	34.67 ab	9.07 bcd	8.63	9.80	6.60	98 a-d
M5-21	KPS 1, Co-60	39.53	34.33 ab	9.70 ab	9.47	11.57	6.77	141 a
M5-22	KPS 1, Co-60	39.67	34.33 ab	9.90 a	8.63	11.60	6.73	142 a
M5-23	KPS 1, Co-60	33.47	35.33 ab	9.40 abc	6.50	10.40	6.77	90 a-d
M5-24	KPS 1, Co-60	35.07	32.33 c	9.67 ab	7.43	11.10	6.13	44 d
M5-25	KPS 1, Co-60	35.53	35.33 ab	8.63 d	7.50	9.70	6.60	104 abc
M5-26	KPS 1, Co-60	35.00	34.33 ab	9.30 a-d	8.27	10.50	6.53	75 bcd
M5-28	CN 36, Cs-137	32.67	34.00 b	9.17 bcd	6.57	10.60	6.97	64 cd
M5-29	CN 36, Co-60	38.53	34.67 ab	9.23 a-d	8.90	10.33	7.03	124 ab
CN36	check	39.73	34.67 ab	9.63 ab	9.00	11.27	7.30	139 a
KPS1	check	36.67	35.33 ab	9.23 a-d	7.37	11.17	6.63	118 abc
F-test		1.10 ns	3.11**	2.83 **	1.74 ns	1.19 ns	<1	2.59*
C.V. (%)		11.5	2.2	3.8	17.1	8.3	6.5	29.1

ns = non-significance

Table 3 Yield and agronomic data of mungbean mutant lines in group I, early rainy season 1997.

Mutant line/variety	Pedigree	Plant height (cm)	Days to 50% flowering	Pod length (cm)	No. pods/plant	No. seeds/pod	100 seed wt (g)	Yield (kg/rai)	CLS	PM
M 5-1	CN 36, Cs-137	61.10	34.00	8.93	15.53	10.10	7.13	330	MR	MR
M 5-2	CN 36, Cs-137	57.17	34.67	7.63	11.77	9.83	7.10	270	MR	MR
M 5-3	KPS 1, Co-60	66.73	33.00	7.68	13.87	11.03	7.10	341	MR	MR
M 5-5	KPS 1, Co-60	56.00	35.33	7.53	11.50	9.83	6.80	244	MR	MS
M 5-6	KPS 1, Co-60	64.47	33.00	7.83	14.03	9.43	6.43	318	MR	MR
M 5-7	KPS 1, Co-60	58.50	35.33	7.88	12.07	10.47	6.57	291	MR	MR
M 5-8	KPS 1, Co-60	59.70	35.67	8.03	12.77	8.90	6.73	273	MR	MR
M 5-13	CN 36, Cs-137	61.57	35.00	8.23	12.90	9.77	6.63	323	MR	MR
CN 36	check	66.20	33.67	8.47	12.97	11.00	6.83	306	MR	MS
F-test		1.56 ns	2.34 ns	1.36 ns	2.40 ns	1.23 ns	<1 ns	1.20 ns		
C. V. (%)		8.7	3.4	8.4	10.9	10.9	7.7	17.0		

CLS = cercospora leaf spot
 PM = powdery mildew
 MR = moderately resistant
 MS = moderately susceptible
 ns = non-significance

Table 4 Yield and agronomic data of mungbean mutant lines in group II, early rainy season 1997.

Mutant line/variety	Pedigree	Plant height (cm)	Days to 50% flowering	Pod length (cm)	No. pods/plant	No. seeds/pod	100 seed wt (g)	Yield (kg/rai)	CLS	PM
M 4-2	CN 36, EMS	69.17	33.33	7.71	15.43	9.90	6.73 ab	339	MR	MR
M 5-15	CN 36, Cs-137	63.10	35.33	7.87	13.27	10.07	6.83 a	306	MR	MS
M 5-16	CN 36, Cs-137	65.80	34.00	8.30	15.10	9.57	6.33 bc	321	MR	MR
M 5-17	KPS 1, Co-60	65.20	34.33	8.23	11.79	10.77	6.63	292	MR	MR
M 5-18	KPS 1, Co-60	67.13	33.33	7.87	15.00	10.73	6.37 ab	338	MR	MR
M 5-19	KPS 1, Co-60	71.13	33.00	7.90	13.53	10.43	6.47 bc	338	MR	MR
M 5-20	KPS 1, Co-60	61.30	34.67	7.91	13.67	10.10	6.47 ab	289	MR	MS
M 5-21	KPS 1, Co-60	57.43	34.67	8.10	11.77	10.67	6.60 ab	279	MR	MR
M 5-22	KPS 1, Co-60	60.53	34.33	8.10	13.17	10.50	6.37 bc	306	MR	MR
M 5-23	KPS 1, Co-60	69.70	33.33	7.71	14.90	9.43	6.53 ab	343	MR	MR
M 5-24	KPS 1, Co-60	70.53	32.33	7.63	16.00	10.47	5.97 c	333	MR	MR
M 5-25	KPS 1, Co-60	67.27	34.33	7.77	14.73	10.57	6.53 ab	313	MR	MR
M 5-26	KPS 1, Co-60	67.90	34.00	8.27	12.70	10.17	6.40 ab	288	MR	MR
M 5-28	CN 36, Cs-137	62.47	34.33	7.93	12.17	10.27	6.53 ab	282	MR	MR
M 5-29	CN 36, Co-60	71.10	33.33	8.10	16.23	10.03	6.57 ab	331	MR	MS
CN 36	check	62.23	34.00	7.50	15.27	10.30	6.40 ab	331	MR	MR
KPS 1	check	62.77	34.33	7.61	14.63	8.97	6.70 ab	312	MR	MR
F-test		1.13 ns	<1 ns	1.64 ns	1.39 ns	1.86 ns	2.14*	<1 ns		
C.V. (%)		10.2	4.1	4.1	15.0	6.1	3.6	17.3		

CLS = cercospora leaf spot
PM = powdery mildew
MR = moderately resistant
MS = moderately susceptible
ns = non-significance

lines in group 1 grown during dry season of 1997, there was no statistical difference in almost every character, except days to 50% flowering of M4-1 and M5-4 being earlier than those of the 2 controls (Table 1). In group 2, M5-24 was found to have the earliest days to 50% flowering while M5-22 possessed the longest pod length and higher yield than the other tested lines including the controls (Table 2).

The tested lines in group 1, grown in early season of 1997, were found not to be differed in any character from one another (Table 3). The resistant levels to cercospora leaf spot of every tested line and those to powdery mildew of most lines were moderately resistant, except M5-5 and CN 36 moderately susceptible to powdery mildew.

Table 4 shows M5-15 to have the greatest seed size, with statistical difference from the other lines but not from the controls. Every tested lines was found to be moderately resistant to cercospora leaf spot. M5-15, M5-20, M5-29 had moderate susceptibility against powdery mildew, whereas the others moderate resistance.

The agronomic characteristics assessment under field conditions found M5-8 with high pod setting, M5-21 and M5-22 with good plant type and high pod setting, M5-19 with pods protruding up above the canopy, and M5-22 with early flowering. According to preliminary test of mungbean mutant lines during both growing seasons, the variation of agronomic characteristics of tested lines was rather high, yet the difference in disease resistance was not apparent due to mild disease infection. The assessment of resistant levels to powdery mildew and cercospora leaf spot by artificial inoculation should be furtherly investigated. However, many mutant lines were found to have potential development into promising lines, such as M5-8, M5-19, M5-21, M5-22 and M5-24.

CONCLUSION

The application of radiation and chemical to 2 mungbean varieties, KPS 1 and CN 36, find many kinds of chlorophyll mutation in M2 generation and such mutation implies that other kinds of mutation could occur as well. Plant selected in M2 and M3 generations carried characters of low powdery mildew infection, early maturity, pods protruding up above the canopy, and purple-red hypocotyl. The preliminary yield trial indicated the selected lines namely M5-5, M5-15, M5-20 and M5-29 and CN 36 to be moderately susceptible to powdery mildew while the others moderately resistant. The elite mutant lines are M5-8, M5-19, M5-21, M5-22 and M5-24 which possessed characters of high pod setting, good plant type, pod protruding up above the canopy, and early flowering. Artificial inoculation will be furtherly undertaken to assess yield damage in severe infection. The mutant lines with purple-red hypocotyl and leaf petiole will be used in a genetic study and compare with CN 36.

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