EFFECTS OF ETHYL METHANE SULFONATE AND Co^{60} GAMMA IRRADIATION IN WINGED BEAN

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ABSTRACT

Mutation and other effects of Co^{60} gamma radiation and ethyl methane sulfonate (EMS) in winged bean were determined at the M_1 generation. Seed germination was significantly reduced by irradiation at all levels used but only at 0.15% and above for EMS. Delayed seedling emergence, high lethality and pronounced morphological and probably physiological damage due to chromosomal aberrations resulted with the radiation treatments but not with EMS at the doses used. Chlorophyll mutations were observed as yellowish emergent seedlings resulting from EMS treatments and yellow variegations and leaf streaking associated with leaf deformations with irradiation. Violet flowers and seeds were also observed in both mutagen treatments but could not be ascertained as true mutations since the seeds used were not tested for purity. On the yield components, significant reduction in number of seeds per pod, and yields of pods, seeds and tubers were observed in the radiation treatments. Doses of 15 and 25 kr, however, resulted in a higher tuber yield compared with the control signifying a likely induction of favorable gene mutations. No significant effects were induced by EMS treatments on any yield parameter.

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KEY WORDS: Winged bean. *Psophocarpus tetragonolobus.* Mutation. Co^{60} gamma radiation. Ethyl methane sulfonate. Mutagens. Irradiation. Chlorophyll mutation. Gene mutation. Seed purity. Yield components.

INTRODUCTION

Winged bean *(Psophocarpus tetragonolobus* (L) DC) has been considered a very promising crop because of its multiple uses and high protein value. However, crop improvement programs for this underexploited legume have been lagging behind with only few researches undertaken to improve its use as a horticultural or agronomic crop. Existing genetic variability on which breeding programs are based has been found to be low for this crop (Khan and Brock, 1975). The desired breeding goals need the development of a specialized crop for seed, pod or tuber use and a multipurpose variety producing high pod, seed and tuber yield with the accompanying improved potentials (National Academy of Science, 1975). To produce the variability needed to pursue these different goals, mutagenesis has been looked into as a more rapid and effective means. Characters commonly found as induced mutations include early maturity, resistance to various diseases, higher protein content and adaptability to soil and climate conditions (Koo, 1972). Gamma radiation, an ionizing radiation, and ethyl methane sulfonate (EMS), an alkylating agent, are two mutagens found effective for use in winged beans and related crops (Brock *et al.* 1972; Gaul *et al.* 1972; Santos, 1969; Khan and Brock, 1975; Senanayake, 1978). This study, therefore, reports on mutations induced by gamma irradiation and EMS treatment and their effects on the growth, gross morphology and agronomic characters of winged bean.

MATERIALS AND METHODS

Uniformly sized seeds of winged bean, PRCRTC Accession 28, known to be pod and tuber producing were used in the study. Seeds were brought to the Philippine Atomic Energy Commission (PAEC), Quezon City, for Co^{60} gamma irradiation at doses of 0, 15, 20, 25, 30 and 40 kr and returned immediately for planting. For the chemical treatment, the seeds were presoaked for 12 hr in water and immersed in EMS solutions for 8 hr with constant shaking at concentrations of 0, 0.05, 0.10, 0.15, 0.20 and 0.25% and then rinsed and blotted dry before planting into the field. Seeds were sown at 25 cm between hills and 100 cm between rows. The 2 experiments were laid out in randomized complete block design in 3 replications with 100 seeds used per replication.

The irradiation experiment was conducted earlier with the irradiated seeds sown on August 12, 1979. The EMS-treated seeds were planted on September 3, 1979 just at the onset of the rainy season. All plants were given proper care and management including trellising. Germination, mutation, and agronomic yield components were determined for the different treatments. Examination of mutants, growth rate, and gross morphology was also done. Efficiency of the mutagens at the different levels was determined using the formula:

$$\text{Efficiency} = \frac{\text{mutation}}{\text{lethality}} \times 100$$

At the peak of the rainy season in mid-October, excessive water resulted in the exclusion of the third block in each experiment since the area was formerly a rice paddy with poor drainage.

RESULTS AND DISCUSSION

Germination and Seedling Emergence.

Irradiation significantly reduced the germination rate of winged bean seeds with the lowest rates observed at doses of 30 and 40 kr while that of EMS at doses of 0.15% and above (Table 1). The reduction in germination at certain treatment levels reflects the lethality of the treatment. With over 50% reduction in germination, the 30 and 40 kr already approached very lethal levels. Lethality of the radiation treatment was also evidenced by delay and slowing down of seedling emergence especially at the 30 and 40 kr doses (Table 2). Slow seedling emergence was also observed at the higher levels of EMS. In the irradiation experiment, however, the control plants emerged later than those in the EMS probably due to the absence of rainfall at planting time in the latter. In all the EMS treatments, seedling emergence was almost completed within 15 days, whereas in the other group, emergence was observed even beyond 20 days. Available moisture may have influenced this trend which favored the EMS experiment.

The lethal effects of the mutagens such as decrease in germination and delayed emergence may be due to genetic damage or cell death. In the case of the gamma radiation, the disruptive effects on the chemical and biological system of the seed were high because of the choice of high doses with high ionizing energy. Injury was thus proportional to dosage since according to Luse (1970), the degree of survival or vigor of seed depends upon the degree of cell damage or the number of inactivated proteins. The EMS may not be very lethal since the effect of this alkylating agent is more on the various specific enzymatic reactions leading to breakdown, repair and misrepair of the genetic material without much cellular disruption (Davis and Evans, as cited by Smith, 1972).

Table 1. Percentage germination of winged bean seeds irradiated with Co^{60} gamma radiation and treated with ethyl methane sulfonate (EMS).[1]

Treatment	Germination (%)
Co^{60}(kr)	
0	86.33 a
15	69.00 b
20	67.67 b
25	56.67 b
30	23.67 c
40	6.67 d
EMS (%)	
0.	86.67 a
0.05	83.33 a
0.10	81.00 a
0.15	51.67 b
0.20	51.67 b
0.25	52.33 b

[1]Means under a treatment group followed by a common letter are not significantly different at 1% level, DMRT.

Growth Rate of Seedlings.

Slow growth and height reduction were the significant effects

Table 2. Effect of Co^{60} gamma irradiation and ethyl methane sulfonate (EMS) on percentage seedling emergence of winged bean within 15 days.

Treatment	Days from sowing							Total	Final
	3	5	7	9	11	13	15		Count
Co^{60}(kr)									
0	—	2.00	29.34	23.67	8.67	4.00	5.67	76.33	86.33
15	—	—	17.67	17.67	12.00	6.67	3.67	58.00	69.00
20	—	1.00	18.67	16.67	14.33	3.67	2.00	56.33	67.67
25	—	—	7.67	16.34	16.33	5.33	3.33	50.00	56.67
30	—	—	1.67	3.00	9.33	3.00	3.00	20.00	23.67
40	—	—	—	0.67	0.67	1.34	0.33	3.00	6.67
EMS (%)									
0	7.67	27.00	18.00	11.34	9.67	4.66	4.67	83.00	86.67
0.05	8.00	25.00	17.00	16.33	6.67	4.00	1.67	79.33	83.33
0.10	4.33	19.67	15.67	15.00	11.00	8.00	2.34	76.00	81.00
0.15	2.00	11.33	13.00	8.00	7.67	4.33	1.67	48.00	51.67
0.20	1.67	10.67	12.33	8.00	6.33	5.00	2.67	46.67	48.33
0.25	1.00	9.33	13.67	8.66	8.00	5.00	2.00	47.67	52.33

caused by radiation treatments with height reductions most pronounced at the 30 and 40 kr doses (Table 3). Seedlings at these levels had stunted growth at different degrees and sometimes had hardly developed leaves and shoots for several days. Production of twin stunted shoots at the higher dosage (30 and 40 kr) was also observed in a few cases, probably another manifestation of lethality at the embryonic stage. While slow growth rate was clearly distinct in the irradiated seedlings, no pronounced growth impairment was observe in the EMS treatments; hence, no measurements were taken since twinning took place very early due to the availability of abundant moisture. Stunting was observed in a few seedlings under 0.20 and 0.25% treatments. In general, the EMS treatments did not result in very noticeable growth damage.

Physiological and Morphological Damage in Seedlings.

Stunted growth, double shoots, dwarf leaf, leaf netting, bifoliate and deformed leaves and stems may well be considered signs of physiological and morphological damage due to irradiation effects. Stunting due to inability of terminal bud to resume growth after emergence was very severe at levels of 30 and 40 kr. Irradiation resulted in pronounced netting and wrinkled appearance of first set of leaves which were smaller at higher doses. This effect was due to enlargement of veins resulting in a chlorotic appearance of the leaves. Leaf

Table 3. Plant height and growth rate of winged bean seedlings as affected by Co^{60} gamma irradiation.[1]

Irradiation	Plant height (cm)			Growth rate (cm/day)		
dose (kr)	5 DAE[2]	10 DAE	15 DAE	5 DAE	10 DAE	15 DAE
0	6.80	13.32	21.24	1.36	1.30	1.58
15	5.12	11.58	19.00	1.02	1.29	1.48
20	5.47	11.21	17.88	1.09	1.15	1.33
25	4.78	10.67	16.18	0.96	1.18	1.10
30	3.36	6.52	11.24	0.67	0.63	0.94
40	3.42	6.22	10.50	0.68	0.51	0.86

[1] Means of 3 replications.

[2] Days after emergence (DAE).

deformations associated with abnormal anastomosing veins and unusual texture or sometimes petiole and stem alteration occurred with the next sets of leaves. Many plants subjected to low doses immediately reverted to normal morphology. Two leaflets instead of three were also observed although the characteristics did not persist. Lateral branching on the second or third node produced healthier shoots which were sometimes free from leaf deformation. Most stunted plants recovered from injury through lateral branching. Under lower doses, seedlings recovered from injury earlier.

The observed injuries may be attributed to chromosomal breakage which is an outcome of irradiation according to Kondo, as cited by Nilan *et al.* (1969) and Luse (1970). The recovery at later stages among the injured plants may be a result of chromosomal repair since facilitated depolymerization of DNA and stickiness due to partial dissociation of nucleoproteins may sometimes result after sometime (Luse, 1970). Physiological and morphological injury resulting from EMS treatments comparable to that of irradiation was not observed.

Chlorophyll Mutations.

Mutations in leaf pigmentation were observed for both mutagen treatments. Chlorophyll mutants due to irradiation were of the streaking and variegation type. The leaf streaks of the mutants were characterized by white or pale lines in the lamina with deep green pigmentation. This type was usually associated with deformed leaves and probably a result of physiological injury due to chromosomal aberration. The variegation type was characterized by yellow leaf areas and stripes signifying mutations in chlorophyll synthesis. The EMS chlorophyll mutants showed wrinkled bright yellow leaves upon emergence. The yellow pigmentation usually reverted to normal green pigmentation after one or more days but newly-formed leaves were always yellow upon initiation.

These types of chlorophyll mutations which may be considered point or gene mutations and a test for viable mutations (Gaul *et al.*, 1972) were considered in the determination of mutation frequency. Higher mutation frequencies were observed for the 25 kr level and 0.25 and 0.10% EMS (Table 4). The efficiency of the mutagens was thus determined considering the survival and lethality rates of the treatments (Table 4). The 20 and 25 kr and the 0.05 and 0.10% EMS treatments which are median doses had higher efficiency probably because they result in considerable mutagenic reactions with less damaging effects. Also, with the absence of some needed precautions, post-handling damage and post-treatment effects might have affected the treated seeds and increased the lethality of some doses. Thus, a lower frequency of mutants was obtained. According to some researchers, a great portion of physiological damage is due to extra treatment effects like injury during post-handling, storage, and post irradiation treatments (Conger *et al.*, 1970) and pre-soaking, infusion of mutagen, post-treatment drying and washing, and application of scavenger for EMS (Konsak *et al.*, 1970).

Violet pigmentations in seeds and flowers were also observed in both mutagen treatments and this might be due to a mutation in

Table 4. Mutation frequency, survival, lethality and efficiency of Co^{60} gamma irradiation and ethyl methane sulfonate (EMS) in winged bean.

Treatment	Mutation frequency[1]	Survival[1] (%)	Lethality[2] (%)	Efficiency (%)
Co^{60} (kr)				
0	—	85.67	0	—
15	0.67	69.00	19.46	3.44
20	2.33	67.67	21.01	11.09
25	6.67	56.67	33.85	19.70
30	2.67	18.67	78.21	3.41
40	2.67	4.67	94.55	2.82
EMS (%)				
0	—	86.67	0	—
0.05	0.67	83.33	3.85	17.40
0.10	1.33	81.00	6.54	20.34
0.15	0.33	51.67	40.30	0.81
0.20	0.67	51.67	40.30	1.66
0.25	2.00	52.33	39.67	5.05

[1]Means of 3 replications.

[2]Percentage reduction in survival adjusted to the control.

anthocyanin synthesis. Induction of brown, yellow, or white seed coat color through EMS or gamma radiation treatment had been reported in black bean varieties (Moh, 1972). Since this was rather a special case, flower or seed coat color was not included in determining mutation frequency. It is also possible that this trait was a natural variation in the seeds since no test for purity was previously done.

Effect on Yield Components.

EMS treatments showed no significant reduction in the different yield parameters studied, while gamma irradiation gave significant effects on length of pods, seed number per pod, and yield of pods, seeds, and tubers (Table 5). Tuber yield in EMS treatments were generally lower probably due to occasional waterlogging which did not favor tuber formation. Reproductive parameters for EMS were, however, generally higher compared to the irradiated ones and this might also be attributed to waterlogging effect. Reduction in yield parameter values of the materials subjected to radiation treatments increased with the dosage and this may be a result of the physiological damage to the treated seeds affecting cellular metabolism and protein synthesis.

Highest tuber yield per plant was obtained in the 25 kr treatment although this was not significantly different from the control. This response, however, might have been the result of high flower drop and low production of pods and seeds since there was more translocation of assimilates to the tubers, a likely indication of a point mutation involved. The significant decrease in the number of seeds per pod with increasing irradiation dose (Table 5) may also indicate that the fertility rate was reduced correspondingly which in turn affected the length of pods and yield of seeds.

Since the study was done on the M_1 generation only, micro-mutations in yield components could not be isolated. However, there is a great possibility of their occurrence due to the observance of great variability in yield characters. Also, in the case of EMS, since no significant reduction in yield parameters was observed despite the probable treatment injury, it is possible that certain treated plants had been improved genetically in some traits. According to Gaul *et al.* (1972), mutants may exert deleterious pleiotropic effects which can result in general decreasing vitality. In the radiation treatment, a prospective micromutation might be present in the tuber component since higher values than the control were observed when the materials were subjected to the 25 and 15 kr doses. The difference may not be significant from the control considering the average values although some individual plants produced unusually large tubers.

Table 5. Effect of Co^{60} gamma irradiation and ethyl methane sulfonate (EMS) on the yield components of winged bean.[1]

Treatment	Green pod length (cm)	Seed number per pod	Shelling percentage	Twenty-seed weight (g)	Yield per plant (g) Dry pod	Seeds	Tuber
Co^{60} (kr)							
0	21.26	11.16a	47.93	8.10	45.10a	18.18a	92.10ab
15	16.38	9.48ab	44.26	7.32	42.25ab	14.38ab	98.12a
20	18.33	10.04ab	46.18	9.04	37.12bc	11.75bc	88.38ab
25	17.19	8.82bc	43.44	8.39	34.75c	10.06bc	117.00a
30	14.29	7.00c	43.92	6.58	21.88c	7.06c	51.75bc
40[2]	—	—	—	—	—	—	40.12c
EMS (%)							
0	19.59	11.86	50.01	8.08	57.86	22.93	47.72
0.05	20.52	12.15	48.85	8.31	56.82	20.08	47.15
0.10	20.62	12.72	48.05	7.69	55.90	22.90	43.08
0.15	20.78	11.98	46.66	8.26	58.53	22.00	50.37
0.20	19.78	11.92	45.18	7.81	65.67	24.85	45.05
0.25	19.88	11.64	46.76	7.80	49.94	19.90	41.08

[1]Means under each treatment group followed by a common letter are not significantly different at 5% level, DMRT.

[2] The 40 kr treatment did not bear fruit.

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