RESEARCH ARTICLE
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Gamma Radiation Induced Mutations in Mungbean

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ABSTRACT: Seeds of mungbean varieties KPS 2, VC 6468-11-1B, their F_1 and F_2 were treated with gamma rays (Cs-137 source) at the dose of 500 Gy. The M_1 seeds were sown in the field with the controls (non-irradiated seeds) and bulk-harvested. The M_2 seeds were sown to observe their characters and number of mutants in each population. Among over 430,000 plants observed, irradiated F_1 population gave the highest frequency of mutants at 0.168%, followed by F_2, VC 6468-11-1B, and KPS 2 at 0.165%, 0.152%, and 0.142%, respectively. Mutant characters were grouped as chlorophyll, leaf, flower, and pod mutants. Chlorophyll mutations included albino, coppery leaf, light-green leaf, variegated leaf, waxy leaf, white streak leaf, and xantha leaf. Leaf mutations were lanceolate leaf, narrow-rugose leaf, multiple leaflet, round-cuneate leaf, unifoliate leaf, and wrinkled leaf. The flower mutant was cock's comb raceme while the pod mutant was a lobed one. All mutants were purified for genetic study and possible uses of the traits.

KEYWORDS: *Vigna radiata*, mungbean, gamma rays, mutants.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) (2n=2x=22) is a self-pollinated legume originated in South Asia. It is an economically important crop in India, Pakistan, Thailand, Vietnam, Myanmar, and China with the combined planted area of over 5 million ha. The crop is considered rather wild as it still gives low seed yield (<1 t/ha), with uneven maturity. This opens an ample room for mungbean breeders to improve the crop. Besides natural genetic variation available in mungbean germplasm collections, mutation techniques are proven useful in obtaining novel traits and creating genetic variability. Gamma irradiation as a mutagen can induce useful as well as harmful mutation in plants[1, 2]. Singh and Sharma[3] isolated a few pentafoliate and tetafoliate mutants from the gamma rays- and ethyl methanesulphonate (EMS) - treated mungbean. These mutants showed a significant increase in dry matter production, total chlorophyll content and yield, as compared to their parents in M_2 and M_3 generations. Santos[4], and Bahl and Gupta[5] described the mutant characters and their inheritance in mungbean and reported that variegated, multifoliata, xantha, chlorina, albino, unifoliata were each controlled by a recessive gene. Variation in quantitative traits by mutation breeding was also reported by several scientists[6-11]. The major traits were seed yield, seed size, pods per plant, seeds per pod, days to maturity, and plant height. Additionally, Wongpiyasatid *et al*[12] reported an improvement in resistance to powdery mildew, Cercospora leaf spot, and cowpea weevil through gamma radiation induced mutation.

The objective of this study is to induce mutation in four mungbean populations using gamma radiation to determine the mutation frequency, observe the mutant traits and purify them for possible uses.

MATERIALS AND METHODS

Seeds of the parental lines, 'Kamphaeng Saen 2' (KPS 2) designated as P_1, and VC 6468-11-1B designated as P_2 were obtained from the Asian Region Center of the Asian Vegetable Research and Development Center (ARC-AVRDC), Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. KPS 2 is a popular Thai mungbean cultivar sown over 150,000 ha annually, owing to its high yielding, shiny seed coat with moderately large seed size (~66 g per 1000 seeds), green hypocotyl, and moderately resistant to powdery mildew and Cercospora leaf spot diseases. VC 6468-11-1B is an elite breeding line with a dull seed coat and a large seed size (~70 g per 1000 seeds), purple hypocotyl, and resistant to both diseases.

Crosses were made using KPS 2 as the female parent. The parents and F_1 seeds were sown in the successive season. All F_1 seedlings had purple cotyledons confirming that they were derived from crossed seeds, since the purple hypocotyl is dominant to the green one. Another set of F_1 seed was also made in parallel to the production of F_2 seeds. Thus, all four mungbean populations (P_1, P_2, F_1 and F_2) were finally obtained in that same season. The initial M_0 seeds were determined for germination percentage in each population and converted to the seed weight of 156, 187, 159, and 212 g for KPS 2, VC6468-11-1B, F_1 and F_2 respectively. Each amount is equivalent to ~2500 seeds that can readily germinate.

The gamma irradiator used in this study is installed at the Gamma Irradiation Service and Nuclear Technology Research Center (GISC), Kasetsart University, Bangkok. It was manufactured by J.L. Shepherd & Associates, under the Model MARK 1-30, Serial No. 1116, loaded with 4500 Curies of Cs-137 having a half-life of 30.12 years. The gamma irradiator was calibrated to irradiate 500 Gy of gamma rays to the seed lots for 82 minutes. The rate of 500 Gy was found to produce much variance while leaving over 60% of the surviving plants[13]. The M_1 seeds were sown in the field surrounded by non-irradiated population as the control. The M_2 seeds were bulk-harvested in each population. There were 7.76, 5.12, 11.02, and 8.72 kg from KPS 2, VC6468-11-1B, F_1, and F_2, respectively. The seeds were drilled in rows, after which the mutants were periodically observed right after germination. In each visit to the field, the mutant plants were marked with bamboo sticks for subsequent observations. Data were recorded on characters and number of the

Table 1. Amount of M_2 mungbean seed sown, number of seedlings germinated, and number of mutants found in the populations of KPS 2, VC6468-11-1B, their F_1 and F_2.

Populations	M_2 seeds sown (kg)	No. of seedlings germinated	Mutant Type					Total	Percent of mutants
			Albino(lethal)	Chlorophyll	Leaf type	Flower	Pod		
KPS	27.76	127,880	113	27	35	0	7	182	0.143
VC6468-11-1B	5.12	81,708	45	26	45	0	8	124	0.152
F_1	11.02	134,607	164	16	35	1	10	226	0.168
F_2	8.72	89,647	105	8	29	0	6	148	0.165
Total	**32.62**	**433,842**	**427**	**77**	**144**	**1**	**31**	**680**	**0.157**

Table 2. Types and number of mutants found in M_2 plants of the four mungbean populations.

Mutant characters	Populations				Total
	KPS 2	VC 6468-11-1B	F_1	F_2	
1. Chlorophyll mutation					
Albino	113	45	164	105	427
Coppery leaf	1	0	0	0	1
Light green leaf	2	2	3	0	7
Variegated leaf	2	3	4	3	12
Waxy leaf	2	6	5	0	13
White streak leaf	1	2	2	3	8
Xantha leaf	19	13	2	2	36
2. Leaflet mutation					
Lanceolate leaflet	2	2	2	0	6
Multiple leaflet	29	37	29	27	122
Narrow-rugose leaflet	2	1	0	0	3
Round-cuneat leaflet	0	0	0	1	1
Unifoliate leaf	2	0	0	1	3
Wrinkled leaf	0	5	4	0	9
3. Flower mutation					
Cock's comb raceme	0	0	1	0	1
4. Pod mutation					
Lobed pod	7	8	10	6	31
Total	**182**	**124**	**226**	**148**	**680**

Table 3. Description of the mutant characters found in M_2 plants of the four mungbean populations.

Mutant characters	Character descriptions
1. Chlorophyll mutation	
Albino	Entirely white leaves. Seedlings survived for less than 2 weeks after germination
Coppery leaf	Copper-like color leaflet beginning from flowering till harvesting
Light-green leaf	Lighter green leaves as compared to normal leaves
Variegated leaf	Persistent variegated yellow-green leaves
Waxy leaf	Normal leaf shape with pale waxy leaflet
White streak leaf	White streak from edge to middle vein
Xantha	Orange yellow to light yellowish white, survived for only 2-3 weeks after germination
2. Leaflet mutation	
Lanceolate leaf	Elongated middle leaflet with broader lateral leaflets
Multiple leaf	Compound leaf with 4 - 9 leaflets per leaf
Narrow-rugose leaf	Narrow and elongated leaflet
Round-cuneat leaf	Short petiole, round leaf, did not set pod
Unifoliate leaf	Single leaf, did not set pod
Wrinkled leaf	Leaf has wrinkled character
3. Flower mutation	
Cock's comb raceme	Raceme look like cock's comb, did not set pod
4. Pod mutation	
Lobed pod	Distinct lobes on pod possibly due to semi-sterility

mutants. At maturity, each mutant plant was individually harvested. The remaining plants were bulk-harvested for M_3 seeds and sown for further observation.

Field cultural practices on this experiment were conducted based on standard management for mungbean grown in Thailand. Briefly, the seeds were drilled in rows of 50 cm apart at the rate of 20 seeds per a meter. Weeds were controlled by pre-emergence spraying of Imazathapyr at 250 g(ai)/ha. Late weeds were eradicated by hand weeding twice at 15 and 30 days after sowing. Insects were controlled by spraying with triazophose (Hostathion 40% EC) at the rate of 40 cc per 20 liters of water when the insect population was building up beyond the threshold level. Irrigation water was applied during the cropping season as needed.

Results and Discussion

Since the gamma rate of 500 Gy was almost at Lethal Dose-50 (LD-50) for mungbean[13], the M_1 seed lost its germination up to 20-30% from the effect of irradiation. Some seedlings showed either albino or xantha leaf, and died prematurely. A number of mutant plants were identified in M_2 generation and the mutation percentages in KPS 2, VC6468-11-1B, F_1 and F_2 population were 0.142, 0.152, 0.168, and 0.165, respectively (Table 1). The percentages were much smaller than that reported by Srichot[13] and Thongpimyn[14] who found the mutant rate of up to 1-4% in both qualitative and quantitative traits. In our experiment, no distinct mutant plants were found regarding yield components, possibly due to such a low mutant rate.

The mutants found were mainly of leaf chlorophyll mutation such as albino, coppery leaf, light-green leaf, variegated leaf, waxy leaf, white streak leaf, and xantha leaf. Leaf mutations were lanceolate leaflet, narrow-rugose leaflet, multiple leaflet, round-cuneat leaflet, unifoliate leaf and wrinkled leaf. Flower mutation gave looks like cock's comb with pollen sterility. Similar mutants were also reported by Lamseejan *et al*[15], Santos[4], and Srichot[13]. A lobed pod mutation with fewer seeds per pod was also found. This trait may associate with partial sterility, causing constriction at the point where there was undeveloped seed. The number of mutants found and their descriptions are shown in Table 2 and 3. These mutants were not found in the control populations. Therefore, they were considered the real mutants and not the results of genetic recombination between the parental lines.

Characteristics of leaflet mutants are shown in Fig 1, while those of the other types are given in Fig 2. The unifoliate leaf mutant was also sterile, in agreement with that reported by Santos[4]. The mutant produced numerous flower buds but failed to open. The round-cuneat leaflet mutant produced flowers but its pollen scattered all over the corolla and thus expressed partial sterility. However, coppery leaf, variegated leaf, waxy leaf, white steak leaf, lanceolate leaflet, narrow-rugose leaflet, multiple leaflet, and wrinkled leaf were fertile

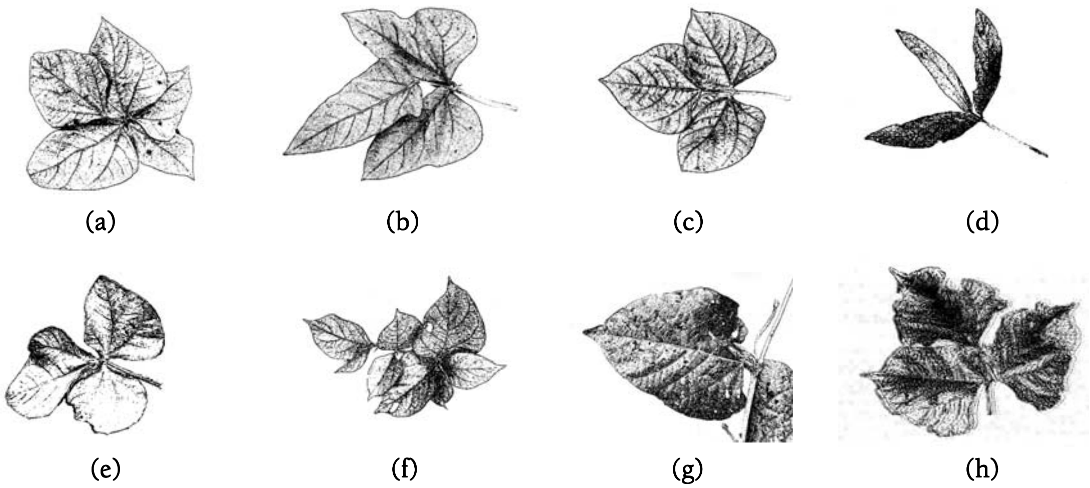

Fig 1. Leaf mutant variation found in the M_2 plants: (a) five multiple leaflet, (b) lanceolate leaf, (c) normal trifoliate leaf, (d) narrow-rugose leaf, (e) round-cuneat leaflet, (f) seven multiple leaf, (g) unifoliate leaf, (h) wrinkled leaf.

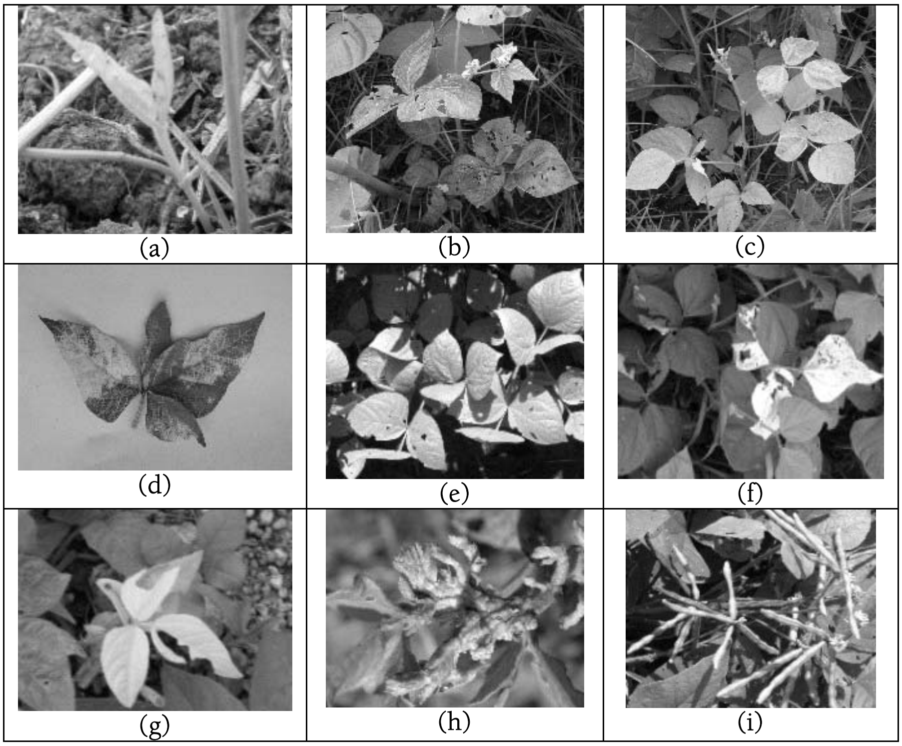

Fig 2. Chlorophyll, flower, and pod mutations found in the M_2 plants: (a) albino, (b) coppery leaf, (c) light-green leaf, (d) variegated leaf, (e) waxy leaf, (f) white streak leaf, (g) xantha leaf, (h) cock' s comb raceme, (i) lobed pod due to sparse seed set.

with low yield. The variegated leaf and narrow-rugose leaf mutants produced only few pods while waxy leaf produced pods with lean seeds. These mutants have been reported by a number of scientists,[1,3-6,13,15] but we have found them all in one experiment, possibly due to the high population used (up to 433,842 seedlings).

Although not statistically significant, the rate of mutation was slightly higher in F_1 and F_2 as compared to the parents, since the progenies are more heterozygous than the parents. The heterozygous genotypes have more possible target alleles to mutate than the pure line parents. However, the mutation rate in this experiments is rather low and thus the result needs to be confirmed in more experiments. The mutant plants were individually harvested for 2 consecutive generations to establish pure mutant lines for further studies. All mutants were bred-true and can be utilized in breeding and genetic study. Some multiple leaflet lines set profuse pods that might be useful as a marker for mungbean yield improvement in the future.

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