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Mutation breeding of *Phaseolus vulgaris* L.: Studies on the effects of irradiation dosage to resolve a suitable procedure of handling M_1 and M_2 generations

C.H. CHEAH and E.S. LIM
Department of Agronomy and Horticulture, Faculty of Agriculture, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia.

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RINGKASAN

Kajian tumbesar bibit bijihbenih Phaseolus vulgaris *yang dirawat dengan radiasi gamma telah dijalan di dalam rumah kaca dan di petak luar. Peratus percambahan di antara bijibenih yang dirawat dengan 10, 25, 25, 30, 35, 40 Krads radiasi gamma tidak berbeza daripada peratus percambahan bijibenih yang tidak dirawat (kawalan). Bagaimanapun, rawatan dengan 100 Krads adalah parah kepada meristem mercu. Pemanjangan epikotil bagi kajian di rumah kaca terdapat sangat sensitif kepada radiasi gamma.*

Berdasarkan kepada 30% penurunan dalam pemanjangan epikotil, dos yang optimum untuk irradiasi adalah 30 Krads. Pada rawatan ini peratus pokok yang hidup pada masa mengutip hasil adalah 50% dari populasi yang tidak dirawat (kawalan). Juga, rawatan dengan 30 Krads menghasilkan bilangan mutan klorofil pejal yang paling tinggi.

Sungguhpun bukan semua mutan klorofil yang didapati daripada populasi B_s (gumpalan terdiri daripada sebiji), % berlakunya adalah tiap-tiap kali lebih daripada nilai yang didapati pada populasi B_p (gumpulan terdiri daripada biji selenggai).

Berdasarkan kepada keputusan-keputusan ini, adalah disyorkan bahawa:

a) radiasi gamma yang paling sesuai untuk program pembaikbiakan secara mutasi adalah dos 30 Krads;

b) bijibenih M_1 patut ditanam di luar dengan menggunakan kepadatan pokok yang dua kali kepadatan yang biasa digunakan untuk kawalan;

c) pokok-pokok M_1 patut dipetik supaya populasi B_s (gumpalan sebiji) dapat diuji untuk kehadiran mutan.

SUMMARY

Seedling growth studies on gamma-irradiated seeds of Phaseolus vulgaris *were conducted in the greenhouse and the field. No significant differences in germination scores between the unirradiated control and the seeds exposed to 10, 20, 25, 30, 35 and 40 Krads of gamma radiation were obtained. Exposure to 100 Krads, however, was lethal to the apical meristem. Growth of the epicotyl under greenhouse conditions was the most affected by gamma radiation.*

Based upon a 30% reduction in epicotyl length the optimum dose for irradiation was 30 Krads. At this level of irradiation the survival score at harvest under field conditions was 50% of that of the unirradiated control. Treatment with 30 Krads also gave the highest number of solid chlorophyll mutants.

While not all the chlorophyll mutants were scored in the B_s (si gle seed bulk) populations, the % occurrence was consistently higher than that for the corresponding B_p (single pod bulk) populations.

Based on these results, it is recommended that :

a) the most suitable level of gamma radiation to use in a mutation breeding programme is 30 Krads;

b) the M_1 seeds should be field-planted at double the planting density of the control;

c) the M_1 plants should be harvested such that the B_s (single seed bulk) population be used for screening of the mutants.

INTRODUCTION

The cultivar Nicaragua 209-480 (U.S.A. *Phaseolus vulgaris* Accession No. 209-480) was among 27 white navy bean varieties introduced in 1977. In the preliminary trials conducted during the period 1977 – 1979, it was the best yielding variety. The desirable agronomic characters of this cultivar include good growth, overall high tolerance to pests and diseases, short maturation period (35 days from sowing to flowering and 99 days from sowing to final harvest), high podding ability, uniform seeding, high shelling percentage and non-shattering, parchment-type pods. The plant habit is indeterminate and twining and capable of supporting three substantial harvests. However, this habit is undesirable as it requires large labour input for maintenance and harvesting.

An essential improvement desired of this cultivar is the modification of its plant architecture. One method is by the induction of a mutant with the desirable erect habit. This technique has been used successfully in the breeding of navy beans in the United States of America. A bush mutant was obtained through the use of X-irradiation on the variety Michelite (Down and Andersen, 1956). This mutant was subsequently used in breeding programmes which resulted in the introduction of four commercial varieties (Sigurbjomsson and Micke, 1974).

In a mutation programme of a seed propagated crop, the appropriate irradiation dose and the method of handling the M_1 and M_2 populations need to be determined. This is because the mutant is expected to be recessive and can be detected only as a homozygote in the M_2. The dose applied should give the highest spectrum of mutations. The progeny size of each contributing M_1 plant should be adequate to give the highest probability of screening the maximum number of cell iniitals exposed to the mutagen. The response of the M_1 plants under existing field conditions should also be known – to facilitate the planning of field requirements. Such information has not been established for the mutation breeding of legumes particularly for *Phaseolus vulgaris* in the tropics. Preliminary studies in the greenhouse and in the field were conducted for this purpose.

MATERIALS AND METHODS

Freshly harvested seeds of Nicaragua 209-480 with approximately 12% moisture were irradiated with gamma rays from a cobalt^{60} source. The rate of irradiation was 1 Krad per 4.930 seconds. A gammatron located in the Fakulti Sains, Universiti Kebangsaan Malaysia, was used.

The first study was carried out in the greenhouse. Seed lots of 200 seeds each were irradiated at 10, 20, 30, 40 and 100 Krads. These irradiated seeds together with the unirradiated control were grown in nursery boxes containing a soil mixture. Germination scores and growth measurements were taken when the cotyledons of the control seedlings abscissed.

A subsequent study based on the results of the first study was conducted in field plots in raised beds 1 m wide and 10 m long. Each treatment consisted of 200 seeds and these were planted in two rows for each bed. The four radiation treatments and the unirradiated control were arranged in a randomised complete block design with three replications. Germination and survival scores at flowering and at harvest were recorded. At harvest the seeds were collected in the following manner:

a) Single seed bulk (B_S) : a single seed randomly taken from the fourth pod (counting from the base of the stem);

b) Single pod bulk (B_P) : the remaining seeds from the fourth pod;

c) Single treatment bulk (B_t) : Seeds from the remaining pods on all the plant in the same treatment.

A third study was on the M_2 population using the seed bulks harvested from the field plots. Each bed was planted with 100 seeds in two rows. For each radiation level, there were one bed of the single seed bulk, two beds of the single pod bulk and three beds of the single treatment bulk. Complete randomisation was applied throughout. The B_s populations were germinated under greenhouse conditions before transplanting into their respective field plots.

RESULTS

The percentage germination of seeds irradiated at 10, 20, 30 and 40 Krads was between 97 – 100% and this was not significantly different from that of the unirradiated control. Irradiation at 100 Krads, however, resulted in a germination score significantly lower than those of the other treatments (Table 1). In addition, this level of irradiation caused damage to the apical meristem resulting in failure of germinated seeds to develop beyond the open cotyledons stage. On the other hand, seedlings from the other lower irradiation doses grew normally and were similar to the control seedlings in their twining behaviour.

Visual differences were observed in the uniformity of green colouration of the primary leaves. Leaves of irradiated seedlings were uniformly covered with fine, pale specks, the intensity of which increased progressively with increase in irradiation level (Plate 1 and 2).

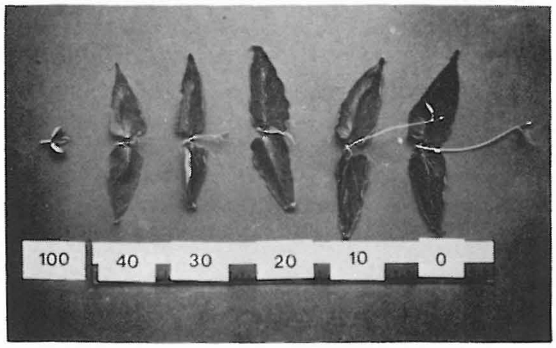

Plate 1: Shoot tips and primary leaves of control and irradiated seedlings

TABLE 1

Seedling Growth Measurements under Greenhouse Conditions

Gamma Radiation (Krads)	% Germination	Seedling Length Mean (cm)	Hypocotyl Length Mean (cm)	s.d.	C.V.	Epicotyl Length Mean (cm)	s.d.	C.V.
0	98	25.0	10.7	0.78	7.3	14.3	1.17	8.2
10	99	25.3	11.5	0.85	7.4	13.8	1.19	8.6
20	100	23.9	10.4	0.74	7.1	13.5	1.30	9.6
30	99	19.9	9.4	0.93	9.9	10.5	1.96	18.7
40	97	12.3	7.3	1.36	18.6	5.0	2.36	47.2
100	21	0	0	–		0	–	–

Gamma Radiation (Krads)	Primary Leaf Area Mean (cm²)	s.d.	C.V.	Fresh Weight Mean (gm)	s.d.	C.V.	Dry Weight Mean (gm)	s.d.	C.V.
0	16.0	0.67	4.2	41.7	0.58	1.4	4.26	0.18	4.3
10	14.4	0.74	5.1	40.7	0.58	1.4	3.86	0.04	1.1
20	14.5	0.53	3.6	40.0	1.00	2.5	3.91	0.08	2.1
30	13.2	0.16	1.2	37.3	1.15	3.1	3.86	0.07	1.8
40	10.6	0.15	1.5	3.1	1.00	3.2	3.35	0.11	3.2
100	0	–	–	0	–	–	–	–	–

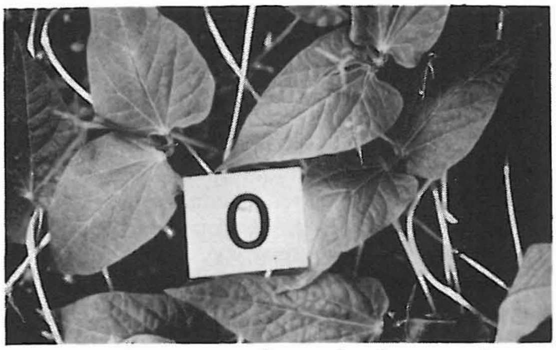

Plate 2: Left – primary leaves showing normal green colouration (control) *Right – primary leaves showing the speckled effect due to gamma irradiation (30 Krads)*

In general, growth of the seedlings was depressed by irradiation. This was evident from the progressive reduction in the total length of seedlings, the hypocotyl length, the epicotyl length, the primary leaf area and the fresh weight of seedlings as the irradiation dosage was increased (Table 1 and Figure 1).

In the field, the percentage germination for each treatment was considerably lower than that

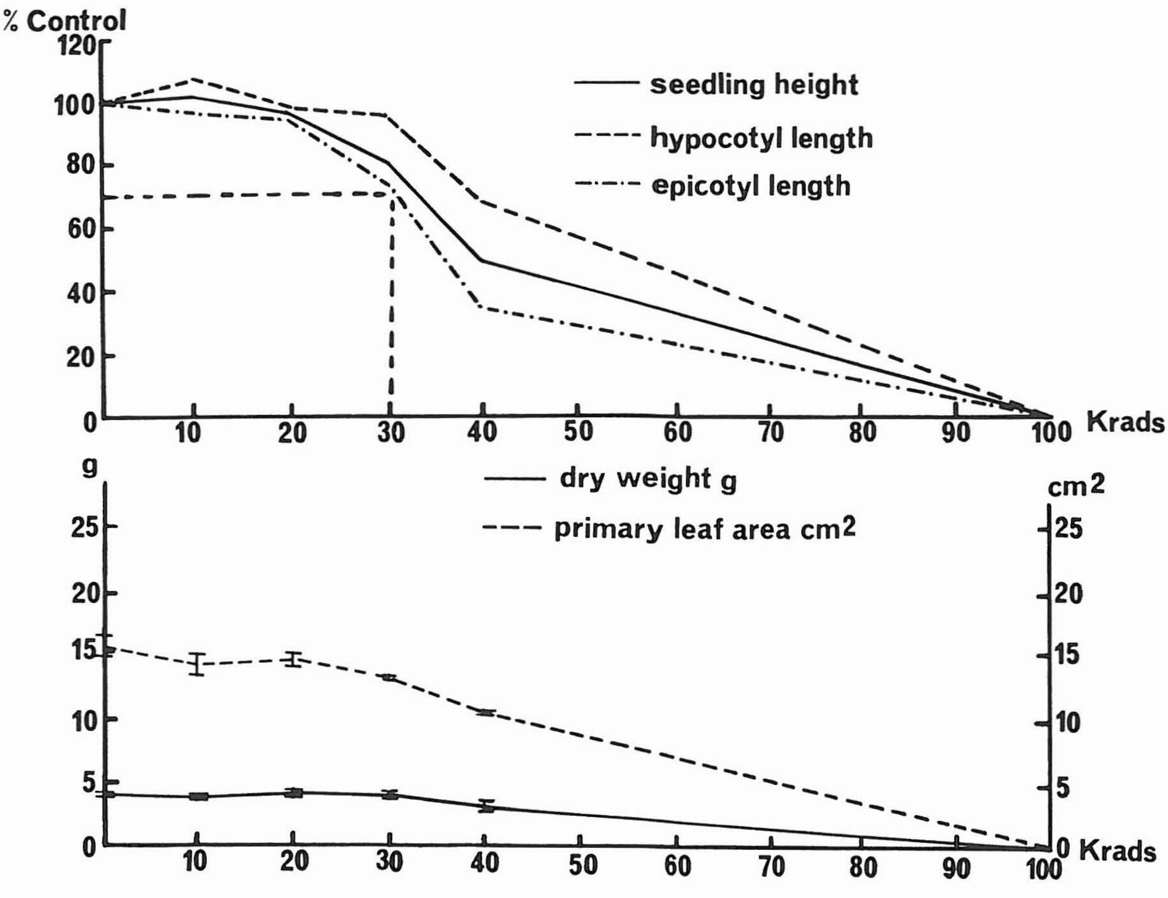

Figure 1. Effects of gamma radiation on seedling growth

obtained in the greenhouse for the same treatment. This is attributed to the heavy soil and excessive moisture content in the field. No significant difference was found, however, between the unirradiated control and the various irradiated populations. Differences in field conditions were expressed in replicate differences which were significant for survival scores at germination, flowering and at harvest. Many of the seedlings that emerged either failed to survive to maturity or if they did, failed to produce seeds. The general average % survival for the whole field trial was low (23%). There was significantly lower survival of the irradiated material at flowering and at harvest than the unirradiated control. Depression in growth by irradiation was expressed in a delay in maturation of the irradiated plants. Irradiation at 30 Krads gave a survival rate of 50% of that of the control (Table 2).

In the field screening of chlorophyll mutants in the M_2, the population irradiated with 30 Krads produced the highest total number of chlorophyll mutants (Table 3). Of this total, the highest number was recovered from the Single Pod Bulk population. All the chlorophyll mutants scored were solid mutants and they did not survive beyond the primary leaf stage (Plate 3).

Plate 3: A solid, lethal chlorophyll mutant found in the M_2 generation

TABLE 2

Germination and Survival Scores under Field Conditions

Gamma Radiation (Krads)	0	25	30	35
Germination Mean %	49.8	52.7	52.8	54.8
Flowering Mean %	36.5	28.3	22.3	19.5
At Harvest Mean %	34.5	23.7	17.0	16.0
Average Seed No per 4th pod harvested	4.5	2.4	3.0	3.1

ANOVA

Sources	df	F values Germination	Flowering	At Harvest
Treatment	3	0.36ns	5.67*	5.33*
Replicate	2	6.10*	27.32**	7.36*
Error	6			
Total	11			

DISCUSSION

Gamma radiation depressed growth irrespective of the parameter used to express the growth of the seedlings. However, growth expressed in terms of epicotyl length appeared to be the most sensitive to gamma radiation (*Figure 1*). In view of the lack of significant differences in germination scores between different irradiated seed lots, the choice of a suitable dose cannot be based on the LD_{50}. A reasonable alternative to the use of LD_{50} as a basis in this case would be the epicotyl length. Gaul (1959) found that there is a correlation between seedling height as measured by the length of the first leaf and the % survival in barley. In his programme on soya beans, Guhardja (1980) based his choice of the most appropriate dose on a 30% reduction in seedling height. Thus based on 30% reduction in epicotyl length, the most appropriate level of irradiation would be 30 Krads (*Figure 1*).

Under the existing field conditions, seeds irradiated with 30 Krads produced a survival score of 17% at harvest compared with the score of 34.5% for the unirradiated control population. This is comparable to 50% survival at harvest taking into consideration the significant environmental effects.

In mutation breeding, seedling chlorophyll mutants are used frequently as a practical and convenient measure of the effectiveness of a particular dose (Gaul, 1964; 1965; 1979). In terms of the recovery of seedling chlorophyll mutants from the different seed bulks, M_2 plants derived from seeds irradiated with 30 Krads gave the highest number of such mutants (total of seven out of 602 M_2 seeds sown).

Based on the seedling performance in the greenhouse and the field, survival under field conditions and the recovery of seedling chlorophyll mutants in the M_2, the dose of 30 Krads of gamma irradiation appears suitable for the induction of mutants.

With regard to the handling of the M_1 at harvest and the size of M_2 population, there are various standard methods such as

a) the tiller, branch or plant progeny method;

b) the single seed bulk method;

TABLE 3

Seedling Chlorophyll Mutant Scores in the M_2 Populations

Gamma Radiation (Krads)		25	30	35	Combined Total
Population					
B_s	Mutant No.	1	2 (5*)	0	3 (7*)
	Total Sown	142	102	96	340
	% Occurrence	0.7	2.0 (1.66*)	0	0.9 (0.75*)
B_p	Mutant No.	0	3	1	4
	Total Sown	200	200	200	600
	% Occurrence	0	1.5	0.5	0.7
B_t	No. Mutants	1	2	0	3
	Total Sown	300	300	300	900
	% Occurrence	0.3	0.7	0	0.3
Total	No. Mutants	2	7	1	10
	Total Sown	642	602	596	1840
	% Occurrence	0.3	1.2	0.2	0.5

(*) : Combined value for Bs and Bp

c) the multiple seed bulk method;

d) the mass bulk method;

as well as modifications of these. The choice of the method would depend on the crop, its pattern of ontogenetic development, the kind of mutation desired and the field requirements and their cost.

The method of collection of seeds from the M_1 plants must be such that the highest possible number of mutated cell initials are represented by their progeny in the M_2 population being screened. Based on the recovery of seedling chlorophyll mutants in the M_2, studies in cereals have shown that the first five tillers produced a higher number of factor mutations than the others (Gaul, 1965). Such information is lacking for leguminous crops. In this case the variety of *Phaseolus vulgaris* used has an indeterminate habit of growth and a very different pattern of ontogenetic development. The fourth pod was arbitrarily selected to be representative of the development region equivalent to the first five tillers in cereals.

If this is a correct reflection of the true situation, then the number of chlorophyll mutants recovered in the M_2 from the B_s and B_p populations combined should be higher than that recovered from B_t (seeds originating from pods other than the fourth pod).

With reference to Table 3, scores for the total number of seedling chlorophyll mutants and for the % occurrence of such mutants, are higher for M_2 progeny from the B_s and Bp populations combined than from the B_t population. The total number of mutants recovered from the B_s and B_p populations combined for the three levels of gamma radiation was seven compared with the total of three in the B_t population. The % occurrence based on the total number sown was respectively 0.75 and 0.3. Of the three radiation levels studied, treatment at 30 Krads produced the highest number of mutants. Again the combined total of the number of mutants from the B_s and Bp populations was higher than that of the B_t population being five and two respectively and with 1.66 and 0.7% occurrence respectively.

Based on these results, the fourth pod can be assumed to be a good sample of the region of the plant with the most number of mutated cell initials.

In cereals, the spike can be of a mixed orogin, that is, more than a single cell initial went into the formation of the spike (Eriksson, 1965). In this case, if it is assumed that a single pod was derived from only one cell initial, the number of cell initials that would be sampled for screening in the M_2 would be the same whether the B_s or the B_p population is used. The difference between these two populations is in the family size (where the pod is represented by one seed in the B_s population while the pod is represented by more seeds usually an average of three seeds in the B_p population). Since the family size would affect the probability of occurrence (a large family size would have a higher probability of the mutant occurring and a smaller family size would mean a reduction in that probability), the level of probability of occurrence would therefore be different for the B_s and B_p population respectively. A theoretical consideration of this probability based on the following formula

$$m = \frac{\log(1-p)}{\log(1-a)}$$

where m is the family size, p is the level of probality of occurrence of the mutant in the form of the homozygote and a is the segregation ratio of the homozygote in the M_2, is as follows.

The segregation ratio for a homozygote of a recessive factor mutation in the M_2 is 0.25 (¼). When the B_s population is used, the family size is one and the p value is calculated to be equal to 0.25 i.e. the probability of finding the one seed growing into the mutant is only 0.25. But when the B_p population (average family size of three) is used, the p value is calculated to be 0.42 i.e. the probability of finding that one of these three seeds growing into the mutant is now increased to 0.42. However, from the practical point of plant maintenance, while using the B_p population ensures a higher probability level of recovering the mutants in the M_2, the triple-fold increase in labour and field requirements would not justify using the B_p in preference to the B_s.

The chlorophyll mutant scores of the three radiation levels studied do bear out this fact that the lower probability inherent in the B_s population would result in the recovery of only some of the possible mutants. However, except for the radiation level of 35 Krads, the % occurrence obtained in practice from the B_s was higher than that from the B_p (Table 3). It is reasonable to conclude that the B_s population would be the best to use for the screening of the M_2 generation for the mutants. The high scores obtained for the radiation level of 30 Krads further support the choice of 30 Krads as a suitable dose.

Based on the mutant scores for 30 Krads of gamma irradiation, the mutation rate for the chlorophyll mutation induced by that level of gamma radiation is calculated to be $10^{-2.05}$ or approximately one in 111.

CONCLUSION

In general, it can be concluded from these studies that :

a) based on 30% reduction in epicotyl length under greenhouse conditions the most appropriate dose to apply is 30 Krads;

b) under existing field conditions only 17% (50% of control) of seeds treated with 30 Krads gamma radiation would survive to contribute to the next generation (or in other words, the LD_{50} at harvest under existing field conditions is 30 Krads);

c) 30 Krads gamma irradiation expressed the highest mutagenic potential of the three levels studied and this treatment produced 2% of chlorophyll mutants in the B_S population;

d) the fourth pod produced the higher % of chlorophyll mutants in the M_2 as compared to the rest of the pods on the plant.

These results specifically indicate that the level of gamma radiation that is suitable is 30 Krads, that the M_1 seeds should be field-planted and harvested in such a manner that the B_S population is used for screening of the mutant in the M_2. Irradiated seeds should be planted at double the normal planting density of the control in the field.

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