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The Influence of Gamma-rays on the Injury and Chromosomal Aberrations of Long Bean (*Vigna sesquipedalis*, Fruw.)

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ABSTRAK

*Kesan-kesan sinar gama ke atas tiga jenis kacang panjang (**Vigna sesquipedalis**, Fruw) iaitu Melaka, Local Black dan Local Long telah dikaji dengan menggunakan 5 takaran mulai 10 hingga 50 KR. Aberasi kromosom dan ciri-ciri kerosakan fisiologi telah digunakan untuk mengkaji kepekaan radiasi varieti-varieti. Pada amnya, radiasi gama tidak mengubah kadar percambahan bijibenih tetapi menyebabkan pengurangan yang signifikan dalam sifat-sifat berkait dengan terushidupan, pertumbuhan dan kesuburan. Kadar aberasi kromosom juga bertambah jika takaran bertambah. Oleh sebab ketinggian pokok dapat diukur dengan cepat dan senang dan juga berkait dengan sifat-sifat lain yang dikaji, ciri ini menjadi satu parameter yang baik untuk mengkaji kesan radiasi ke atas kacang panjang. Berdasarkan pengurangan lebih kurang 30% daripada sifat ketinggian pokok atau 50% kemautan, maka kekuatan dos memanjang yang dicadangkan ialah 30 hingga 50 KR bagi kerja mutasi aruhan dalam kacang panjang.*

ABSTRACT

*The effects of gamma-rays on three varieties of long bean (**Vigna sesquipedalis**, Fruw), namely Melaka, Local Black and Local Long were studied using five doses ranging from 10 to 50 kR. Both Chromosomal aberrations and characteristics related to physiological damage were used to study radiation sensitivity of the varieties. In general, gamma radiation did not affect % seed germination but caused a significant reduction in characteristics related to survival, growth and fertility. Percentage chromosomal aberrations also increased with increasing dose. As the measurement of seedling height is simple, quick and highly correlated with most characteristics studied, it could be a useful parameter in the study of radiation effects on long bean. Using a criterion of approximately 30% reduction in seedling height or 50% lethality, it is suggested that doses ranging from 30 to 50 kR would be suitable for mutation induction in long bean.*

INTRODUCTION

Long bean, a tropical climbing annual, is grown in Malaysia mainly for its long, fleshy and tender pods for use as a vegetable. As the existing cultivars are climbers, staking is required to keep the pods from touching ground and rotting. This often involves extra cost to growers and restricts the area of cultivation to where the stakes are available.

As the determinate plant type has been known to be a recessive trait (Singh *et. al.* 1976), the prospect of using induced mutation to alter the climbing habit seems promising. Furthermore, the use of induced mutation breeding techniques has been shown to result in many new varieties of different crops (Singurbjornsson, 1983).

In Malaysia, mutation work on long bean is unknown. However, there are a few reports on the genetics of some quantitative traits (Mak and Yap, 1977, 1980) and conventional breeding for yield improvement (Yap *et. al.*, 1977). The present paper reports a pilot experiment conducted to examine a number of characteristics reflecting the effects of gamma-rays on M_1 plants and their usefulness as a guide to determine the appropriate dose suitable for mutation induction in long bean.

MATERIALS AND METHODS

Three commmonly cultivated varieties of long bean were used for the radiation treatment. The varieties were Local Long (large, dark brown seed), Local Black (small seed, black with white patches) and Melaka (light brown seed, medium size). These varieties were found to breed true consistently after two generations of seed multiplication through natural selfing.

A cobalt-60 source (at the Universiti Kebangsaan Malaysia, Bangi) was used to treat the seeds. Each variety was treated with 5 different doses ranging from 0 kR to 50 kR with a difference of 10 kR between any two consecutive doses. For each treatment, 200 healthy and uniform seeds were used. The moisture content of the seeds was 13 ± 0.4%. After radiation treatment, the seeds were planted within three days for the following studies:

Seed Germination and Chromosomal Aberrations

For each treatment and variety, 50 seeds were soaked overnight and placed in a petri-dish with two layers of moist filter-paper and left at room temperature (23 ± 4°C) in the laboratory. Radicles of pre-determined length (1.0 – 1.8 mm) were harvested. These were fixed at 3:1 alcohol-acetic acid fixative for ½ hour at room temperature and then stored at approximately 4°C until further use in anaphase analysis. The last 2 mm of each root tip was squashed and then stained in acetocarmine. For each treatment, 50 anaphase/telophase plates were randomly selected from a set of 6 to 9 slides to score for bridges, laggards and fragments according to the classification of Evans (1962).

Effects on M_1 Seedlings

Another batch of 50 seeds were divided equally into 2 lots and each lot was planted in perforated aluminium trays filled with a mixture of sand and garden soil. Data were collected for

a. *Seedling Height (cm):* the average height of 2-weeks-old seedlings from each tray was used. Height was measured from the base of plumule to the tip of the first leaf (i.e. shoot length).

b. *Seedling Dry Weight (g):* the average dry-weight of five 3-weeks old seedlings from each replicate was taken.

c. *Leaf Surface Area (cm^{-2}):* the average of five leaves from each replicate was calculated. The leaf area was determined by tracing the first fully matured leaf onto a graph paper.

Effects on M_1 Adult Plants

For each treatment, 50 seeds per radiation dose per variety were planted in raised beds of 20 cm in height, 60 cm in width and 7 m in length. The beds were spaced at 60 cm apart. The distance between planting points was 30 cm. A randomized complete block design with two replicates were used. Supporting sticks were put up two weeks after planting. The characteristics measured included:

a. *% Survival:* survival count was taken at seed emergence and at harvest again. Survival was expressed as % of the control (i.e. 0 kR) for the respective varieties.

b. *Pollen Abnormality:* Two flowers were collected for each treatment and variety (preferably at the same stage) to obtain pollen grains for staining with acetocarmine. Two hundred pollen grains per replicate per treatment for each variety were taken for analysis. This procedure was based on the preliminary observation that flowers collected from the control treatment at different stages (before opening, opening and after opening) showed no difference in pollen abortion.

The average of ten pods from the early harvest (or less for treatment with high doses) in each replicate was taken for the measurements of

(c) Pod length (cm), (d) Fresh pod weight (g) and (b) Number of seeds per pod.

RESULTS

Germination, Survival and Chromosomal Aberrations

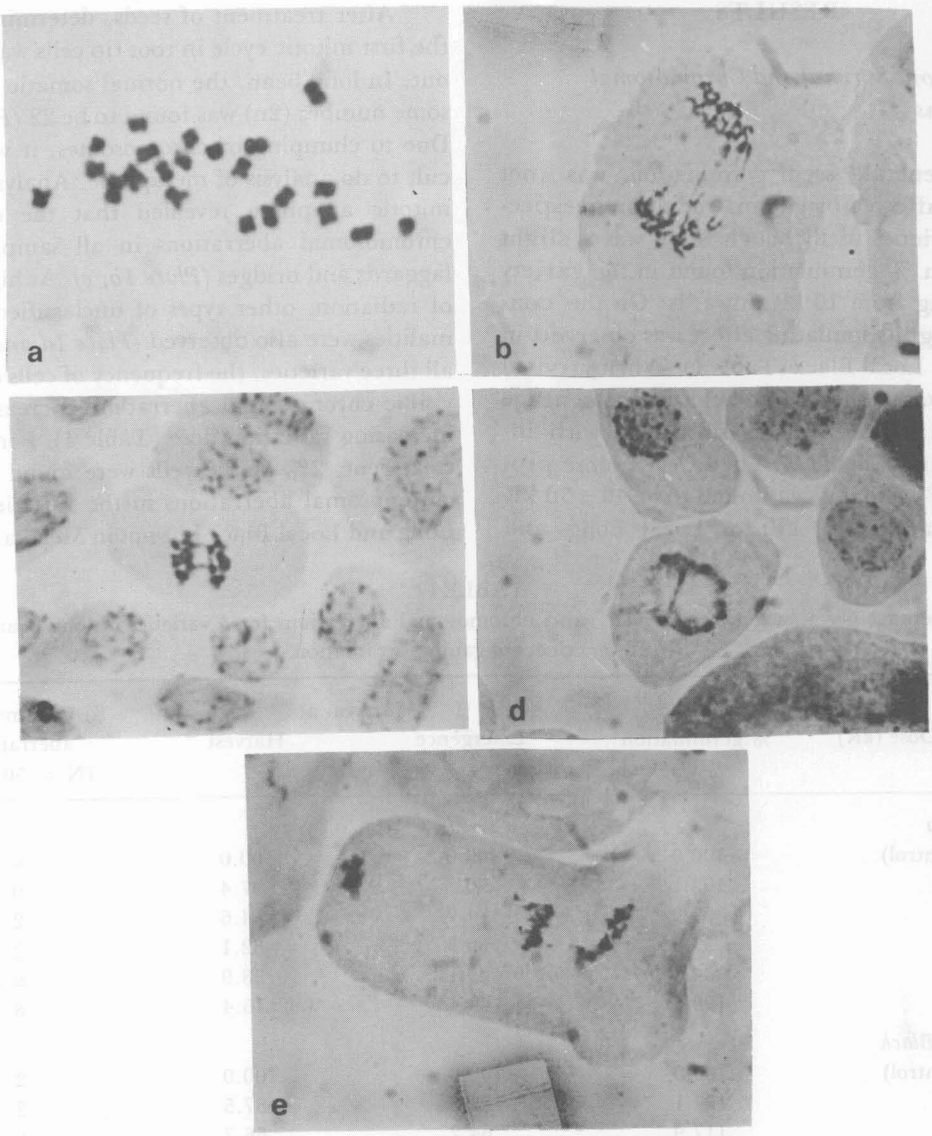
In general, seed germination was not adversely affected by gamma radiation irrespective of varieties used though there was a slight decrease in % germination found in the variety Local Long from 10 kR onwards. On the contrary, a slight stimulating effect was observed in the variety Local Black (Table 1). When survival of plants was studied in the field, the percentage of survival showed a decreasing trend with increasing doses (Table 1). The LD_{50} i.e. dose producing 50% lethality was found to be 40 − 50 kR for Melaka, 30 − 40 kR for Local Long and Local Black. A dose of 50 kR also proved lethal to Local Long but not for other two varieties.

After treatment of seeds, determination of the first mitotic cycle in root tip cells was carried out. In long bean, the normal somatic chromosome number (2n) was found to be 22 *(Plate 1a)*. Due to clumping of chromosomes, it was difficult to do analysis of metaphase. Analysis of the mitotic anaphase revealed that the common chromosomal aberrations in all samples were laggards and bridges *(Plate 1b, c)*. At high levels of radiation, other types of unclassified abnormalities were also observed *(Plate 1d and e)*. For all three varieties, the frequency of cells carrying visible chromosomal aberrations increased with increasing radiation dose (Table 1). For control treatment, 2% of the cells were found to show chromosomal aberrations in the varieties Local Long and Local Black but not in Melaka.

TABLE 1
Percentage germination ⁺, Survival ⁺ and chromosomal aberrations for 3 varieties of long bean at different doses of gamma-irradiation

Variety/Dose (kR)	% germination	% Survival at Emergence (− Field Planting −)	% Survival at Harvest	% Chromosomal aberration (N = 50 cells)
Melaka				
0 (control)	100.0	100.0	100.0	0
10	102.1	90.7	97.4	0
20	97.9	104.7	84.6	2
30	97.9	95.3	82.1	2
40	104.3	65.1	58.9	6
50	100.0	44.1	15.4	8
Local Black				
0 (control)	100.0	100.0	100.0	2
10	107.1	78.6	87.5	2
20	117.9	64.2	66.7	6
30	135.7	50.0	33.3	6
40	103.6	53.5	45.8	8
50	103.6	25.0	16.7	10
Local Long				
0 (control)	100.0	100.0	100.0	2
10	96.9	78.2	69.5	4
20	87.9	104.3	100.0	4
30	87.9	86.9	86.9	6
40	87.9	34.7	39.1	8
50	93.9	0.0	0.0	14

⁺: In comparison to the control which was taken as 100%.



a: Somatic chromosome number, 2n = 22
b: Laggard; c: Bridge; d and e: unclassified abnormalities

Plate 1: Somatic chromosome number and chromosomal aberrations.

Seedling Characteristics. In general, a trend of reduction with increasing dose was observed for seedling height, seedling dry weight and leaf surface area (Table 2). However, at lower doses such as 10 – 20 kR, the effects were less severe or not significantly different from control. At higher doses, the reduction was distinct and severe (Table 2).

Visual observation showed another interesting radiation effect on the leaves of the irradiated plants. Compared to the control, the leaf surface of the treated plants was rough and the roughness tended to increase with increasing dose. This was generally accompanied by dark coloration and crumpling of leaves. These leaves subsequently senesced prematurely, especially at high doses.

Fertility and Adult Plant Characteristics. Pollen grains collected from fully developed flowers that were fully stained with acetocarmine were taken as normal and fertile while empty, shrivelled and partially stained ones as abnormal

TABLE 2
Mean values of some seedling characteristics

Variety	*Melaka*	*Local Black*	*Local Long*	*Mean*
Dose (kR)				
(a) Seedling height (cm)				
0	15.03	7.14	9.23	10.47
10	13.68	7.40	9.81	10.30
20	12.56	7.12	9.87	9.85
30	11.53	5.80	8.72	8.69
40	9.20	5.90	4.81	6.63
50	6.69	4.19	4.13	5.00
Mean	11.45	6.26	7.76	
$LSD_{.05}$(Var.) = 1.36; $LSD_{.05}$(Dose) = 1.92				
(b) Seedling dry weight (g)				
0	0.363	0.256	0.345	0.321
10	0.347	0.276	0.376	0.390
20	0.254	0.262	0.334	0.283
30	0.235	0.224	0.247	0.292
40	0.220	0.231	0.130	0.194
50	0.177	0.129	0.095	0.134
Mean	0.266	0.230	0.254	
$LSD_{.05}$(Var.) = 0.034; $LSD_{.05}$(Dose) = 0.049				
(c) Leaf surface area (cm^{-2})				
0	24.70	19.25	21.29	21.75
10	22.21	16.75	21.72	20.23
20	19.09	15.71	19.24	18.02
30	18.74	13.08	16.81	16.21
40	13.26	11.14	6.24	10.21
50	11.34	7.50	4.60	7.81
Mean	18.22	13.91	14.98	
$LSD_{.05}$(Var.) = 1.92; $LSD_{.05}$(Dose) = 2.71				

or sterile. Pollen abnormality expressed as a percentage of the total pollen grains counted (N = 400) is presented in Table 3. The increase in the percentage of unstainable pollen was particularly great at 50 kR for the varieties Melaka and Local Black. However, response of Local Long at 50 kR was not known due to its 100% lethality. At lower doses, the trend was rather erratic, probably due to sampling error associated with the fact that only two flowers per treatment were used. For the varieties Local Black and Local Long, the control treatment also showed an exceptionally high level of unstainable pollen.

Sterility was also measured in terms of seed. When the number of seeds per pod was determined, a general trend of decreasing seed set with increasing dosage was apparent (Table 3). The reduction was particularly large at 50 kR. A reduction in seed set was also associated with a corresponding decrease in pod size in terms of pod length and pod weight (Table 3).

DISCUSSION

Mutagens can cause physiological damage besides gene and chromosomal changes. Physiological damage, mainly manifested as growth retardation and death, is generally restricted to M_1 generation. The present study showed that gamma radiation did not have severe effect on % seed germination in long bean. The first phase of germination is swelling of cells by hydration followed by enzymatic activation and metabolism. Seed germination which is simple growth of radicle and shoot, is apparently unaffected by embryo damage caused by irradiation treatment. However, embryo damage might become apparent only at the later stages of ontogenesis. This is evident from the results of the survival count where survival rate decreased with increasing levels of doses. A similar trend of results was obtained in cowpea (Ojomo and Chedda, 1971) and other crops (Anon, 1977).

As expected, the height of M_1 seedlings was significantly reduced after irradiation, especially at higher doses. Such phenomenon has been attributed to changes in hormonal levels such as auxins and ascobic acid; physiological and biochemical disturbances (Gunckel and Sparrow, 1954; Singh, 1974); changes in enzyme activity (Blinks, 1952) and impaired mitosis in the meristematic zone of growing seedlings (Cherry and Hageman, 1961). Woodstock and Justice (1967) also suggested that it might also be due to a decrease in respiratory quotient in the irradiated seedlings. Growth retardation was also manifested in a reduction in dry weight production and leaf surface area. The results are quite similar to the ones reported in *Phaseolus vulgaris* (Cheah and Lim, 1980). In addition, leaf abnormality was also observed as a primary effect of gamma-rays. This resulted in premature shedding of leaves probably due to the evolution of ethylene (Romani, 1966).

Although high doses of ionizing radiation inevitably inhibit growth in plants, the results of many other reports (De Nettancourt and Contan, 1966; Donini *et. al.*, 1964; Sax, 1963; Mikaelson and Aastveit, 1957) showed an apparent growth stimulation by exposure to low doses. However, the mechanism is unknown. Though a slight stimulating effect was observed for low dose irradiation in some traits such as seedling height and dry weight, the difference is non-significant when compared to the control.

Physiological disturbances were also evident in the adult plants where pod size (in terms of pod length and pod weight) was significantly reduced with increasing doses. In Mungbean however, a reverse trend was reported by Shamsi and Sofajy (1978). A common phenomenon i.e. a reduction in reproductive capacity in terms of pollen abortion and seed set, is also observed in long beans. Undoubtedly, physiological effects induced by radiation treatments are of different natures. The causes can be chromosomal and extra-chromosomal. Though a separation of the two causes is generally not possible, an increased frequency of chromosomal aberration with increasing doses signifies that the physiological damage is at least partly chromosomal in origin.

In many mutagenic studies, seedling height has been found to correlate well with lethality and chromosomal aberrations (Anon, 1977). Hence, it is a common parameter used to deter-

TABLE 3
Mean values of some adult plant characteristics

Variety	*Melaka*	*Local Black*	*Local Long*	*Mean*
Dose (kR)				
(a) % aborted pollen grains				
0	4.0	31.3	11.3	15.5
10	12.5	44.3	38.8	31.9
20	11.8	27.8	11.8	17.1
30	14.3	43.0	22.3	26.5
40	27.5	45.3	39.0	37.3
50	93.3	79.8	—	86.6 $^{+}$
Mean	27.2	45.3	24.6 $^{+}$	
LSD $_{.05}$ (Var.) = 8.73; LSD $_{.05}$ (Dose) = 11.27				
(b) No. seeds per pod				
0	18	18	18	18.0
10	18	16	16	16.7
20	15	14	17	15.3
30	13	13	14	13.3
40	12	12	10	11.3
50	5	7	—	6.0 $^{+}$
Mean	13.5	13.3	15.0 $^{+}$	
LSD $_{.05}$ (Var.) = 1.85; LSD $_{.05}$ (Dose) = 2.39				
(c) Pod length (cm)				
0	62.3	53.6	51.8	55.9
10	57.3	48.8	47.6	51.2
20	52.5	48.3	48.7	49.8
30	56.5	43.6	45.8	48.6
40	50.5	42.1	32.9	41.8
50	38.2	35.9	—	37.1 $^{+}$
Mean	52.9	45.4	45.4 $^{+}$	
LSD $_{.05}$ (Var.) = 5.15; LSD $_{.05}$ (Dose) = 6.65				
(d) Fresh pod weight (g)				
0	37.2	19.6	21.5	26.1
10	34.2	16.2	14.1	21.5
20	20.1	17.1	14.3	17.2
30	28.6	14.6	14.0	19.1
40	27.0	14.6	7.1	16.2
50	12.4	9.9	—	11.2 $^{+}$
Mean	26.6	15.3	14.2 $^{+}$	
LSD $_{.05}$ (Var.) = 3.64; LSD $_{.05}$ (Dose) = 4.69				

—: No data due to 100% lethality at 50 kR.

$^{+}$: Average based on existing data.

mine the effect of a mutagenic seed treatment. The present study also pointed to the same conclusion as seedling height in long bean was also highly and significantly correlated with % survival at harvest (r = 0.75), chromosomal aberrations (r = −0.82), fertility such as % pollen abnormality (r = −0.76), number of seeds per pod (r = 0.69) and pod size in terms of pod length (r = 0.88) and pod weight (r = 0.86). Thus, seedling height, being simple and quick to measure, is a useful parameter. The other parameters, though useful as indicators for irradiation effects, are often more difficult, tedious and time consuming for their determination.

To deduce the most suitable dose to use, one has to understand the fact that mutation frequency tends to increase with increasing dose. On the other hand, plant lethality and chromosomal damage often increase with increasing dose. Hence, an intermediate dose has to be taken i.e. between the low dose with lower mutation frequency but higher survival rate and the high dose with higher mutation frequency but lower survival rate. The common measurement taken is the LD_{50} (lethal dose 50) or dose that caused 30 − 50% growth reduction, particularly seedling height. In the present study, approximately 30% reduction in seedling height was obtained between 30 − 40 kR for the varieties Melaka and Local Long; and between 40 − 50 kR for Local Black. In term of % survival at harvest, 50% reduction was observed for Melaka at 40 − 50 kR, for Local Long at 30 − 40 kR and for Local Black at 20 − 40 kR. As variety and dose interaction was found to be not significant for seedling height, the different varieties tended to show similar response though the magnitude might vary. Hence, though the apparent difference between varieties or between parameters used makes it difficult to generalize about the most suitable dose of gamma-rays for inducing mutation in long bean, doses ranging from 30 to 50 kR appear to be appropriate.

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REFERENCES

ANON. (1977): Manual on mutation breeding. 2nd edition. IAEA Technical Reports Series No. 119. FAO/IAEA, Vienna.

BLINKS, L.R. (1952): Effects of radiation on marine algae. *J. cell comp. physiol.* **39:** 11.

CHEAH, C.H. and LIM, E.S. (1980): Mutagenesis applied to the improvement of *Phaseolus vulgaris* as a grain legume crop in Malaysia. In induced mutations for improvement of grain legume production II, A technical document issued by IAEA, Vienna, 1982.

DE NETTANCOURT, D. and CONTANT, R.B. (1966): Comparative study of the effects of Chronic gamma irradiation on *Lycopersicum esculentum* mill and *L. pimpirellifolium* Dund. *Rad. Bot.* **6:** 545 − 56.

DONINI, B., SCARASCIA MUGNOZZA, G.T. and D'AMATO, F. (1964): Effects of Chronic gamma irradiation in durum wheat and bread wheat. *Rad. Bot.* **4:** 387 − 93.

EVANS, H.J. (1962): Chromosome aberrations induced by ionizing radiations. *Int. Rev. Cytology* **13:** 221 − 316.

GUNCKEL, J.E. and SPARROW, A.H. (1954): Aberrant growth in plants induced by ionizing radiations. *Brookhaven Symp. Biol.* **6:** 252 − 77.

MAK, C. and YAP, T.C. (1977): Heterosis and combining ability of seed protein, yield and yield components in long bean. *Crop Sci.* **17:** 339 − 41.

MAK, C. and YAP, T.C. (1980): Inheritance of seed protein content and other agronomic characters in long bean (*Vigna sesquipedalis* Fruw.) *Theor. Appl. Genet.* **56:** 233 − 39.

MIKAELSON, K. and AASTVEIT, K. (1957): Effect of neutrons and chronic gamma radiation on growth and fertility in oats and barley. *Hereditas* **43:** 371 − 80.

OJOMO, O.A. and CHHEDA, H.R. (1971): Mitotic events and other disorders induced in cowpeas, *Vigna unguiculata* (L) Walp. by ionizing radiation. *Rad. Bot.* **11:** 374 − 81.

ROMANI, R.J. (1966): Biochemical responses of Plant Systems to large doses of ionizing radiation. *Rad. Bot.* **6:** 87 − 104.

SAX, K. (1963): The stimulation of plant growth by ionizing radiation. *Rad. Bot.* **3:** 176 − 86.

SHAMSI, S.R.A. and SOFAJY, S.A. (1978): Effects of low doses of gamma-radiation on the growth and yield of two cultivara of broad beans. *Env. Expt., Bot.* **20:** 87 – 94.

SINGH, H.B., MITAL, S.P., DABAS, B.S. and THOMAS T.A. (1976): Breeder's stocks of cowpea. *Indian J. Genet. Plt. Breeding* **36:** 410 – 17.

SINGH, B.B. (1974): Radiation induced changes in catalase, lipase and ascorbic acid of safflower seeds during germination. *Rad. Bot.* **14:** 195 – 99.

SIGURBJORNSSON, B. (1983): Induced mutations. *Acta Agric. Scand. Suppl.* **23:** 62 – 66.

WOODSTOCK and JUSTICS (1967): Radiation-induced changes in respiration of corn, wheat, sorghum and radish seeds during initial stages of germination in relation to subsequent seedling growth. *Rad. Bot.* **7:** 129 – 36.

YAP, T.C., MAK, C. and POH, C.T. (1977). Genetic studies and breeding of long beans in Malaysia. *Mal. Appl. Biol.* **6(2):** 155 – 62.

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