RADIOBIOLOGICAL EFFECTS OF VARYING DOSE LEVELS OF GAMMA RADIATION ON SORGHUM, *Sorghum bicolor* (L.) Moench*

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Introduction

The mutagenic potential of ionizing radiations such as X-rays and gamma radiation has long been widely applied in biological and agricultural researches. As these physical mutagens induce random ingralocus or intergenic mutations, the trend on mutation breeding becomes a practical alternative to the conventional hybridization and recombination methods in plant breeding. With induced mutagenesis, production of new germ plasm is possible through proper identification and selection of the desirable types from the randomly-occurring mutations. Eventually, this new germ plasm would be important in the improvement of some crop plants (Brock, 1972; Haq et. al., 1971).

Sorghum bicolor (L.) Moench. (Family Poaceae) is a cereal and forage crop. It is usually ranked fifth in acreage among the grain crops of the world (Hanna, 1982). Aside from being a part of the human diet as a source of carbohydrates, sorghum is an important source of hog and poultry feeds, fodder, silage and molasses. It is also used as a raw material in the manufacture of alcoholic beverages. Thus, sorghum merits further extensive studies aimed at improving some of its agronomic characters to effect better and higher crop yield.

The objectives of the present study are: (1) to investigate some radiobiological effects of the varying dose levels of gamma radiation on sorghum for two generations; and (2) to characterize and isolate some mutations caused by the different dose levels used in the experiment.

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Materials and Methods

Dormant dry seeds of sorghum, *Sorghum bicolor* (L.) Moench. cultivar BPI-Cosor 3 purchased from the Bureau of Plant Industry, San Andres, Manila were used as the experimental material.

Methodology

A total of 4,000 good seeds were handpicked and were divided into five lots, one lot per treatment making a total of four experimental treatments. One lot was kept unirradiated as control. The seeds were placed in five separate small paper boxes and were kept in a moist desiccator (with a 1:1 mixture of saturated solutions of NH_4C1 and $NaNO_3$) for seven days to bring the seed moisture content to approximately 15 percent. Then, the seeds were laid flat on small rectangular cardboards and were sealed in polyethelene plastic bags ready for gamma irradiation.

Gamma irradiation of samples was done at the Gamma Cell Facility of the Philippine Atomic Energy Commission (PAEC), Diliman, Quezon City. The radiation doses administered ranged from 15 to 60 kilorads (kR) at the dose rate of 188.13 kilorads per hour from a radioactive Cobalt (Co^{60}) source.

Immediately after irradiation, the seed samples were rehydrated in a water-bath maintained at 30°C for two hours. The seeds from each lot were divided into two sub-lots, one sub-lot was germinated using the 'growing rack or blotter sandwich method' in a growth room supplied with 120 ft-candle illumination at room temperature for seedling height data; the other sub-lot was germinated in moist blotting paper in the petri dish, one dish per sub-lot.

Seven days after treatment, the seedlings from the second sub-lot were transplanted to the field plots provided at the Experimental Station, U.P. Department of Botany following a dose-to-row pattern, two replications per treatment in a completely randomized block (design (CRB). Fifty seedlings per treatment were planted, 12 seedlings per sown meter in furrows spaced at 50 cms. as recommended (PCARRD, 1975).

Data Gathering

Five parameters were used to determine and assess the effects of varying dose levels of gamma radiation in the first (M_1) generation. Data on seedling height were scored by measuring the shoot length of the seedlings germinated in the 'blotter sandwich method' one week after treatment. Plant height was measured at maturity. To determine earliness or lateness of maturity, flowering time was determined by counting the number of days the plants had taken to anthesis from the time of germination. Sterility effect was determined based on the percentage reduction of the

seed-set of the main spikes, and the average dry weight per 1,000 kernels taken at random from the main spikes was likewise scored.

Seeds obtained from the main spikes of the M_1 plants were processed, incubated in a drying oven (60°C) for three hours, and were germinated. Seven days after germination, chlorophyll-deficient seedling mutations were screened. Other morphological mutations were likewise scored. Sixty seedlings per treatment per replicate were transplanted to the field plots and were grown to maturity. Except on seedling height, the same parameters were used to determine the radiation effects in the second (M_2) generation.

Analysis of Data

Data on the effects of varying dose levels of gamma radiation in both the M_1 and M_2 generations were statistically analyzed using Analysis of Variance (ANOVA) — one-way classification following Gomez and Gomez (1976). To determine which dose level had caused significant effect on the M_1 seedling height, Duncan's Multiple Range Test (DMRT) was done following Walpole (1974).

Results and Discussion

Effects on the M_1 Plants

Table 1A summarizes the effects of varying dose levels of gamma radiation in the M_1 plants. As shown in Figure 1, Table 1B and Plate 1, there is a significant reduction in the seedling height caused by 45 and 60 kR treatments and a curvilinear dose response relationship is observed. This curvilinear dose response results from multiple hit events characteristic of the low linear energy transfer (LET) radiations (Brock, 1970). The significant reduction in the main seedling height may be due to radiation-induced physiological and genetic effects (Gaul, 1977). Radiation has been known to alter plant metabolism. Low doses have been found to cause stimulatory effects (Sax, 1963; Skok *et al.*, 1965) while high doses cause a marked inhibition of growth (Bajay, 1968; Bajay *et al.*, 1970) and to some extent the death of the plants (Koo et al., 1972; Ungson, 1975; Inoue et al., 1975, 1980). The observed impairment of the seedling height with 45 and 60 kR can be ascribed to the effects of irradiation on the auxin supply (Skoog, 1935) caused by inactivation of the auxin-synthesizing system (Gordon and Weber, 1955). Since auxin affects cell division and elongation, irradiation might have caused mitotic delay (Neary *et al.*, 1959) or loss of the capacity of the cells to proliferate and elongate (Evans, 1965). It is also most likely that irradiation affects the biosynthesis of gibberellins, another growth regulatory hormone in plants (Machaiah *et al.*, 176; Sideris *et al.*, 1971); on the nucleic acid synthesis (Yeally and Stone, 1975) and nucleic acid profile (Tano, 1971). Alteration in the nucleic acid synthesis and profile may stem from irradiation

induced formation of chromosomal aberrations (Conger and Stevenson, 1966; Natarajan and Maric, 1961). As shown in Figure 1, the 58 kR dose is considered as the LD_{50} dose since it caused 50 percent reduction in the seedling height.

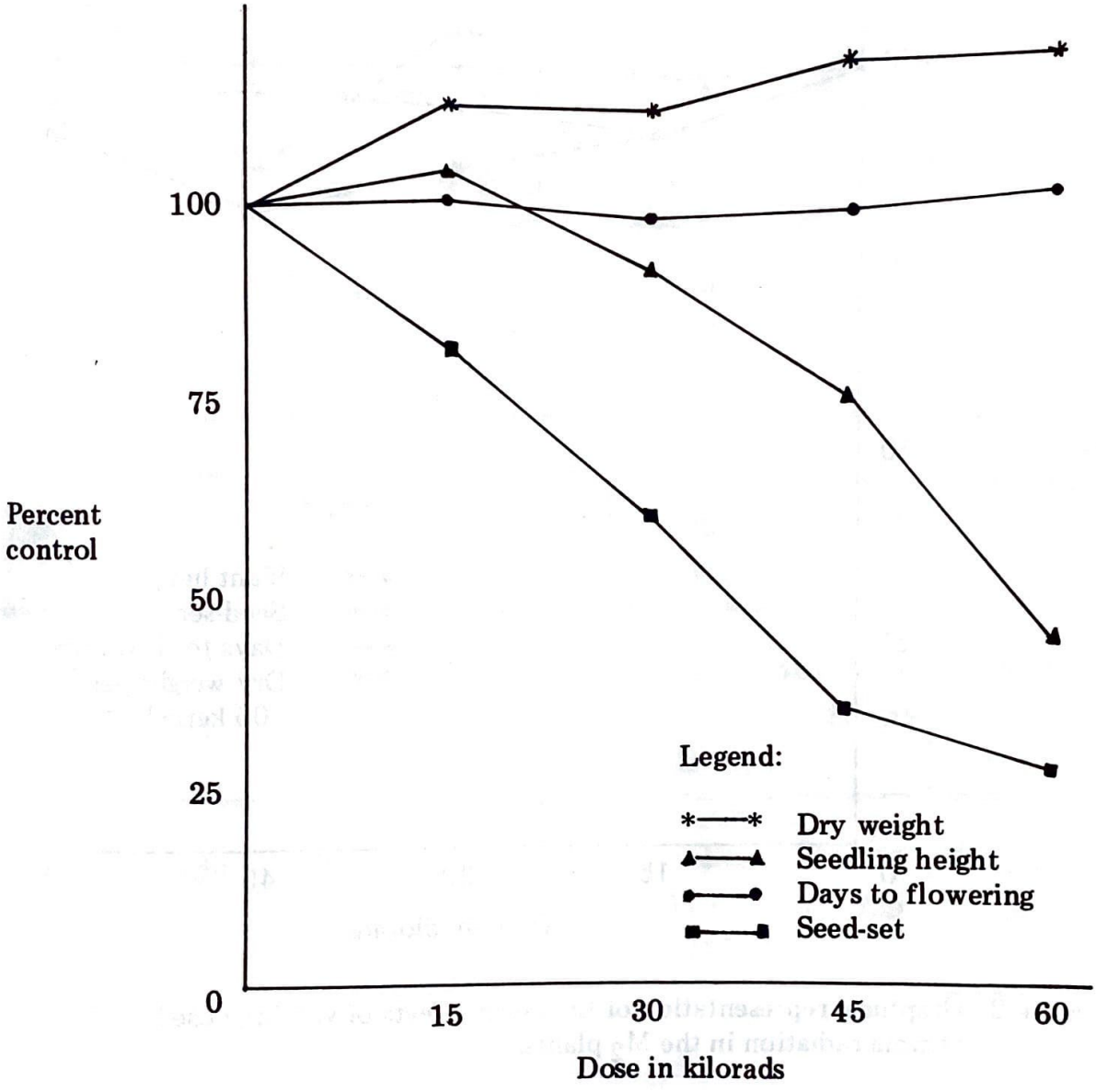

Figure 1. Graphical representation of the mean effects of varying dose levels of gamma radiation in the M_1 plants.

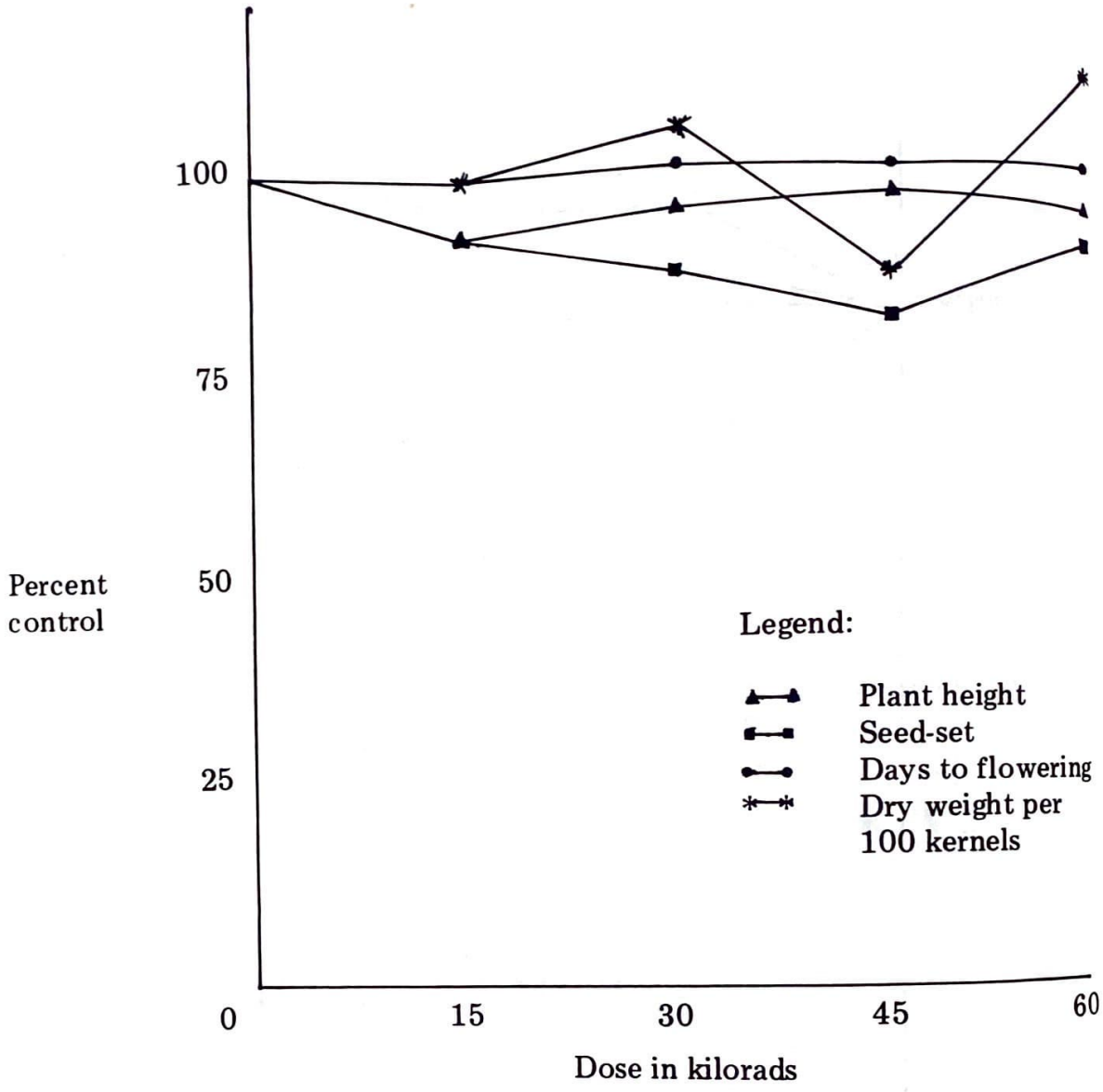

Figure 2. Graphical representation of the mean effects of varying dose levels of gamma radiation in the M_2 plants.

Table 1B. Analyses of Variance of the mean effects of different dose levels of gamma radiation on the M_1 plants.

Parameter	Source of variation	Sum of Squares	Degrees of freedom	Mean square	f (computed)
1. Seedling height	Treatment	1029.78	4	257.445	38.942**
	Error	1950.24	295	6.611	
	Total	2980.02	299		
2. Plant height	Treatment	8224.66	4	2056.145	2.1547^{ns}
	Error	229013.41	240	954.223	
	Total	237238.07	244		
3. Number of days to flowering	Treatment	287.53	4	71.8825	2.3352*
	Error	7141.42	232	30.78198	
	Total	7428.95	236		
4. Seed-set (percent)	Treatment	161642.42	4	40410.605	82.92**
	Error	115273.28	231	489.449	
	Total	274215.70	235		
5. Dry weight per 1,000 kernels	Treatment	701.065	4	175.266	46.865**
	Error	653.763	170	3.739	
	Total	1336.828	174		

* significant at 5% only
** highly significant
ns nonsignificant

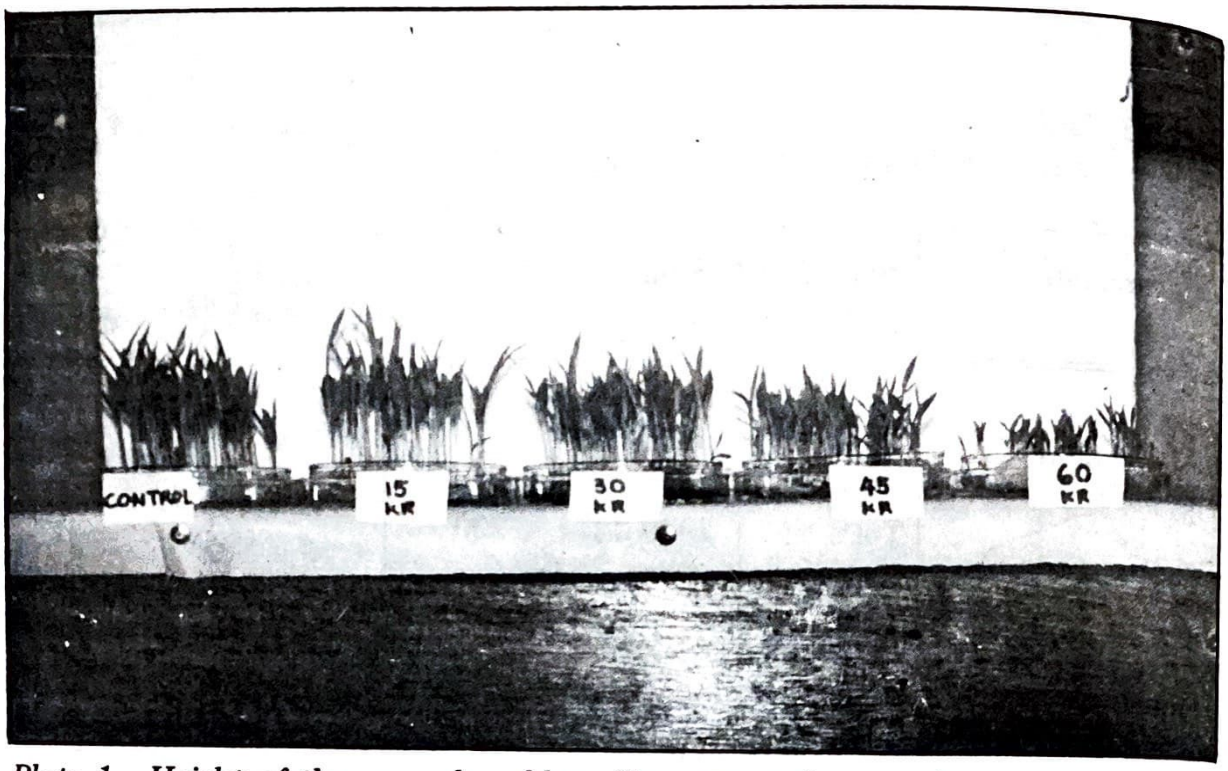

Plate 1. Height of the seven-day old seedlings grown from seeds exposed to varying dose levels of gamma radiation.

Flowering time was slightly delayed in plants exposed to 60 kR dose (significant at 5% only) as shown in Table 1B. In *Arabidopsis thaliana*, flowering is conditioned by two genetic systems; one controlling the flowering process such as flower initiation and the other controlling the rate of development from initiation to emergence (Brock, 1967). If similar genetic systems control flowering in sorghum, it is highly plausible that irradiation might have affected any one or both of the systems brought about by the radiation-induced chromosomal aberrations. Furthermore, as irradiation causes metabolic disturbance in plants, this slight delay in the flowering time may be attributed to its effects in the production of florigen — a hormone which controls flowering.

All the dose levels caused significant effects on the mean percent seed-set (Table 1B and Plate 2). As shown in Figure 1, a linear dose response curve is shown indicating that sterility is directly proportional to the increasing radiation dose. Moreover, the dose of 38 kR is the LD_{50} as it caused 50 per cent reduction in the mean fertility of the treated plants. The pronounced M_1 sterility as indicated by the marked reduction in the percentage seed-set may be chromosomal, genic or physiological in nature (Ehrenberg, 1960). However, it is believed that such is chromosome-conditioned referred to as translocation sterility (Ehrenberg, 1959); or caused by invisible chromosomal aberrations or point mutations (Sato and Gaul, 1967). Translocations are believed to be the type of chromosomal aberration that had

caused sterility since this is the only type readily recognizable during meiosis (Gaul, 1977). As a consequence of chromosomal translocation, meiotic irregularities in both the microspore and megaspore mother cells are most likely to occur. This eventually leads to the formation of non-functional or sterile gametes which may contain duplications and/or deficiencies (Ehrenberg, 1959).

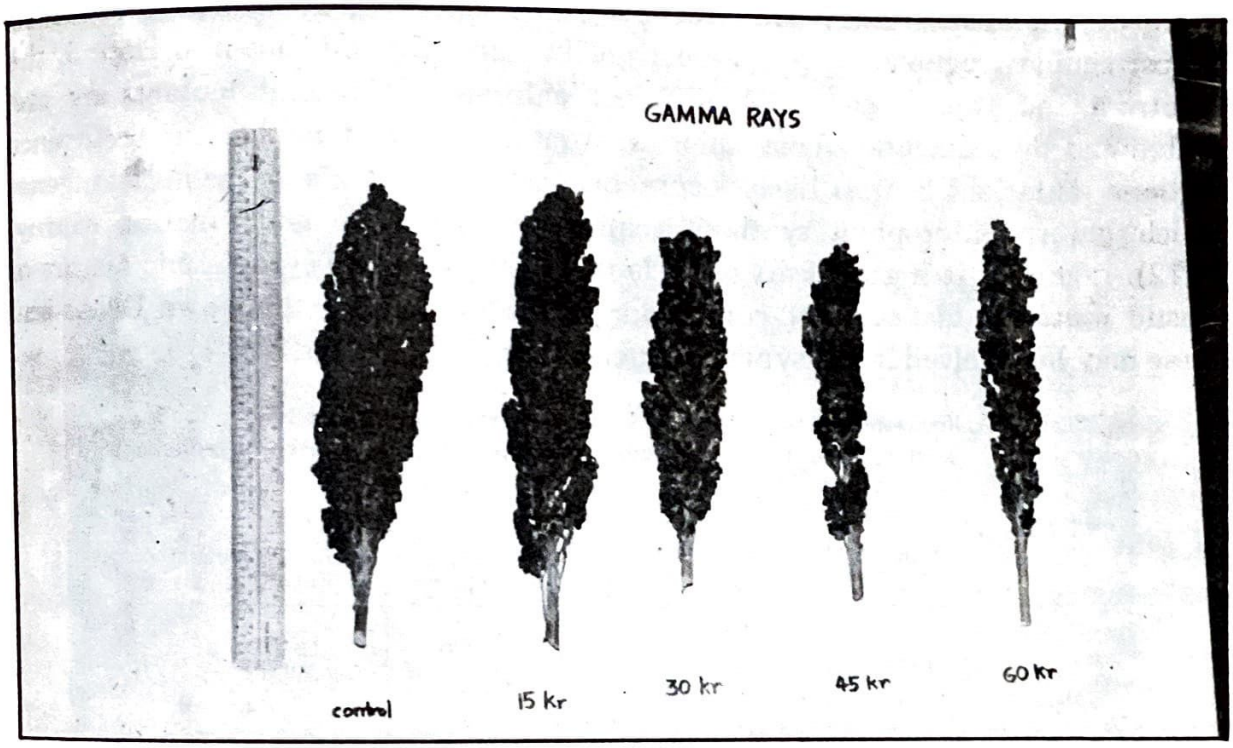

Plate 2. Representative samples of the seed-set of the main M_1 spikes.

The different dose levels caused a significant increase in the average dry weight per 1,000 kernels taken at random from the main spikes ranging from 3.23 to 5.44 grams more than the control. No definite account was given by Darvies (1968) to explain the correlation of this parameter with the radiation effects. However, a positive correlation was reported by Natarajan and Maric (1961) between the immediate morphological observations like dry weight, wet weight and seedling height with relatively low dose levels. In the present experiment, since not one dose level was found to induce total sterility to the plants, fertile, although few, gametes were expected to be produced and this would eventually result in normal fertilization. As there were only few kernels growing on the spikes, it is expected that these kernels would be bigger hence heavier than the control.

Effects on the M_2 Plants

The frequency of chlorophyll-deficient seedling mutations observed in the M_2 generation is presented in Table 2A. The 45 kR dose yielded the most number of chlorophyll-deficient mutations with a frequency of 12.77 per 1,000 seedlings. While 60 kR caused 10.22 mutation per 1,000 seedlings, 15 and 30 kR yielded almost similar frequencies. As summarized in Table 2B and shown in Plate 3, the spectrum and percentage of the different chlorophyll-deficient mutants are presented and these include albina, chlorina, striata, viridis and xantha. The occurrence of these mutations is most likely a consequence of mutation/s on the nuclear genes which govern chlorophyll synthesis, chloroplast structure and function (Sprey, 1972). Likewise, it is also highly possible that change/s in the cytoplasmic factors or plastid mutation had occurred considering that plastids have their own DNAs and these may be involved in the synthesis of chlorophyll (Sprey, 1d72).

Table 2A. Frequency of chlorophyll-deficient seedling mutations observed in the M_2 seedlings grown from M_1 seeds exposed to varying dose levels of gamma radiation.

Radiation dose (kR)	No. of seedlings analyzed	No. of mutated seedlings	Frequency per 1,000 M2 seedlings
control	11,590	—	—
15	8,180	52	6.300
30	7,180	41	5.696
45	6,968	89	12.770
60	5,282	54	10.223

Table 2B. Spectrum and percentage of the cholorophyll-deficient seedling mutations observed in the M_2.

Radiation dose	Spectrum and percentage of chlorophyll-deficient mutations					No. of mutated seedlings
	albina	chlorina	striata	viridis	xantha	
control					26 50%	
15	9 17.3%	10 19.2%		7 13.5%		52
30	14 34.2%	24 58.5%			3 7.3%	41
45	49 55.05%	25 28.08%	15 16.85%			89
60	31 54.4%	5 9.3%			18 33.3%	54

Plate 3. Spectrum of chlorophyll-deficient mutations observed in the M_2 seedlings. A — albina (white with arrow) and chlorina (yellow-green with arrow); B — xantha (yellow with arrow) and C — striata.

Furthermore, with 45 kR, morphological mutants were isolated in the frequency of 0.861 per 1,000 seedlings (Plate 4). These mutants had greatly reduced heights with deep-green leaves. In a follow-up study conducted, it was found that at the 6-leaf stage (i.e. 18 days after emergence), the mutants had the average height of 3 centimeters and these mutants died 45 days after emergence.

Plate 4. Morphologically-dwarfed M_2 mutation (with arrow) induced by 45 kr gamma radiation.

In sorghum, four genes have been known to exert a major effect in the height of the plant (Bhaskara Rao and Reddi, 1975). The 45 kR might have incidentally caused mutation among any or all of the genes and this mutation brought about pleiotropic effects on the height components and leaf characteristics of the plants. Although it is premature to conclude at this stage, the workers suspected that such was a case of lethal mutation.

The radiobiological effects in the M_2 plants are summarized in Table 3A. Analysis of variance (Table 3B) shows that the varying dose levels caused no significant effects in the mean plant height and flowering time. With the slight delay in the flowering time in the plants treated with 60 kR in the M_1, it can be deduced that such effect was caused by the damaging effect of radiation and these plants might have recovered through the inherent repair system (Patil and Goud, 1985). It is also possible that environmental conditions favored recovery by the plants.

The sterility effects of irradiation must have persisted through the second generation as evidenced by the significant reduction in the average percentage seed-set in the main spikes, hence bigger and heavier kernels are expected to develop.

Summary and Conclusion

The radiobiological effects of varying dose levels of gamma radiation were investigated for two generations in sorghum using a number of agronomic traits of the plants as the parameters. In the M_1, seedling height and percentage seed-set were significantly reduced by certain dose levels giving each a curvi-linear and linear dose response respectively. On the other hand, a slight delay in the flowering and increase in the average dry weight per 1,000 kernels were observed in the plants treated with 60 kR.

That the different dose levels of radiation induced mutations in sorghum is supported by the observed frequency and spectrum of chlorophyll-deficient mutants obtained in the M_2 seedlings. These mutations result from changes in either or both the nuclear genes and cytoplasmic factors which are directly involved in the synthesis of chlorophyll and chloroplast structure and function. Furthermore, lethal morphological mutants were isolated from the M_2 population treated with 45 kR, and this mutation may be a manifestation of a change or changes in the genes governing the height of the plants.

Of the significant effects in the M_2, the reduction in the fertility of the main spikes and the height mutations were of utmost importance as these represented the type of heritable changes that had occurred following M_1 irradiation. These mutations could have some practical applications in some plant breeding programs.

Since most of the M_1 effects are found to be chiefly physiological and non-genetic, it is therefore recommended that further experimentations be made with more advanced generations (up to M_5 if possible) since most of the genetic changes appear in these generations.

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Table 1A. The effects of different dose levels of gamma radiation on the M_1 plants.

Parameter	Radiation Dose (kR)	Sample size	Mean ± S.E.	Range	Percent control
1. Seedling height* (in cm.)	control	60	10.33± 2.28b	2.7 – 14.2	100
	15	60	10.89± 1.93ab	7.5 – 15.3	105.42
	30	60	9.64± 2.07bc	4.4 – 14.1	93.32
	45	60	8.05± 2.50d	1.3 – 11.8	77.93
	60	60	4.68± 2.23e	0.6 – 9.5	45.50
2. Plant height (in cm.)	control	51	198.45± 23.68	159.00– 263.00	100
	15	42	201.05± 26.55	121.25– 266.00	101.29
	30	51	191.47± 31.25	110.25– 266.75	96.45
	45	56	186.99± 390.90	119.00– 256.50	94.23
	60	45	186.48± 40.90	85.00– 286.50	93.96
3. Number of days to flowering	control	51	72.72± 5.09	64 – 68	100
	15	42	73.95± 4.41	66 – 83	101.68
	30	49	72.59± 6.31	63 – 86	99.81
	45	52	72.87± 4.72	62 – 82	100.19
	60	42	75.62± 6.95	63 – 89	103.97
4. Seed-set (percent)	control	50	96.30± 2.65	81.15– 99.55	100
	15	41	80.76± 18.44	21.19– 98.33	83.86
	30	48	59.82± 26.64	9.83– 97.48	62.12
	45	51	34.94± 25.44	1.69– 90.97	36.28
	60	42	27.57± 27.48	0 – 97.88	28.63
5. Dry weight per 1,000 kernels (in grams)	control	60	26.17± 3.39	23.5 – 32.4	100
	15	42	29.65± 1.74	25.8 – 31.8	113.30
	30	36	29.40± 1.94	25.2 – 35.5	112.36
	45	22	31.17± 0.63	25.2 – 32.9	119.11
	60	15	31.61± 1.60	27.4 – 33.8	120.81

*Mean values with similar letter/s show no significant difference as determined by Duncan's Multiple Range Test (DMRT).

Table 3A. The effects of different dose levels of gamma radiation on the M_2 plants.

Parameter	Radiation Dose (kR)	Sample size	Mean ± S. E.	Range	Percent control
1. Plant height (in cm.)	control	81	219.46 ± 36.28	154.00 – 307.5	100
	15	75	203.95 ± 45.66	124.00 – 413	92.93
	30	78	208.83 ± 41.00	126.00 – 323	95.16
	45	77	214.41 ± 35.47	134.00 – 312	97.70
	60	56	212.14 ± 43.29	123.00 – 312	96.66
2. Number of days to flowering	control	77	90.43 ± 4.29	82 – 101	100
	15	74	90.18 ± 3.90	82 – 100	99.12
	30	67	91.00 ± 4.06	82 – 98	100.63
	45	71	90.79 ± 3.85	82 – 101	100.40
	60	66	91.98 ± 3.89	82 – 101	101.72
3. Seed-set (percent)	control	36	92.89 ± 3.52	80.94 – 97.94	100
	15	35	85.59 ± 16.45	37.24 – 97.47	92.36
	30	40	81.59 ± 15.45	19.41 – 96.38	87.83
	45	38	76.80 ± 28.81	0 – 99.04	82.68
	60	27	85.69 ± 16.54	23.28 – 97.48	92.25
4. Dry weight per 100 kernels	control	51	2.439 ± 0.368	1.8 – 3.2	100
	15	31	2.437 ± 0.555	1.5 – 3.4	99.91
	30	37	2.572 ± 0.437	1.4 – 3.4	105.43
	45	32	1.139 ± 0.623	0.8 – 3.3	87.69
	60	26	2.779 ± 0.472	1.7 – 3.8	113.93

Table 3B. Analyses of Variance of the mean effects of different dose levels of gamma radiation on the M_2 plants

Parameter	Source of variation	Sum of squares	Degrees of freedom	Mean square	computed
1. Plant height	Treatment	10758.544	4	2689.639	1.6566^{ns}
	Error	587729.326	362	1623.562	
	Total	598487.87	366		
2. Number of days of flowering	Treatment	134.0897	4	33.522	2.08755^{ns}
	Error	5620.3892	350	16.058	
	Total	5754.4789	354		
3. Seed-set (percent)	Treatment	5217.062	4	1304.2655	3.939**
	Error	56610.020	171	331.0527	
	Total	61827.082	175		
4. Dry weight per 100 Kernels	Treatment	6.4298	4	1.6074	6.775**
	Error	38.6128	158	0.2444	
	Total	45.0425	162		

** highly significant
ns nonsignificant