THE RADIOBIOLOGICAL EFFECTS OF FAST NEUTRONS ON SORGHUM, *Sorghum bicolor* (L.) Moench.

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Introduction

An increasing number of mutagens, both chemical and physical, has been reported by many investigators recently. Some of these mutagens have been popularly used in some applied fields, especially in agriculture. Of the different physical mutagens, the effects of ionizing radiations such as gamma rays and fast neutrons have been extensively investigated using a great number of agricultural crops. The mutations induced by such mutagenic agents could be potential sources of germ plasm for breeding purposes in some crop plants (Brock, 1972).

The radiogenetic effects of fast neutrons have been widely reviewed by Bender (1972) who commented that early investigations on neutron-induced mutagenesis suffered from some inaccuracies. These include the uncertain neutron dosimetry since it has been reported that it would be difficult to separate the core radiation from the contaminating radiations (Mikaelsen and Brunner, 1968). However, the installation of the Standard Neutron Irradiation Facility solved the problem on uncertain neutron dosimetry.

Fast neutrons are a type of particulate radiation with an average Linear Energy Transfer (LET) of 9.5 keV/micron (Neary et al., 1959). The choice of fast neutrons as the mutagen used in the present study is based on the findings that fast neutrons cause low physiological toxicity and a relatively high genetic efficiency (Mac Rey, 1968).

The objectives of the present study are to investigate the radiobiological effects of the varying dose levels of fast neutrons on sorghum using the different parameters, and to isolate some mutations caused by the mutagen used.

Materials and Methods

Sorghum bicolor (L.) Moench. cultivar BPI-Cosor 3 purchased from the Bureau of Plant Industry, San Andres, Manila was used as the experimental material.

The procedures and methods used in the pre-and post-irradiation processing of sorghum seeds, transplanting, and gathering and analysis of data are discussed in detail elsewhere (Doyungan and Arañez, 1986).

Neutron Irradiation of Seed Samples

Irradiation of seed samples was done at the Standard Neutron Irradiation Facility (SNIF) located in a swimming pool-type reactor in the Philippine Reactor Research (PRR) at the Philippine Atomic Energy Commission (PAEC) in Diliman, Quezon City. Fast neutron doses administered were 0.5, 1.0, 1.5, and 2.0 kilorads (kR) at the dose rate estimated in chamber D=1.875 rads per sec, and the neutron flux estimated from the activity of sulfur pellets at 4.71 x 10^8 n/cm/sec (Soriano et al., 1972).

Results and Discussion

Table 1A summarizes the mean effects of the different dose levels of fast neutrons on the M_1 plants.

A drastic reduction on the seedling height was observed (Plate 1 and Figure 1) showing a curvi-linear dose-response relationship. Based on Duncan's Multiple Range Test, the heights of the seedlings grown from seeds exposed to 1.0 to 2.0 kR differed significantly from each other and from the control. As shown in Fig. 1, the dose of 1.32 kR was considered the LD_{50} (Lethal Dose 50%) as it caused 50 percent reduction on the seedling height. Estimation of the relative biological effectiveness (RBE) of fast neutrons using gamma radiation as the standard radiation with LD50 at 58kR as previously reported (by Doyungan and Arañez, (1986) showed that fast neutrons were 43.94 times more effective in reducing the seedling height compared with the effects of gamma radiation.

As shown further in Table 1A and Fig. 1, survival of the plants grown from seeds exposed to the highest radiation dose (2.0 kR) was totally affected as all the plants died one week after transplanting. Likewise, a significant delay in the flowering time among the plants exposed to 1.5 kR and a significant reduction on the panicle length of the main spikes were observed. Furthermore, a drastic reduction in the fertility of the main spikes was observed as scored by the percentage seedset. As graphically shown (Fig. 1), the dose of 0.51 kR caused a 50 percent reduction on the fertility of the main spikes (LD_{50}), and this gave an estimated RBE value of 74.51 based on the LD_{50} caused by gamma radiation (Doyungan and Arañez, 1986)

TABLE 1a. Mean effects of the different dose levels of fast neutrons on the M1 plants

Parameter	:	Radiation Dose (in KR)	:	Mean +		S.E.	:	Percent Control
Seedling height* (in cm)	:	control	:	10.33	+	2.28b	:	100
	:	0.5	:	10.24	+	1.56ab	:	99.13
	:	1.0	:	6.71	+	2.09c	:	64.94
	:	1.5	:	4.39	+	1.12d	:	42.50
	:	2.0	:	2.58	_	0.52e	:	21.98
Number of days to flowering	:	control	:	72.72	+	5.09	:	100
	:	0.5	:	71.76	_	5.87	:	98.53
	:	1.0	:	74.27	+	5.06	:	100.25
	:	1.5	:	79.50	-	5.73	:	107.85
	:	2.0	:	--		--	:	--
Panicle length (in cm)	:	control	:	24.51	+	2.86	:	100
	:	0.5	:	22.32	_	4.03	:	91.06
	:	1.0	:	21.79	+	3.89	:	88.90
	:	1.5	:	19.63	_	3.16	:	80.09
	:	2.0	:	--		--	:	--
Seed-set (percent)	:	control	:	96.30	+	2.65	:	100
	:	0.5	:	49.46	+	27.73	:	51.36
	:	1.0	:	23.13	+	23.52	:	24.02
	:	1.5	:	9.15	+	11.41	:	9.50
	:	2.0	:	--		--	:	--
Dry weight per 1,000 kernels (in gm)	:	control	:	26.17	+	3.39	:	100
	:	0.5	:	30.56	+	1.79	:	116.78
	:	1.0	:	32.59	+	1.90	:	124.53
	:	1.5	:	xx		xx	:	xx
	:	2.0	:	--		--	:	--

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

-- No data were obtained because all the plants died one week after transplanting.

xx Only a total of more than 1,000 kernels were produced by the treated plants.

TABLE 1B.

Analyses of variance of the mean effects of the different dose levels of fast neutrons on the M_1 plants.

Parameter	Source of variation	Sum of squares	Degrees of freedom	Mean square	f value
Seedling height	:Treatment	: 28.72.76	: 4	: 718.19	: 255.53**
	: Error	: 829.12	: 295	: 2.81	:
	:				
	: Total	: 3701.88	: 299	:	:
Number of days to flowering	:Treatment	: 1,219.03	: 3	: 406.34	: 13.85**
	: Error	: 5278.80	: 180	: 29.33	:
	:				
	: Total	: 6497.83	: 183	:	:
Panicle length	:Treatment	: 447.07	: 3	: 149.03	: 11.70**
	: Error	: 2215.87	: 174	: 12.74	:
	:				
	: Total	: 2662.94	: 173	:	:
Seed-set (percent)	:Treatment	:189167.29	: 3	: 63055.76	: 170.72**
	: Error	: 62792.28	: 172	: 369.37	:
	:				
	: Total	:251959.57	: 175	:	:
Dry weight per 1,000 kernels	:Treatment	: 642.38	: 2	: 321.19	: 64.23**
	: Error	: 460.10	: 96	: 5.00	:
	:				
	: Total	: 1102.48	: 98	:	:

** highly significant

Analyses of variance (Table 1B) showed that certain dose levels of fast neutrons had caused significant effects on the different parameters used in the study.

Fast neutrons have the same mechanism of action in affecting the living system with any other ionizing radiations. These M_1 effects caused by the fast neutrons may therefore stem from both radiation-induced physiological and genetic effects (Gaul, 1977). It has been widely known that ionizing radiations affect the physiology of the plants by impairing the biosynthetic processes of some growth hormones such as auxins and gibberelins (Skoog, 1935; Gordon and Weber, 1955; Machaiah et al., 1976; and Sideris et al., 1971), or affecting the nucleic acid synthesis and profile (Yeally and Stone, 1975 and Tano, 1981). Likewise, ionizing radiations hit and eventually damage the chromosomes thereby causing some forms of structural as well as numerical chromosomal aberrations, or they may affect the nitrogen bases or any part of the DNA, thus causing point mutations. These combined physiological and genetic effects inflicted on the plant by fast neutron irradiation account for the significant effects on the seedling height, flowering time, panicle length, and sterility of the main spikes.

As observed, fast neutrons yielded the estimated RBE values of 43.94 and 74.51 for seedling height and seedset, respectively, compared with the standard radiation used. This can be ascribed to the fact that fast neutrons have much higher linear energy transfer (LET) compared with gamma radiation. As such, fast neutrons therefore have the ability to produce more chromosomal breaks and aberrations than gamma radiation. The death of all the plants treated with 2.0 kR may be due to the lethal effects caused by the formation of much higher incidence of chromosomal aberrations (Natarajan and Maric, 1961).

As observed further, the average dry weight per 1,000 kernels taken from the main spikes significantly increased with the increasing dose. This is due to the fact that as the fast neutron dose was increased, the percentage seedset was concomitantly reduced. As few seeds were developing, it was expected that bigger and eventually heavier kernels would be produced.

Fast neutrons were capable of inducing chlorophyll-deficient mutations among the M_2 seedlings. As shown in Table 2, the frequency of chlorophyll-deficient seedlings increased with the increasing fast neutron doses as analyzed per 1,000 M_2 seedlings. The spectrum of these chlorophyll-deficient mutants, as presented in Table 3, included albina, albo-striata, chlorina, viridis and xantha.

It is most likely that these mutations occurred as a consequence of the radiation-induced effects on the nuclear and/or cytoplasmic factors which govern chlorophyll synthesis, chlorophyll structure, and function (Sprey, 1972).

TABLE 2.

Frequency of chlorophyll-deficient mutation observed in the M_2 seedlings grown from seeds exposed to different levels of fast neutrons.

Radiation dose (in KR)	No. of seedlings analyzed	No. of mutated seedlings	Mutation per 1,000 M_2 seedlings
control	11,590	----	---
0.5	8,356	68	8.137
1.0	2,426	30	12.360
1.5	685	10	14.599

TABLE 3.

Spectrum and percentage of chlorophyll-deficient M_2 seedlings grown seeds exposed to different levels of fast neutrons.

Radiation dose	albina	albo-striata	chlorina	striata	viridis	xantha
Control	-	-	-	-	-	-
0.5	73.5	-	22.1	-	2.9	1.5
1.0	3.3	6.7	83.3	-	-	6.7
1.5	30.0	-	50.0	-	-	20.0

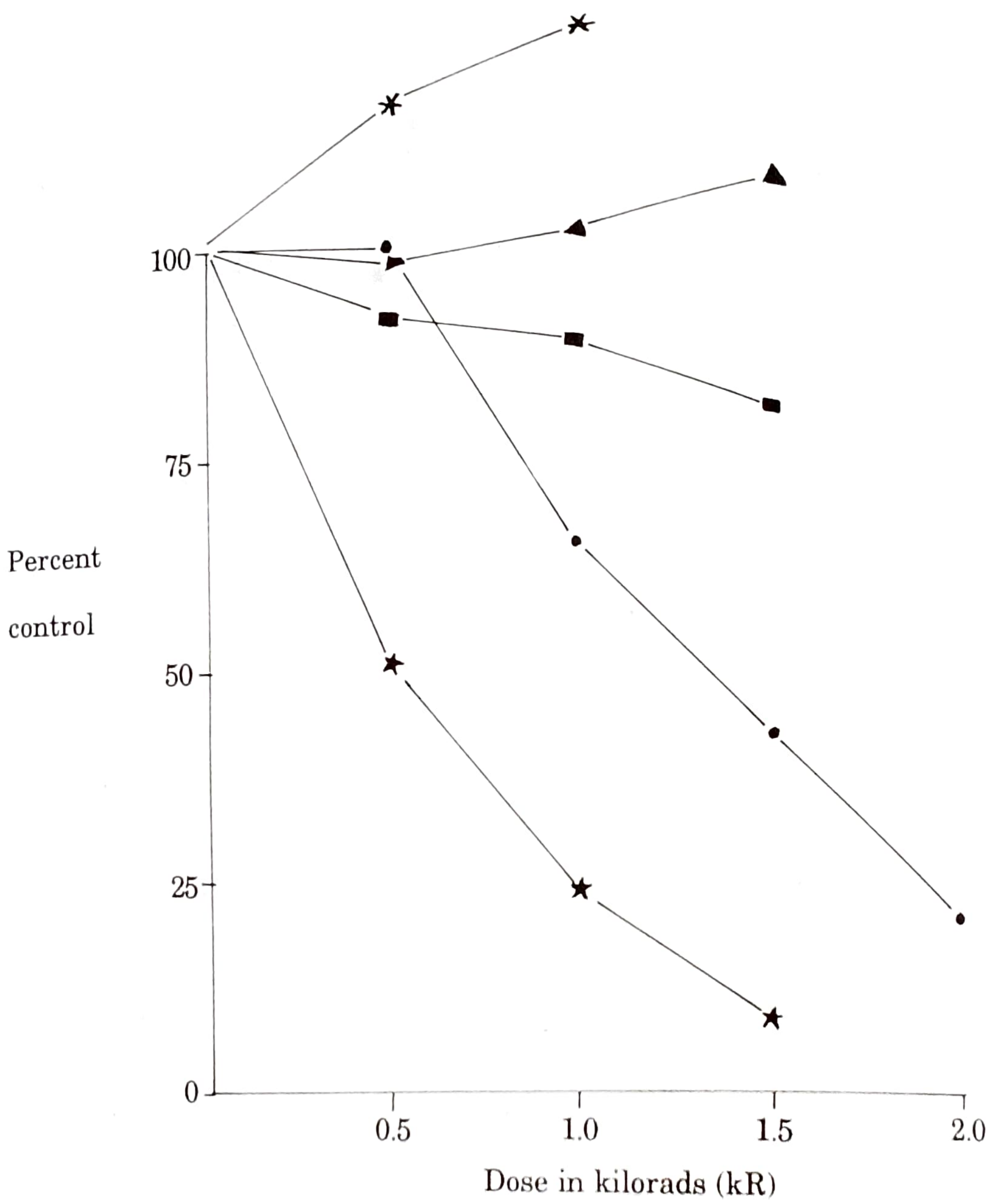

FIGURE. 1 Graphical representation of the mean effects of the different dose levels of fast neutrons on the M_1 plants

Legend:
- ●—● seedling height
- ▲—▲ flowering time
- ■—■ panicle length
- ★—★ seedset
- ✶—✶ dry weight

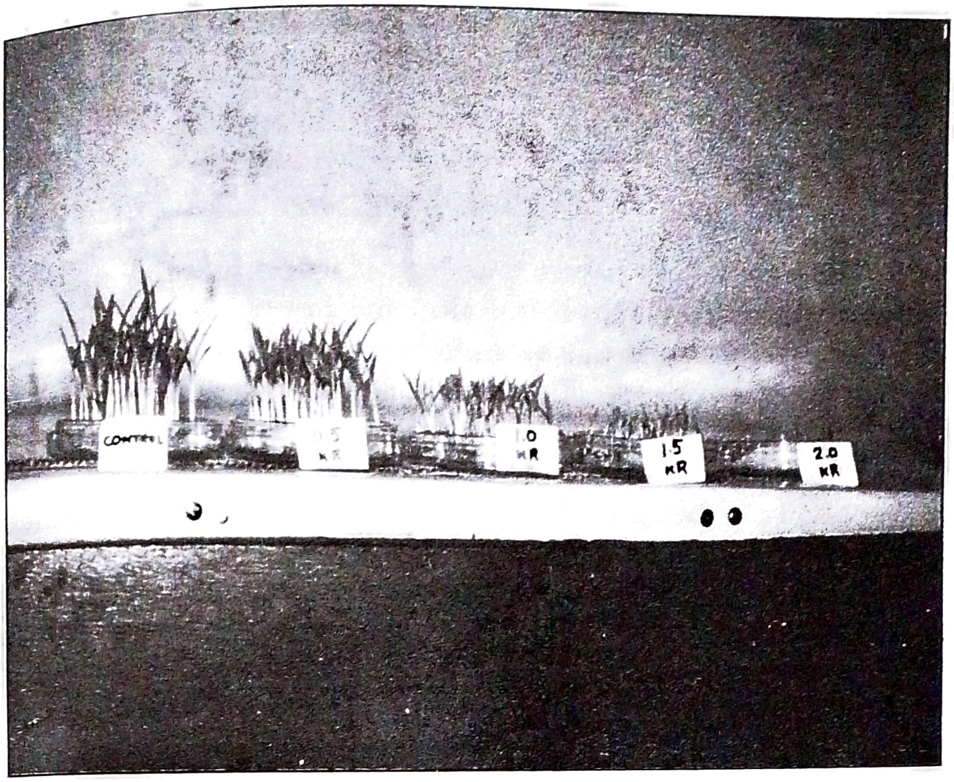

Plate 1. Height of the seven-day old seedlings grown from seeds exposed to varying dose levels of fast neutrons.

Summary and Conclusion

A study was conducted to determine the effects of increasing fast neutron doses on some agronomic traits of sorghum, *Sorghum bicolor* (L.) Moench., and to isolate some mutations in the second generation.

Results of the study showed that certain dose levels of fast neutrons caused drastic and significant reduction on the seedling height with the estimated RBE value of 43.94 compared with the standard radiation used. Furthermore, the flowering time was slightly delayed among the plants treated with 1.5 kR, and the panicle lengths of the main spikes were significantly reduced. Sterility effects, as determined by the percentage seedset of the main spikes, were very pronounced. Fast neutrons had the estimated RBE value of 74.51 compared with the standard radiation used in affecting this particular parameter. Concomitant, however, with the drastic reduction on the seedset, heavier and larger kernels were produced from the main spikes among the treated plants.

Mutations isolated from this study included only the chlorophyl-deficiency among the M_2 seedlings as the study was conducted only until the seedling stage in the M_2 generation. The frequency of mutation per 1,000 M_2 seedlings analyzed increased with the increasing fast neutron doses, and the spectrum included albina, albo-striata, chlorina, viridis, and xantha.

As has been widely known, the effects of ionizing radiation on the M_1 generation may stem from both physiological and genetic effects. It is therefore possible that fast neutrons cause significant reduction on the seedling height, panicle length and seed-set of the main spikes, and a slight delay in the flowering time by presumably the combined radiobiological effects on the metabolism, particularly in the biosynthesis of some growth hormones and on the genes and chromosomes of the plants. The results of the present study conform with several similar studies using different plant materials.

Just like any other ionizing radiation, fast neutrons induce chlorophyll-deficient seedling mutations, and this may be a consequence of radiation-induced alteration or changes on the genes, nuclear or cytoplasmic, which govern chlorophyll synthesis, and chloroplast structure and function.

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