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INDUCTION OF MICRONUCLEI IN BEAN ROOTS

BY 250 GeV HADRONS

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ABSTRACT

Vicia Faba bean roots were irradiated with 250 GeV hadrons and the induction of micronuclei was evaluated. A Relative Biological Efficiency (RBE) of about 1.8 was found using ^{60}Co γ -rays as reference radiation. For the production of multiple micronuclei the RBE value was determined to be 2. Cytological anomalies were detected after the hadron irradiation.

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1. INTRODUCTION

From the dependence of the Relative Biological Efficiency (RBE) on the mean Linear Energy Transfer (LET) ¹⁻³⁾ of radiations, the effects produced by very high energy hadrons are expected to be the same as those produced by ⁶⁰Co γ -rays.

However, Smith et al.^{4,5)} reported RBE values between 3.5 and 5.2 for induction of cytogenetic damage in maize, by 28 GeV protons. Anomalies in biological responses, leading to RBE values between 2 and 23 were reported recently by Akoev and Yurov⁶⁾ for a 70 GeV hadron beam. Those observations have obvious implications in radioprotection and prompted a preliminary investigation on the biological effects of a 250 GeV/c hadron beam of the CERN Super Proton Synchrotron (SPS). Micronuclei induction in *Vicia Faba* root tips was one of the biological end-points selected, because it represents a rapid, easy, and reliable way of evaluating chromosome damage produced by noxious agents. As the radiosensitive area is located in the last 3 mm of the root tip, this system is very suitable for exposures where the diameter of the beam available for irradiations is very small.

2. MATERIALS AND METHODS

Vicia Faba seedlings of the variety "Fillbacker" were used for the experiment. The dry seeds were grown following a standard procedure previously described⁷⁾. The nine-days-old seedlings were selected on the basis of the length and growth. Bean roots with a three-day growth or a length exceeding the mean value by more than one standard deviation were discarded. The beans selected for irradiation were positioned with their root meristems enclosed in a circle of 15 mm diameter.

The beans were irradiated at a depth of 32250 mg/cm² in a water absorber. During the irradiations the temperature of the aerated water was kept at 19°C $\pm$ 0.2. The beam consisted mainly of protons with about 10% positive pions. Details of the beam composition and radiation dosimetry have been given elsewhere⁸⁾. The meristems were irradiated with doses of 0.48, 0.9, and 0.96 Gy given at a dose rate of 3.9 Gy/hour. Following irradiations the beans were transferred to the

growing tanks where aerated water was continuously added at a temperature of 19°C. Three root tips were fixed in an alcohol:acetic acid solution (3:1), at various time intervals up to 72 hours after exposure. The roots were stained using the Feulgen technique, and permanent slides were made from the last 1½ mm of the meristem, using the dry ice method. One thousand cells were scored per sample. Cobalt-60 γ-rays were used as reference radiation, where beans were irradiated with doses of 0.55, 0.8, 1.1, and 1.6 Gy at a dose rate of 5 Gy/hour.

3. RESULTS

Chromosome and chromatid aberrations which give rise to acentric fragments during mitosis are expelled from the cell nucleus and form one or several micronuclei. The mean number of cells containing micronuclei after 250 GeV hadron irradiation is reported in Table 1. The total amount of micronuclei per 1000 cells scored is given in Table 2. Results obtained after ^{60}Co γ irradiations are reported in Tables 3 and 4.

To evaluate the RBE the average number of cells containing micronuclei observed in the period between 24 and 48 hours after irradiation has been calculated and compared with the γ-ray results (Fig. 1). The line represents the best linear least-squares fit and the errors have been calculated from the standard errors of the mean for each fixation point.

In comparing Tables 1 and 2 with Tables 3 and 4 differences can be observed between γ-rays and hadrons for the production of multiple micronuclei in irradiated cells. Therefore the average number of cells containing more than one micronucleus in the period between 24 and 48 hours after irradiation was analysed in the same way as the total number of cells containing micronuclei. The data obtained have been plotted in Fig. 2. The dose dependence for production of multiple micronuclei is clearly visible.

4. DISCUSSION

For the different doses the RBE evaluated from the average number of cells containing micronuclei was found to be about 1:8, whereas the RBE calculated from

the production of multiple micronuclei gave values of about 2.1. This test was carried out in parallel with other tests, one of which was the 10 days growth reduction in bean roots, where the RBE was found to be of the order of unity⁸⁾.

The theoretical predictions of RBE values near unity do not seem to be confirmed by the evaluation of chromosome damage induction. The results presented here agree rather well with those reported by Akoev and Yurov⁶⁾ after 70 GeV/c hadron irradiations for the evaluation of chromosome aberrations in *Vicia Faba*. They observed that the RBE mean values for the total number of aberrant cells with fragments or bridges were between 1.52 and 1.91, whereas the RBE values for the number of fragments or bridges per 100 aberrant cells varied between 1.8 and 2.20. Observations on the anaphase aberrations induced by 250 GeV hadrons are still in progress.

Some anomalies were observed, such as the presence of clusters of heavily damaged cells (see Fig. 3), denaturation of the nuclear material of the cells, and presence of very minute micronuclei. More material is needed to quantize and clarify these phenomena.

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Table 1

Mean number of cells with micronuclei (per 10^3 cells)
for different doses and various times after irradiation
with 250 GeV hadrons

Fixation time (hours)	Mean number of cells with micronuclei $\pm$ S.E.		
	0.48 Gy	0.9 Gy	0.96 Gy
12	23.0 $\pm$ 2.9	47.0 $\pm$ 5.6	43.3 $\pm$ 3.7
18	36.5 $\pm$ 4.7	62.0 $\pm$ 5.5	65.8 $\pm$ 4.0
24	44.3 $\pm$ 4.1	107.0 $\pm$ 10.9	109.3 $\pm$ 6.5
36	44.7 $\pm$ 4.2	100.3 $\pm$ 5.0	123.6 $\pm$ 5.9
42	40.2 $\pm$ 2.8	107.3 $\pm$ 8.4	104.3 $\pm$ 7.0
48	43.3 $\pm$ 4.6	122.3 $\pm$ 8.6	98.0 $\pm$ 6.3
60	39.5 $\pm$ 4.7	-	94.5 $\pm$ 9.5
72	-	69.0 $\pm$ 5.1	75.5 $\pm$ 6.8

Table 2

Mean number of micronuclei (per 10^3 cells)
for different doses and various times after irradiation
with 250 GeV hadrons

Fixation time (hours)	Mean number of micronuclei $\pm$ S.E.		
	0.48 Gy	0.9 Gy	0.96 Gy
12	24.3 $\pm$ 3.2	50.0 $\pm$ 6.2	44.7 $\pm$ 3.8
18	38.5 $\pm$ 5.8	68.7 $\pm$ 6.7	70.8 $\pm$ 4.2
24	47.0 $\pm$ 4.6	118.5 $\pm$ 12.8	120.5 $\pm$ 7.4
36	47.3 $\pm$ 4.5	113.7 $\pm$ 5.9	140.2 $\pm$ 7.3
42	43.6 $\pm$ 4.5	113.7 $\pm$ 5.9	113.7 $\pm$ 8.1
48	49.0 $\pm$ 5.4	145.0 $\pm$ 12.5	113.0 $\pm$ 8.8
60	44.0 $\pm$ 5.4	-	102.0 $\pm$ 11.6
72	-	77.7 $\pm$ 5.7	82.5 $\pm$ 8.5

Table 3

Mean number of cells with micronuclei (per 10^3 cells)
after ^{60}Co γ irradiation

Fixation time (hours)	Mean number of cells with micronuclei $\pm$ S.E.			
	0.55 Gy	0.8 Gy	1.1 Gy	1.6 Gy
0	1.0 $\pm$ 0.7	0.5 $\pm$ 0.5	0	3.0 $\pm$ 0.9
12	6.0 $\pm$ 1.6	9.7 $\pm$ 0.2	14.3 $\pm$ 2.4	27.3 $\pm$ 2.8
18	-	-	-	-
24	28.0 $\pm$ 3.6	39.0 $\pm$ 4.6	54.7 $\pm$ 4.1	130.0 $\pm$ 4.3
36	24.3 $\pm$ 3.0	42.0 $\pm$ 5.1	38.0 $\pm$ 3.8	95.0 $\pm$ 5.0
42	23.0 $\pm$ 2.6	41.0 $\pm$ 4.5	62.0 $\pm$ 5.4	109.0 $\pm$ 5.3
48	22.7 $\pm$ 3.4	31.0 $\pm$ 3.4	43.0 $\pm$ 4.0	120.0 $\pm$ 4.6
60	15.7 $\pm$ 3.0	24.0 $\pm$ 3.8	27.0 $\pm$ 3.2	-
72	10.0 $\pm$ 1.6	21.0 $\pm$ 3.4	21.0 $\pm$ 3.0	74.0 $\pm$ 5.2

Table 4

Mean number of micronuclei (per 10^3 cells)
after ^{60}Co γ irradiation

Fixation time (hours)	Mean number of micronuclei $\pm$ S.E.			
	0.55 Gy	0.8 Gy	1.1 Gy	1.6 Gy
0	1.0 $\pm$ 0.7	0.5 $\pm$ 0.5	0	3.6 $\pm$ 0.1
12	6.0 $\pm$ 1.6	9.7 $\pm$ 0.2	16.0 $\pm$ 2.7	32.0 $\pm$ 3.6
18	-	-	-	-
24	28.0 $\pm$ 3.8	41.0 $\pm$ 4.9	57.3 $\pm$ 4.4	147.0 $\pm$ 5.3
36	24.3 $\pm$ 3.0	44.3 $\pm$ 5.6	39.0 $\pm$ 3.9	102.0 $\pm$ 5.7
42	23.0 $\pm$ 2.6	41.7 $\pm$ 4.5	66.0 $\pm$ 6.6	118.0 $\pm$ 6.3
48	23.3 $\pm$ 3.5	32.5 $\pm$ 3.7	45.3 $\pm$ 4.8	138.0 $\pm$ 5.7
60	15.7 $\pm$ 3.0	26.5 $\pm$ 4.5	29.0 $\pm$ 3.4	-
72	10.0 $\pm$ 1.6	22.0 $\pm$ 3.8	22.0 $\pm$ 3.2	82.0 $\pm$ 6.2

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Figure captions

- Fig. 1 : Average number of cells with micronuclei between 24 and 48 h after irradiation as a function of dose.
- Fig. 2 : Average number of cells with multiple micronuclei between 24 and 48 h after irradiation as a function of dose.
- Fig. 3 : Cells showing multiple micronuclei after irradiation with a dose of 0.9 Gy of 250 GeV hadrons.

Fig. 1

Fig. 2

Fig. 3