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Effect of Gamma and Ultraviolet Radiation on Survival Pattern of *Spirogyra verrucosa*

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Irradiation studies by gamma and UV rays were conducted on *Spirogyra verrucosa* and the survival pattern, screened. A delay in the onset of mitosis was significant. The alga was resistant to low doses (10 kR, 20 kR and 30 kR) but was damaged beyond repair by high doses (40 kR, 50 kR and above) which were lethal. Fragmentation of the filaments was a primary consequence of irradiation. However, repair mechanisms seemed to operate during the lag phase of growth after ultraviolet irradiation.

Key Words Alga Gamma rays Irradiation Mitosis UV

Compared to higher plants, irradiation studies on eucaryotic algae particularly with reference to their cytology are very scanty. Godward (1954) was the first to observe the cytological effects of radiation (x-rays) on *Spirogyra crassa*. Vedajanani & Sarma (1979a,b,c) reported certain effects of ionizing and non-ionizing radiations on *S azygospora* and ultraviolet survival pattern of some Conjugales. The present work was undertaken to study the survival pattern of the gamma irradiated and ultraviolet irradiated filamentous green alga *S.verrucosa* (Rao) Kolkwitz & Krieger characterized by small rod like polycentric chromosomes.

MATERIALS & METHODS Uni-algal cultures were maintained and clonal cultures raised as per the method of Pringsheim (1946) and cultured in soil extract solution and pH of 7.5 under controlled conditions (15 + 1 C and 16/8 hr LD cycle). Materials were fixed in carnoy's fluid and stained following iron-alum acetocarmine method (Godward 1948), for obtaining stages of nuclear division.

The material was exposed to a 60 C gamma-irradiation source (doses of 10, 20, 30, 40 and 50 kR). They were then inoculated into sterile culture solution and the flasks placed under optimum conditions. Similarly, material was exposed to a U.V.source of 15 Watt Philips germicidal lamp. The exposure rate was 87 ergs/mm2/sec at a distance of 15 cm from the source. The different doses were obtained by increasing the time of exposure while keeping the distance of 15 cm constant (5, 10, 15, 20 and 25 min). The material after treatment, was transfered to sterile culture medium, placed in the dark for 24 h to exclude any photo reactivation and returned to normal culture condition. The various parameters employed to estimate growth and survival on the last day of every week for a period of 3 weeks were:

Mitosis The filaments were fixed at 1 hourly interval for 24 h to locate the mitotic peaks (primary and secondary) where maximum mitotic divisions occurred. Percentage mitosis was obtained by estimating the proportion (percentage) of cells exhibiting any stage of mitosis at the given instant of the primary mitotic peak, on the last day of the week.

Survival It is the proportion (percentage) of healthy or unaffected cells out of the total number of cells observed at random on the last day of the week.

Dry Weight The filaments are picked up on the last day of the week with a forceps, air dried, placed on a previously weighed filter paper, folded and placed in an oven at 100 C for 3 h. The filter paper was weighed and difference in weight was recorded.

Chlorophyll content On the last day of each week, 5 mg of the algal filaments were weighed after shaking off the moisture sticking to them. They were placed in a test tube containing 5 ml methanol and kept at 50 C for 10 minutes. The contents were shaken well and left for a few min. The clear green extract was decanted and optical density was scanned from 400 to 700 µm. These were plotted on a graph to obtain the absorption spectra of the chlorophyll. By utilising the formula given by Holden (1965), the total chlorophyll content was calculated.

OBSERVATIONS The chromosome number of the alga was, n=14 and the chromosomes were polycentric with diffuse centromeres deduced from the fact that a parallel separation of the chromatids occured at anaphase of mitosis.

The mitotic percentage reached maximum by the first week and decreased by the third week in the control. In the 10 kR and 20 kR gamma irradiated cultures, the maxi-

Table-1 **Percentage Mitosis Values of Cultures of *Spirogyra verrucosa* Recorded During the Course of Three Weeks After the Gamma/U.V. Irradiation.**

Type of Irradiaton	Dosage/ Duration	% Mitosis values after I Week	II Weeks	III Weeks
Gamma irradiation	Control	12	10	8.2
	10 Kr	7	8.8	8.5
	20 Kr	6.7	9.1	7.6
	30 Kr	3.8	1	0.9
	40 Kr	Nil	Nil	0.2
	50 Kr	Nil	Nil	Nil
U.V. irradiation	Control	12	11	8
	5 min	8.1	6.2	4.2
	10 min	4.5	2	1
	15 min	3	1.8	1
	20 min	1.1	Nil	Nil
	25 min	Nil	Nil	Nil

Table-2 **Percentage Survival Values of Cultures of *Spirogyra verrucosa* Recorded During the Course of Three Weeks After the Gamma/U.V. Irradiation.**

Type of Irradiaton	Dosage/ Duration	% Survival values after I Week	II Weeks	III Weeks
Gamma irradiation	Control	97	100	96
	10 Kr	58	65	91
	20 Kr	40	50	72
	30 Kr	21	32	50
	40 Kr	10	2	Nil
	45 Kr	Nil	Nil	Nil
U.V. irradiation	Control	97	98	100
	5 min	97	97	100
	10 min	90	94	100
	15 min	60	48	51
	20 min	22	28	38
	25 min	10	14	11

mum was by the 2nd week indicating tnat the filaments recovered by the end of the first week and resumed normal division (Table 1). In 30 kR irradiated culture, no recovery could be traced and almost no mitotic stages could be located after 40 kR and 50 kR gamma irradiation. The percentage mitosis values of 5 min and 10 min U.V.exposed cultures were similar to the control but the number of mitotic divisions decreased in 15 min exposed culture. The 20 and 25 min exposure was deleterious and no division was recorded (Table 1).

The percentage survival values of gamma irradiated culture indicated that there was tremendous recovery in 10 kR to 30 kR irradiated cultures (Table 2). The higher doses were lethal. The percentage survival values of 5 min and 10 min U.V.exposure were not affected whereas U.V.exposed cultures for 15 to 25 min showed a sharp decline, though a slight recovery by the end of the third week occurred (Table 2).

A gradual increase occurred in dry weight from first week to the third week in case of 10 to 30 kR gamma irradiated cultures (Table 3). But, a decrease in dry weight was recorded in case of 40 and 50 kR gamma irradiated cultures. The values of dry weights of 5 and 10 min U.V.exposed cultures matched those of the control but there was a slight fall in case of 15 min U.V.exposed culture. The values of the 20 min and 25 min exposed cultures declined sharply indicating the damaging effect of irradiation (Table 3).

There was a general increase of chlorophyll content from first week in 10, 20 and 30 kR gamma irradiated cultures, but the values decreased in case of 40 and 50 kR irradiated cultures (Table 4). The total chlorophyll content decreased with increase of U.V.exposure duration (Table 4). In 5, 10 and 15 min exposed cultures, the values increased from first week to third week similar to the control although they were considerably lower; the least being the 15 min exposed culture. The values decreased throughout the three week period in case of 20 and 25 min U.V.exposed cultures. There was a general bleaching of colour in U.V.irradiated cultures due to the effect on chloroplasts, the paleness increased with increased duration of exposure.

DISCUSSION Though the survival pattern of gamma and U.V. irradiated cultures resembled each other, the sites and modes of action of the two types of radiation were quite different even in the material. Vadajanani & Sarma (1979a,b,c) reported similar observations such as a high tendency of the filaments to break into a few celled or one celled fragments in case of gamma rays while fragmentation of filaments was not a very common occurrence in case of U.V.rays. In case of U.V. treatment, whole intact filaments turned colourless immediately in a day or two after exposure. It might be due to the fact that U.V.rays bring about immediate and direct effects on chloroplasts of cells, by affecting the chloroplast DNA, as was established by Kemp *et al* (1972).

The most frequent consequence of gamma irradiation is that there was a delay in the onset of mitosis and the maximum mitotic percentage of irradiated cultures was recorded by second week unlike the control, which normally yielded maximum mitotic percentage by first week. In general, the algae was remarkably resistant to lower doses (10, 20 and 30 kR), but at 40 and 50 kR, the cultures were damaged beyond repair. The adverse effects of gamma irradiation can be termed as delayed due to the initial abortive nuclear division involved. According to Nybom (1953) the lethal effects of U.V. irradiation are immediate because they result from protein denaturation or photo-oxidation of the enzymes. Evidences by Arnold (1933), Holt *et al* (1951) and Redford & Myers (1951) indicated that the U.V.rays bleach and destroy the chlorophyll, and its ultimate action is the inhibition of Hill reaction. This might perhaps be the reason for the immediate decolourisation of whole filaments that occurred more significantly in case of high doses of U.V. irradiation. Comparatively, the most significant effect of gamma irradiation is the tendency of fragmentation of the filaments into a few or one celled units as was also claimed by Vedajanani & Sarma (1979a).

Kemp & Wentworth (1971) suggested that repair mechanisms operate during the lag phase of growth after U.V. irradiation and provided a satisfactory explanation for the remarkable recovery observed in cultures treated with lower doses of U.V. The damaged cells were eliminated and the slightly damaged cells recovered during the lag phase. Similar mechanisms of repair seem to operate in case of gamma irradiated cultures where the lethal dose kills almost all the cells. In lower doses, the slightly affected cells tend to recover during the mitotic delay, to commence normal mitotic division and the damaged cells being eliminated from the culture. The polycentric nature of the chromosomes, and their minute size might have helped the algae to escape the damaging effects of irradiation.

Table-3 Dry Weights of Cultures of *Spirogyra verrucosa* Recorded During the Course of Three Weeks After the Gamma/U.V. Irradiation.

Type of irradiation	Dosage/ Duration	Dry weight (in grams) values after I Week	II Week	III Week
Gamma irradiation	Control	0.07	0.07	0.09
	10 Kr	0.03	0.03	0.06
	20 Kr	0.01	0.02	0.04
	30 Kr	0.01	0.01	0.03
	40 Kr	0.009	0.008	0.006
	50 Kr	0.009	0.006	Nil
U.V. irradiation	Control	0.09	0.09	0.1
	5 min	0.08	0.09	0.09
	10 min	0.08	0.08	0.09
	15 min	0.04	0.06	0.09
	20 min	0.009	0.01	0.01
	25 min	0.006	0.002	0.002

Table-4 Total Chlorophyll Content of Cultures of *Spirogyra verrucosa* Recorded During the Course of Three Weeks After Gamma/U.V. Irridiation.

Type of irradiation	Dosage/ Duration	Total Chlorophyll content (mg/L) values after I Week	II Week	III Week
Gamma irradiation	Control	0.7	0.9	0.9
	10 Kr	0.3	0.4	0.6
	20 Kr	0.2	0.2	0.4
	30 Kr	0.1	0.1	0.2
	40 Kr	0.01	0.007	0.002
	45 Kr	0.007	0.002	Nil
U.V. Irradiation	Control	0.6	0.7	0.8
	5 min	0.0	9 0.1	0.1
	10 min	0.07	0.08	0.09
	15 min	0.06	0.08	0.09
	20 min	0.01	0.009	0.001
	25 min	0.009	0.005	Nil

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