Cytological Effects of Ionizing Radiations (Gamma rays and X-rays) on Two Species of *Oedogonium* (Oedogoniales)

S. B. Agrawal and Y. S. R. K. Sarma

Lab. of Algal Cytology and Cytogenetics, Centre of Advanced Study in Botany,
Banaras Hindu University, Varanasi-221005, India

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A large number of studies have been made on the cytological effects of radiations on higher plants and animals. In comparison, similar studies on algae are very scanty. Godward (1954,b) was the first worker to report the karyological effects of X-rays on a green alga *Spirogyra crassa*. Since then, sufficient work has been added to this field by a number of workers on various groups of algae, particularly the taxa representing the orders, Oedogoniales, Conjugales, Cladophorales and Charales. Contributions made by various workers to irradiation studies of algae are reviewed by Godward (1962) and Sarma (1979).

In the present investigation the effects of ionizing radiations (gamma and X-rays) were seen on the morphology and cytology of two species of *Oedogonium*, *O. gunnii* Wittr. and *O. virceburgense* Hirn. Two species of *Oedogonium* were selected for comparative studies because the former species was characterized by large size of chromosomes, while *O. virceburgense* possessed smaller chromosomes, besides difference in chromosome numbers.

Materials and methods

Both the species of *Oedogonium* were grown in Godward's medium (Godward 1942) supplemented with 8% soil extract. The cultures were maintained in a thermostatically controlled culture chamber maintained at $21 \pm 1^\circ\text{C}$ and illuminated with the help of 40 w fluorescent tubelights with a light intensity of 2000 lux approximately for 16 h daily.

The cultures were transferred to fresh culture media 24 h before irradiation of materials. Just before irradiation these filaments were kept in small petriplates containing 10 ml of Godward's inorganic nutrient medium and were exposed to varying doses of X-rays and gamma rays. In all the experiments, controls were also maintained.

X-ray treatments were given by Picher's therapeutic, X-ray unit runout at 110 kv, 4 mA with a filtration of 1 mm Al at a distance of 10 cm. having the exposure rate 0.2 kr/min. The source for gamma-rays was ^{60}Co from a Colorado unit having the exposure rate of 0.368 kr/min at a distance of 30 cm. The different doses were obtained by increasing the time of exposure. The effective dose range both for X-rays and gamma-rays were 0.25 kr-4.0 kr and 0.5 to 4.0 kr for *O. gunnii* and *O. virceburgense*, respectively. Following irradiation, materials were fixed daily upto 9 days

at the time of maximum mitotic division in a mixture of three parts of absolute alcohol and one part of glacial acetic acid. The fixed samples were studied cytologically by employing iron-alum acetocarmine technique (Godward 1948) and observations were noted both qualitatively as well as quantitatively.

Observations

1. Control materials

The chromosomes of *O. gunnii* ($n=17$), ranged from 6.6 to 10.0 μm in length at metaphase (Figs. 1, 2) while those of in *O. virceburgense* ($n=9$) ranged from 1.4 to 3.4 μm in length at metaphase (Figs. 9, 10).

2. Morphological effects

Filaments irradiated with gamma rays and X-rays revealed certain common morphological effects in all the treatments, such as bulging of cells, shrinkage and degeneration of chloroplasts and appearance of fragmented filaments. The degree of the above effects was found to be linearly increased with the increase in dose, to which the algae were exposed. Materials of *O. gunnii*, exposed to 5 kr did not survive after 4 days following treatment with gamma rays and 5 days with X-rays, while *O. virceburgense* could not survive beyond 5th and 6th day following irradiation with 5 kr to gamma and X-rays, respectively.

3. Mitotic delay

Mitotic delay upto 4–5 days in *O. gunnii* and 5–6 days was noted in *O. virceburgense* as a result of irradiation (Table 1).

4. Cytological aberrations

The main chromosomal aberrations observed in *O. gunnii* and *O. virceburgense* by gamma and X-rays are given in Table 2. From the Table 2, it is clear that the effects observed by gamma rays and X-rays are more or less similar in both the algae. In *O. virceburgense*, the broken dot like chromosomes were seen to be in close vicinity of their respective chromosome portions rendering their photography a very difficult task.

Percentage of chromosomal aberrations on *O. gunnii* was 7.89% at 0.25 Kr whereas at a higher dose of 4.0 Kr it increased upto 47.32% as scored on the 7th day after irradiation with gamma rays (Fig. 16). With X-rays the percentage of cells showing chromosomal aberrations increased from 5.66% at 0.25 Kr to 41.50% at 4.0 Kr on the 7th day (Fig. 18). In *O. virceburgense* the percentage of chromosomal aberrations with gamma rays at 0.5 kr was 5.68% only, whereas with 4.0 Kr the percentage increased to 31.81% as scored on the 7th day treatment (Fig. 17). Likewise, the dose of 0.5 Kr of X-rays showed 5.81%, while the percentage increased to 27.90% at highest dose of 4.0 Kr as scored on the 7th day of treatment (Fig. 19).

Figs. 1-15. Explanation of microphotographs. *O. gunnii*, Figs. 1-8, c. $\times 1500$ and *O. virceburgense*, Figs. 9-15, c. $\times 1800$. 1, metaphase of *Oedogonium gunnii* showing $n=17$ chromosomes. 2, drawing of Fig. 1. 3, chromosome breakage at metaphase. 4, anaphase showing laggard formation. 5, chromosome breakage at anaphase. 6, ring chromosome. 7, anaphase showing formation of bridges. 8, metaphase showing $n=9$ chromosomes in *O. virceburgense*. 9, drawing of Fig. 9. 10, drawing of Fig. 9. 11, clumping of chromosomes at metaphase. 12, micronuclei. 13-14, bridge formation. 15, ring chromosome.

Figs. 16-17. Effects of gamma irradiation. 16, histogram showing percentage of chromosomal aberrations in *O. gunnii*. 17, histogram showing percentage of chromosomal aberrations in *O. virceburgense*.

Figs. 18-19. Effects of X-rays. 18, histogram showing percentage of chromosomal aberrations in *O. gunnii*. 19, histogram showing percentage of chromosomal aberrations in *O. virceburgense*.

Table 1. Showing mitotic delay

Dose in K rads	Gamma rays		X-rays	
	<i>O. gunnii</i> (in days)	<i>O. virceburgense</i> (in days)	<i>O. gunnii</i> (in days)	<i>O. virceburgense</i> (in days)
0.25	4	—	4	—
0.5	4	5	4	4
1.0	4	5	4	4
2.0	5	6	4	4
3.0	5	6	4	5
4.0	5	6	5	5

Table 2. Showing chromosomal aberration in *O. gunnii* and *O. virceburgense* by gamma and X-rays

Type of chromosomal aberrations	<i>O. gunnii</i>	<i>O. virceburgense</i>
1. Stickiness and clumping of chromosomes	Frequent at doses below 2 Kr (Fig. 8).	Frequent at doses 0.5 to 1.0 Kr (Fig. 11).
2. Chromosome breakage	2-4 chromosome fragments with 0.25-1.0 Kr More than 4 or rarely heavy fragmentation of chromosomes with 2, 9, 3.0 and 4.0 Kr (Figs. 3, 5).	1-2, small dot like bits of broken chromosomes at 0.5-1.0 Kr. 2-5 fragments at higher doses of 3.0 and 4.0 Kr.
3. Bridge formation at anaphase	Sticky bridges or single stranded bridges were common at 0.25-1.0 Kr 1-3 long continuous strands rarely with fragments at higher doses of 2, 3 and 4.0 Kr (Fig. 7).	Sticky bridges not very common, mainly single stranded bridges (Figs. 13, 14) were present which were thin and thread like. Sometimes double stranded bridges were observed at 3.0 and 4.0 Kr.
4. Laggards	Observed with 0.25 Kr and above (Fig. 4).	Observed at 1.0 Kr and above.
5. Ring chromosomes	At 0.25 Kr and above (Fig. 6).	At 1.0 Kr and above (Fig. 15).
6. Chromosomes with fuzzy outlines	Observed at 2 Kr.	Not observed.
7. Transverse orientation of daughter nuclei and formation of binucleate cells	Frequently observed at all doses employed.	Frequently observed at all doses.
8. Micronuclei	1-2 in number were observed with 1.0 Kr and above with Gamma-rays while at 3.0 Kr and 4.0 Kr with X-rays.	1-2 with 0.5-1.0 Kr of gamma rays and 0.5 to 2.0 Kr or X-rays while at higher doses 3.0 and 4.0 Kr, the same increased upto 4 (Fig. 12).
9. Degeneration of nuclei (deformation/pycnosis/vacuolization)	Frequent	Frequent

Discussion

The effects of gamma rays and X-rays on algae have been studied by several workers. However, observations on chromosomal aberrations consequent to irradiation on algae are very few. Godward (1962) reviewed the work related to various types of radiations on algae and effects produced by them. The extremely wide range of tolerance towards gamma and X-irradiations demonstrated by different algae was reviewed recently by Sarma (1979). In the present investigation chromosomal aberrations could be induced at doses ranging from 0.25 to 4.0 Kr in *O. gunnii* and from 0.5 to 4.0 Kr in *O. virceburgense*, thus occupying an intermediate position in between algae exhibiting high resistance and those showing less tolerance towards both the radiations.

Another pronounced effect of radiations was delay in onset of first mitosis. These observations are in conformity with the findings reported by Vedajanani and Sarma (1979a) in *Spirogyra azygospora* with gamma rays, Srivastava and Nizam (1974) in *Pediastrum* sp. and Dodge and Godward (1963) in *Prorocentrum micans* by X-rays. Delay in mitosis was also reported by Sarma and Agrawal (1980) in *Rhizoclonium hieroglyphicum* by both the radiations.

In the present investigation, *O. gunnii* showed 2–4 fragments upto the dose of 2.0 Kr and heavy fragmentation at 3.0 and 4.0 Kr while maximum of 1–5 fragments were recorded in *O. virceburgense*. Sarma and Singh (1974a, 1977a) and Singh (1974) reported more than two chromosome fragments above 0.4 Kr in *Nitella acuminata*, *N. opaca* and *N. flagelliformis*, while in *Chara fibrosa* and *C. globularis* they reported 1–2 fragments at a dose of 0.5 kr, but at higher doses of 2.0 Kr chromosome fragments were more. Sarma and Agrawal (1980) reported in *Rhizoclonium hieroglyphicum* that the number of chromosome fragments varied from 1–3 (1–2 with X-rays) within the dose range of 0.25 to 1.0 Kr of gamma rays and heavy fragmentation occurred at higher doses of 4.0 and 5.0 Kr. On the other hand, while occasionally near about 6–8 fragments were observed at 20.0 Kr of gamma rays heavy shattering of chromosomes could not be seen even at higher doses of 130 Kr. in *Spirogyra azygospora* (Sarma and Vedajanani 1979a).

In *O. gunnii*, at lower doses sticky bridges were more common while at higher doses bridges were made up of 1–3 strands. *O. virceburgense* showed generally one stranded bridges. These results are comparable with the reports of Sarma and Singh (1974a, 1977a) and Singh (1974) on some members of Charophytes and Sarma and Agrawal (1980) on *R. hieroglyphicum*.

In the present study the percentage of cells showing chromosomal aberrations increased with the increase in the dose of gamma/X-radiation. These results are comparable with the results of Sarma and Khan (1967), Dodge and Godward (1963), Chatterjee (1971), Singh (1974), Sarma and Singh (1974a, 1977a) and Sarma and Agrawal (1980). Another aspect which becomes clear from the present study is that gamma rays are more effective than X-rays in bringing about a particular type of effect.

From the present investigation it is clear that *O. gunnii* and *O. virceburgense* are sensitive to gamma/X-rays even at a lower dose of 0.25 and 0.5 kr, respectively.

Based on the results of present study and those of Singh (1974), Sarma and Singh (1974, 1977a) and Sarma and Agrawal (1980) obviously a generalization cannot be made that all algae are more resistant to radiations in comparison to higher plants. However, the algae characterized by fairly large size of chromosomes with localised centromeres seem to be more sensitive, since more area is available for absorption of radiation energy. Thus, the chromosomes of *O. gunnii*, being larger in size are much more sensitive to ionizing radiations than *O. virceburgense* having smaller size of chromosomes. On the other hand, the members of Conjugales, with polycentric chromosomes in most cases, euglenoids, dinoflagellates with acentric and atypical chromosomes, Chlorococcales with minute chromosomes are notable ones which show high levels of resistance to radiation in comparison to other algae and higher plants (cf. Sarma 1979). In such cases of algae with polycentric chromosomes as in some species of *Spirogyra* and atypical chromosomes in Euglenineae and Dinophyceae, although large chromosome surface is exposed to irradiation, the fact remains that they are more resistant as compared with long chromosomes with localised centromeres. Some other mechanism must be operating to bestow the capacity of resistance to radiations on the chromosomes of such algae.

Summary

In the present study the effects of gamma rays and X-rays were seen on the karyology of two species of *Oedogonium*, *O. gunnii* Wittr. and *O. virceburgense* Hirn. (Oedogoniales). Various types of chromosomal aberrations were assessed qualitatively and quantitatively. The percentages of chromosomal aberrations and percentages of nuclear degeneration were found to be directly related with the administered doses of gamma/X-rays. Gamma rays were found more effective than X-rays in bringing about a particular type of effect. *O. virceburgense* proved to be more resistant than *O. gunnii* for both the radiations.

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