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Gamma-ray Induced Morphological Changes in Two Species of *Chara*

Ruma Pal
Centre for advanced studies in Botany
University of Calcutta
Calcutta-700 019.

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Gamma-irradiation from Co_{60}/ source was given to *Chara Zeylanics* var. & f.*Zeylanica* (n=28) and *C. vulgaris* var. *inconnexa* f. *hippliana* (n=28). Morphological changes were of diverse types such as increased calcification (including calcite deposits on reproductive structures), heavy epiphytic algal growth on the irradiated thallus, formation of giant cells, variation in cortex and stipulodes, branching patterns.

Key Words Gamma Giant cells calcification Chara Epiphyte

In nature, Charophyta exhibit a bewildering variety of morphological characters, and in classical taxonomy only some key morphological characters are taken into consideration in identification. Morphological variations due to irradiation in charophyte species have been described (Mountschen, 1951; Gillet, 1963). The effect of light quality and quantity on morphogenesis of a few species of *Chara* was investigated by Maeda & Imahori (1968). The present investigation was aimed to find out how far the key morphological characters could be modified by radiation, particularly by gamma-rays. Radiation induced morphological changes that parallel natural morphological variations within intraspecific taxa are also induced in this investigation.

MATERIALS & METHODS

C. zeylanica var. *zeylanica* f.*zeylanica* Klein ex Willd. and *C. vulgaris* var. *inconnexa* f.*hippelliana* (Vihl) R.D.W. were used.

Unialgal cultures were grown in soil-water biphasic medium, in large glass jars near north facing window. Light intensity was 2000 lux on culture surface and the temperature was 15 to 20 C. The specimens cultured in the laboratory were identical in morphological characters to plants collected from natural habitat. Radiation was given to whole plants with rhizoids at the rate of 1 kR per 30 seconds from Co_{60} source. Total doses given were 2,4,6 and 8 kR. Irradiated plants were returned to soil water medium. The experiment was repeated for 5 times. Young shoot tip, portions of main axis and branchlets were mounted in 10% glycerine and examined under a microscope. Extensively calcified materials were treated with N HCl for 2 to 5 minutes to remove superficial $CaCO_3$ Dimensions of the main axis and branchlets were measured.

RESULTS & DISCUSSION

Quantitative data of a few morphological changes induced by gamma-irradiation in two species of *Chara* are summarised in Table 1. Gamma-irradiation resulted in increased growth of both species due to giant cell formation. In algae, irradiation induced giant cell formation has been reported in different genera, such as in *Zygnema* (Petrova, 1962), *Oedogonium* (Horseley et al, 1967), *Mougeotia* (prasad & Godward, 1968), *Chlorella* (Hampl et al, 1971). Tolmach & Marcus (1960) suggested that giant cells may result from the ultimate failure of cell division process. Wells and Hoshaw (1979) obtained 26% giant cells in irradiated species of *Sirogonium*, which did not undergo mitosis, but they continued the synthesis of RNA and enzyme required for entensive cell wall elongation. Cytophotometric readings of giant cell nuclei indicated DNA synthesis, but the karyokinetic process was not initiated.

In irradiated plants, the peripheral cells of the main axis lost the natural phasing of formation, and hapazard cortication or total absence of cortication resulted (Fig. 2-3). This explains the absence of stipulodes, bracts, gametangia etc. (Fig. 6, 12). In both the species, irradiation induced changes in cortication on branchlets were similar to that of the main axis. With lapse of time and in the course of proliferation of new sprigs, the brachlets again returned to their normal structure at lower dosages, whereas, at 6 to 8 kR the changes were drastic Bracts failed to develop and branchlet cortication was entremely haphazard for two months after the date of irradiation (Fig. 2-5). After two months, those plants which survived showed totally ecorticated branchlets devoid of any sex organs (Fig. 3,6,9,12).

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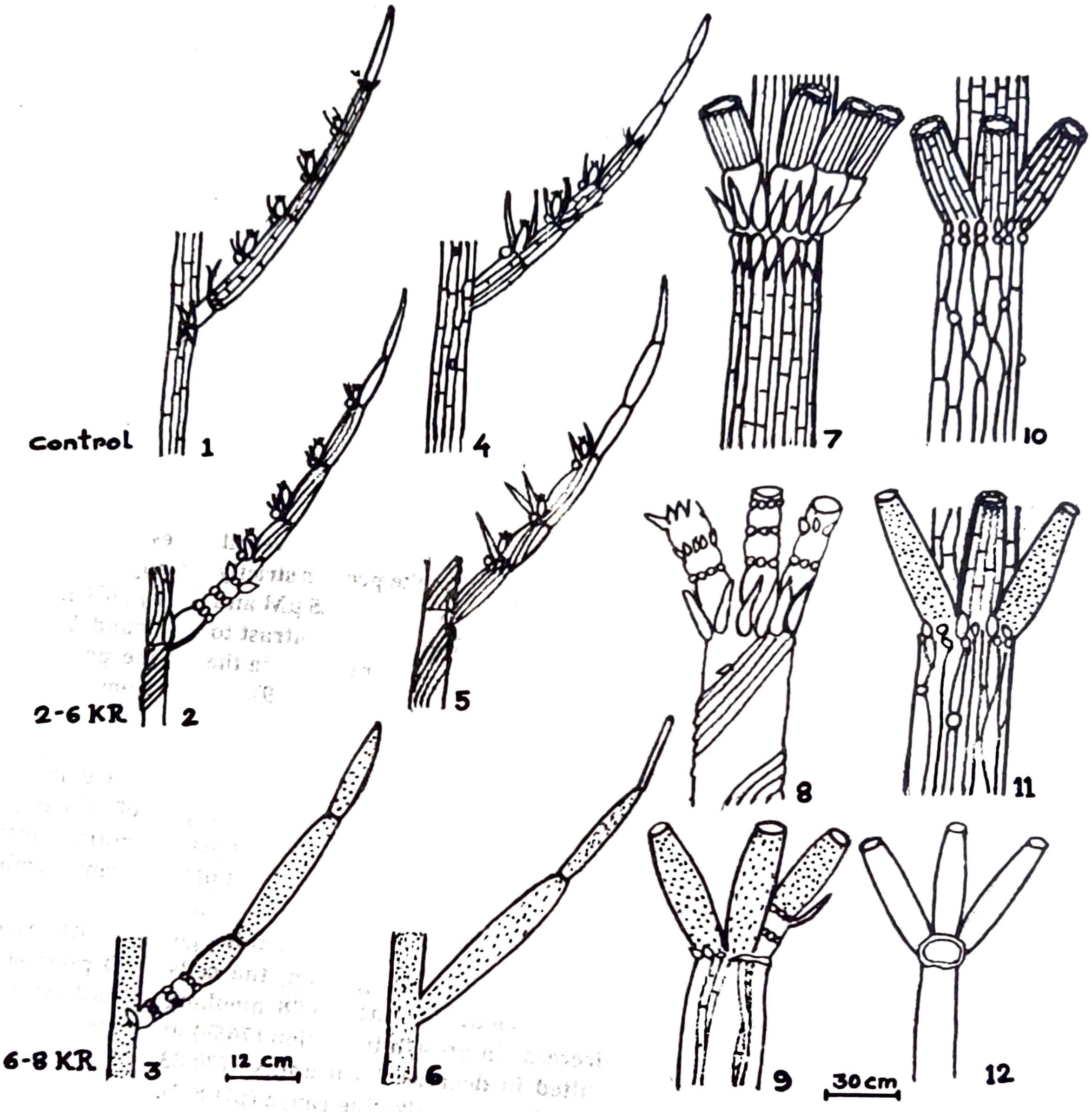

Figs. 1-3 : Camera lucida drawings of branchlets of *C.zeylanica*.

Fig. 1 : Branchlet of control plant with normal cortication.

Fig. 2 : Haphazard cortication, a number of ecor ticated cells at the basal portion with rows of cortex initials.

Fig. 3 : Totally ecorticated branchlet with $CaCO_3$ deposition and reduced cortex initials

Figs. 4-6 : Branchlets of *C.vulgaris*

Fig. 4 : Normal cortication of control plant.

Fig. 5 : Haphazard cortication

Fig. 6 : Totally ecorticated branchlet.

Figs. 7-9 : Nodal region of *C.zeylanica*

Fig. 7 : Control plant showing diplostephanous stipulodes and normal stem cortication.

Fig. 8 : Haplostephanous stipulodes and spiral cortication.

Fig. 9 : Estipulatic condition and haphazard cotication.

Fig.10-12 : Nodal region of *C. vulgaris*

Fig. 10 : Two rows of stipulodes, lower tier globular and normal cortex.

Fig. 11 : Stipulodes irregular in shape, with size variation.

Fig. 12 : Main axis ecorticated and without stiplode.

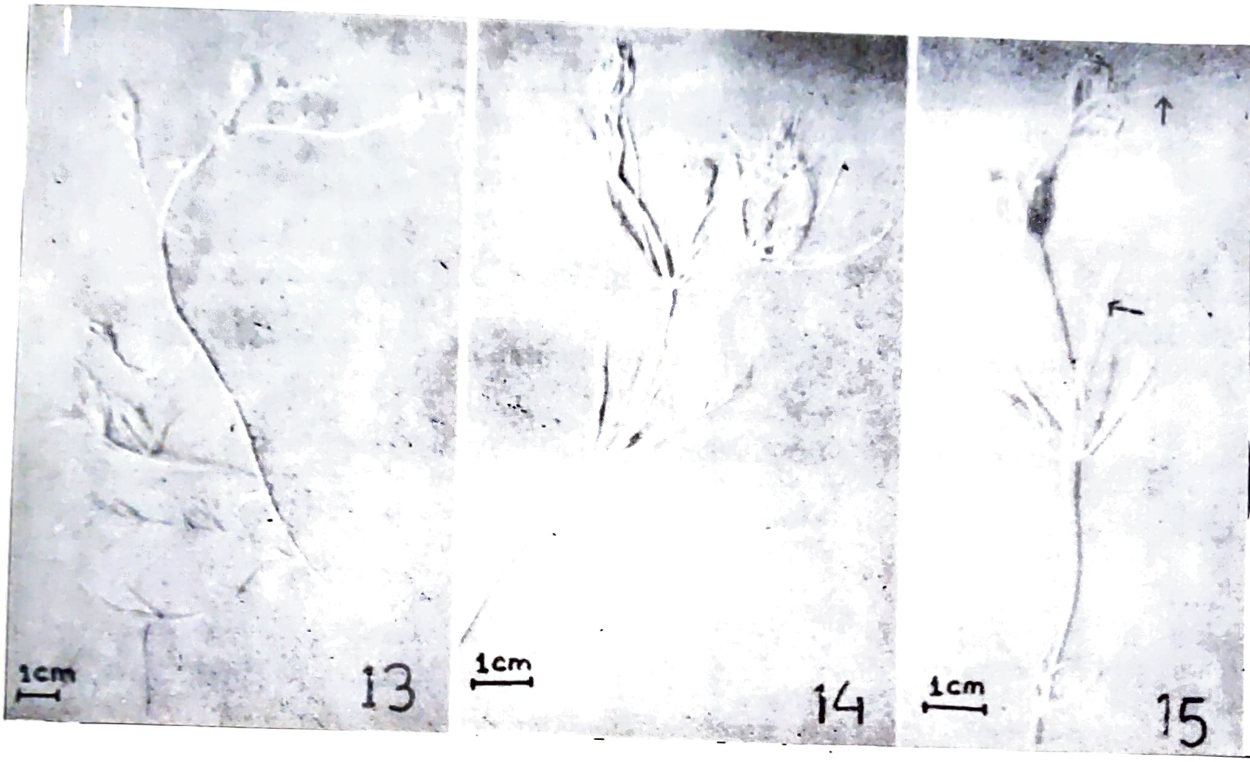

Fig. 13 : Spring showing helicoid branching in radiation recovered plant (one month - 6 kR).

Fig. 14 : Sprig showing development of compact head at the top (after 3 weeks - 6 kR).

Fig. 15 : Sprig showing development of unbranched protonemas from the nodal regions.

In C. zeylanica, Sundaralingam (1954) reported that the nodal initial of the branchlet, by series of vertical curved septa normally forms a node consisting of a number of peripheral cells enclosing a single cell in the centre. These peripheral cells ultimately elongate and form the cortex of the branchlets. In irradiated plants, these peripheral cells lose the capacity of cell division and fail to elongate. They remain rudimentary and form a row of globular cells on the nodes of the branchlets, or they elongate slightly and loosely adhere to the internodal cells of the branchlets (Fig. 2,3,8).

In case of both the species with $1^1/_2$ to 2 months of irradiation at a dose of 6 to 8 kR, new shoot tips appeared with haphazard main cortex and stipulodes, and ultimately the stipulodes disappeared resulting in an estipulodic condition (Fig 3,6,7-12). Clearly irradiation altered the normal morphogenesis of peripheral cells to a great extent.

In *Chara* new branches develop from the basal nodal cell of the main stem (Sundaralingam, 1954). In irradiated plants rapid development of branches occured. Branches developed from almost all main axis nodes giving a very crowded appearance. Variation in branching, development of compact heads at the top etc. (Fig. 13-15). Luxuriant growth of branches was also recorded.

A tendency existed in *Chara* to restore the effect of irradiation by developing a large number of branches from the irradiated plants. At higher dosages (6 kR up) where changes were maximum, plants survived and propagated either by protonemata or atypical branches, which on entering the soil surface again proliferated (Fig. 15).

Within 5-7 days following irradiation, the plants became covered with thick deposits of $CaCO_3$; the calcified layer appeared first as mosaic like pattern on the main axis and branchlets. Gradually they covered the reproductive structures. Calcification increased on plants irradiated with 4 to 8 kR dosages.

According to Horn af Rantzien (1959) calcification of the cosporangia of charophyte species is a highly specific feature, whereas calcification of the vegetative parts of thallus is non specific and is correlated with a high concen-

Table 1 Morphological Changes Following 1 to 2 Months of Irradiation in *Chara*

Species	Dosages (KR)	Length of internodes (mm)	Length of branchlet (mm)	Giant cell formation (%)	Stipulode nature	Branching type
Chara zeylanica	Control	51	32	—	Diplostephanous	Normal whorl arrangement
	2	41	13	—	Hapostephanous - suppression of lower tier	Irregular
	4	72	37	58	Haplostephanous	Irregular
	6	74	45	71	Haplostephanous	Irregular
	8	68	42	61	Haplostephanous	Irregular
C. vulgaris	Control	53	36	—	Diplostephanous	Normal whorl arrangement
	2	52	31	—	Diplostephanous	Whorl arrangement
	4	59	39	47	Diplostephanous	Irregular with unequal branchlet
	6	72	43	67	Diplostephanous	Irregular
	8	60	44	52	Totally absent	Irregular

tration of $Ca(HCO_3)_2$, in the habitat. Borowitzka & Larkum (1976) noted in *Halimeda* that photosynthesis stimulated calcification, by removing CO_2 from intercellular spaces, whereas respiratory CO_2 evolution inhibited calcification by decreasing the pH and CO_3. In this case gamma radiation decreased the rate of respiration by serving as a respiratory inhibitor. Thus the rate of photosynthesis of the giant plant (due to irradiation) increased in comparison to the respiration rate leading to a local rise in pH. This in turn resulted in calcification.

Three to 5 days after irradiation all the irradiated plants became thickly coated with epiphytic algal growth which was absent in control plants. Epiphytic algae included blue greens and green filamentous forms like *Oedogonium*, unicellular green alga *Oocystis* and various diatoms. The portions of plants directly exposed to irradiation became more heavily coated than the other parts and turned brown in colour. The new shoot tips which appeared after irradiation were also affected by the algal growth but to a reduced extent.

Healthy *Chara* plants excrete two allelopathic substances dithiolane and trithiane which prevent the growth of phytoplankton and other filamentous algae (Wium-Anderson *et al.* 1982). Thus irradiated plants were colonized readily by other algae probably due to the non-secretion of these substances. As a result of irradiation the plants in general became weak so as to permit colonization by other algae.

In the taxonomy of *C.vulgaris*, variation in morphological characters plays an important role. These characters include, varying degree of development of the branchlets, from stout well developed ones to tiny one, nature of spine (solitary or geminate), degree of cortication of the main and branchlet axis, etc. According to Wood and Imahori's concept (1964), these variations in character are to be regarded as providing important diagnostic characters, and therefore these phenotypic characters were given due importance in the construction of keys for taxonomic identification.

From our studies with gamma-rays on *C.vulgaris*, it became clear that the variation in morphology induced closely paralleled the morphological diversity that exists in nature among different constituent varieties and forms of this taxon. For example, *C.vulgaris* var. *inconnexa f.hippeliana* following radiation of 6 to 8 kR became grass-like in appearance with obscure tiny branchlets resembling the appearance of *C.vulgaris* var. *kirghisorum f.filliformis* (after Migula). At a radiation of 4 to 6 kR the taxon developed occasionally geminate spine cells instead of solitary spine cells with irregular cortex - a condition which is frequently shown by C.*vulgaris* var.*vulgaris* f.*kieneri* and C.*vulgaris* var. *vulgaris* f. *pistianensis* Totally ecorticated branchlets were produced in radiation recovered plants at a dosage of 6-8 kR. This condition is analogous to the character exhibited by *C.vulgaris* var. *vulgaris* (Fig..6). Similarly in C.*zeylanica* var. & f.*zeylanica* gamma radiation recovered plants showed small reduced branchlet whorls, closely resembling the general features of C.*zeylanica* var. *diaphana* f.*depauperata*. Irregular cortication which is a character of *C. zeylanica* var. diaphana was mimicked by radiation recovered (4-6 kR) *C. zeylanica* var. & f. *zeylanica* plants (Fig. 2), whereas total ecorication in branchlets and main axis was induced at 8 kR (fig. 3), the latter condition being known in C.*zeylanica* var. *brittonii*.

In both the species, sex organs were never produced by radiation recovered plants (6 kR onwards). Plants multiplied by protonemal branches only.

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