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Effects of UV-Light and γ-Rays on the Survival, Akinete Formation and Akinete Germination in *Stigeoclonium pascheri*

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ABSTRACT. The damage produced by UV light to any of the three different stages of the life cycle of the parent generation of the green alga *S. pascheri*, *i.e.* akinetes, germinating akinetes and vegetative cells remained up to the stage of germination of akinetes of the first generation and no deleterious effect was reported thereafter. Lower doses of γ-rays (25—75 Gy) increased the percentage germination of akinetes and germinating akinetes of parent generation. The percentage germination of akinetes, germinating akinetes, survival of colonies originated from vegetative cells and sporulation of cells of the parent generation decreased with increasing doses from 100 to 300 Gy. The γ-induced effect to any of the three different stages was not transferred to the subsequent stage of algal generation.

Irradiation studies on green algae with reference to their spore germination, survival and sporulation are scanty. An attempt was made by Davies (1965) to establish the UV sensitivity of a series of stages in the cell cycle of a synchronous population of diploid meiotic spores of *Chlamydomonas reinhardi*. Parker and Horsley (1972) have shown the loss of reproductive capacity of cells of UV-irradiated *Oedogonium cardiacum*. The vegetative survival of the different algae was found to be UV-dose-dependent (Redford and Myers 1951; Nybon 1953; Dodge 1964; Kemp and Wentworth 1971; Vedajanani and Sarma 1979). Sarma and Agrawal (1980) reported that UV light delayed and decreased spore germination, colony formation and sporulation of cells of the alga *Stigeoclonium pascheri*.

Considerable work has been done on the effects of γ-rays of the levels of sensitivity of a number of green algae (*cf.* Godward 1962; Horsley and Fucikovsky 1963; Herbert and Sparrow 1964; Srivastava 1969; Vidyavati and Nizam 1974; Vedajanani and Sarma 1979). In the present study three different stages of the life cycle of the alga *S. pascheri*, *i.e.* akinetes, germinating akinetes and vegetative cells were separately irradiated with γ-rays. To observe whether the effects produced by γ-rays to the parent generation were transferred to the different stages of the subsequent generation of the alga, the germination of akinetes, growth of germlings and sporulation of cells of the subsequent generation of the alga were examined.

MATERIAL AND METHODS

The alga *Stigeoclonium pascheri* (VISCHER) COX and BOLD (*Chaetophorales, Chlorophyceae*), isolated from a fresh-water pond at Sarnath, Varanasi (India) and maintained clonally in Bold's basal medium (BBM) (Cox and Bold 1966) at 22 ± 1 °C and illuminated at *ca.* 2 klx light intensity from daylight fluorescent tubes for 16 h/d, reproduces through the formation of akinetes, which commence to appear in 30-d-old culture. Further 30 d were required from the time of initiation to the maturation of an akinete on the agar plates. The akinetes harvested from the basal medium and stored in complete darkness at 21—22 °C in sterilized double-distilled water when transferred to fresh medium, whenever needed germinated directly into new vegetative cells. A single generation of the alga includes all the stages from the germination of an akinete to its formation.

The irradiation was done with three different stages of the parent generation of the alga: (1) Akinetes directly obtained from stored stock; (2) akinetes obtained from stored stock and soaked in BBM at 22 ± 1 °C for 18 h in the presence of light (these akinetes were called germinating akinetes); the term "germinating" as used here implies only that the akinetes were placed under conditions favourable to this event; however, emergence of germlings usually starts 2 d after incubation; (3) 10-d-old actively growing vegetative cells.

The experimental materials were irradiated with UV light by the methods given elsewhere (Sarma and Agrawal 1980). The energy fluence rate of UV light was 3.2 W/m^2. Energy fluence of UV-light which was obtained by increasing the time of exposure from 10 to 40 min, ranged from 1.92 to 7.68 kJ/m^2. Absorbed dose of γ-rays (^{60}Co) administered ranged from 25 to 300 Gy. After irradiation, the irradiated materials were separately centrifuged and then plated on agar plates containing 20 mL of sterilized BBM solidified with 1 % agar. The inoculated plates were examined at intervals from the start of the experiment so as to determine the time taken for initiation of akinete germination, percentage germination of akinetes, growth of germlings, time taken for initiation of sporulation and percentage sporulation.

RESULTS AND DISCUSSION

UV Light

UV irradiation of akinetes, germinating akinetes or vegetative cells of the parent generation delayed and decreased the germination of akinetes and germinating akinetes; delayed the appearance of vegetative colonies and decreased the percentage survival of colonies arising from irradiated vegetative cells of the parent generation (Sarma and Agrawal 1980). The time taken for initiation of sporulation and percentage sporulation of vegetative cells were delayed and decreased in UV-irradiated vegetative cells as well as in the vegetative cells (unirradiated) originated after the germination of UV-irradiated akinetes and germinating akinetes of the parent generation and these were more pronounced in irradiated vegetative cells rather than in those vegetative cells that were not irradiated but obtained after the germination of irradiated akinetes and germinating akinetes (Sarma and Agrawal 1980).

The germination of akinetes of the first generation obtained either from irradiated vegetative cells of parent generation or from vegetative cells formed

after germination of irradiated akinetes or germinating akinetes of the parent generation decreased with an increase in energy fluence of UV light and this was more pronounced in akinetes (of the first generation) obtained from irradiated vegetative cells of the parent generation than in those obtained from germinating akinetes or akinetes of the parent generation (Table I). However, the growth of germlings, the time taken for initiation of sporulation

TABLE I. Percentage germination of akinetes (nonirradiated) of the first generation obtained either from UV or γ irradiated akinetes, germinating akinetes or vegetative cells of the parent generation (mean of three replicate counts, rounded off to the nearest whole number)

Irradiation	Irradiated parent generation		
	Akinetes	Germinating akinetes	Vegetative cells
UV Light (min)			
0	53	50	51
10	50	41	29
20	44	36	17
30	36	24	9
40	30	17	5
γ Rays (Gy)			
0	53	51	50
50	54	49	52
100	51	54	55
200	55	50	57
300	50	53	52

and percentage sporulation of vegetative cells formed after the germination of akinetes of the first generation obtained either from 40 min irradiated akinetes, germinating akinetes or vegetative cells of parent generation were more or less similar to those of nonirradiated vegetative cells of the parent generation (control) (Table II). Therefore, the UV-induced damage to the different stages of a generation of the alga *S. pascheri* existed up to the stage of akinete germination of the next generation and were completely recovered during that period.

γ-Rays

There was no change in the time taken for initiation of germination of akinetes and germinating akinetes of the parent generation irradiated from 25 to 200 Gy as compared with that of the control, where the process began to be initiated on the second day after inoculation; however, a delay of 2 and 1 d were observed in the germination of akinetes and germinating akinetes, respectively, after irradiation with 300 Gy. Irradiation of akinetes and germinating akinetes of the parent generation increased their germination in an ascending order with an increase in absorbed dose from 25 to 75 Gy, the maximum enhancement being at 50 GY. However, germination of akinetes

TABLE II. Growth features of *S. pascheri* after irradiation (rounded means of three replicates)

Irradiated parent generation	Irradiation used	Length of germlings, % celled[a]			Time[b] d	Sporulation
		1–5	6–10	11–15		
	UV-light (min)					
Akinetes	0	82	12	6	30	65
	40	79	14	7	30	63
Germinating akinetes	0	77	10	12	30	66
	40	81	9	10	30	64
Vegetative cells	0	81	11	8	30	65
	40	84	6	10	32	67
	γ-rays (Gy)					
Akinetes	0	78	12	10	32	63
	50	81	13	6	31	60
	300	81	11	8	33	65
Germinating akinetes	0	79	8	13	30	62
	50	81	11	8	30	60
	300	81	10	9	31	63
Vegetative cells	0	82	9	8	28	61
	50	77	10	12	30	65
	300	80	10	10	30	62

[a] For 16–20 all values are zero.
[b] For initiation of sporulation.

and germinating akinetes decreased with an increase in absorbed dose from 100 to 300 Gy (Table III).

γ-Rays beyond 100 Gy, *i.e.* at 200 and 300 Gy delayed the initiation of colony appearance arising from vegetative cells of the parent generation by 1 and 4 d, respectively, as compared to their initiation on the second day after inoculation in nonirradiated vegetative cells of the parent generation (control). No change was observed in any of the remaining treatments. Percentage survival of the colonies formed from the irradiated vegetative cells of parent generation decreased with increasing absorbed dose from 100 to 300 Gy. The length of germlings (nonirradiated) originated from γ-irradiated and nonirradiated akinetes of the parent generation were more or less similar to each other (Table III).

No change was observed in the time taken for initiation of sporulation of irradiated vegetative cells and of nonirradiated vegetative cells formed after the germination of irradiated akinetes and germinating akinetes of the parent generation at any of the absorbed dose administered. Percentage sporulation of irradiated vegetative cells of parent generation declined at 100 to 300 Gy. However, the percentage sporulation of nonirradiated vegetative cells originated after the germination of irradiated akinetes or germinating akinetes of the parent generation was not changed at any of the absorbed dose used as compared with that of control (unirradiated vegetative cells of parent generation).

The akinetes of the first generation formed either from 50 or 300 Gy of irradiated vegetative cells of the parent generation or from nonirradiated

TABLE III. Effects of γ-rays on the germination of akinetes, survival of vegetative cells and sporulation (means of three replicates, rounded off to the nearest whole number)

Observation	Absorbed dose, Gy						
	0	25	50	75	100	200	300
Percentage germination of							
akinetes	58	73	84	64	50	41	28
germinating akinetes	56	76	89	69	47	36	24
Percentage survival of colonies originated from irradiated vegetative cells	100	100	100	100	96	74	38
Percentage of length of germlings (unirradiated) obtained from irradiated akinetes							
1–5 celled	80	80	78	77	79	85	84
6–10 celled	12	14	15	13	12	11	12
11–15 celled	7	6	7	9	8	4	3
Percentage sporulation of							
irradiated vegetative cells	65	65	65	64	53	46	38
unirradiated vegetative cells originated from irradiated							
akinetes	65	65	65	65	63	63	60
germinating akinetes	65	65	65	65	64	64	61

vegetative cells formed after the germination of 50 or 300 Gy of irradiated akinetes or germinating akinetes of the parent generation showed a more or less similar percentage of germination as compared with that of nonirradiated akinetes of the parent generation (Table I). The growth of germlings, the time taken for initiation of sporulation and percentage sporulation of vegetative cells formed after the germination of akinetes of the first generation obtained either from 50 or 300 Gy of irradiated akinetes, germinating akinetes or vegetative cells of the parent generation were more or less similar to those of nonirradiated vegetative cells of the parent generation (Table II).

In the present study, akinetes and germinating akinetes of *S. pascheri* subjected to lower doses of γ-rays showed an increase in percentage germination. A similar observation was also reported in *Bacillus megaterium* (Levinson and Hyatt 1960) and in the slime mold *Dictyostelium discoideum* (Khoury *et al.* 1970; Hashimoto 1971). Hashimoto and Yanagisawa (1970) explained that γ-irradiation may cause some structural changes in the membrane of the slime mold spores which led to an induction of their germination. It was observed by Levinson and Hyatt (1960) that increase in the germination of γ-irradiated *B. megaterium* spores could be due to a rise in oxygen consumption rate. Gould and Ordal (1968) proposed that the activation of spores of *Bacillus cereus* following γ-irradiation is due to some changes in the tertiary structure of protein which might expose previously masked

reactive sites which are important for germination. However, no exact mechanism of activation of akinetes following γ-rays at low levels has been established. A decline in the percentage germination of akinetes and germinating akinetes, decreased percentage survival of vegetative colony and sporulation at higher absorbed dose of γ-rays may be due to γ-induced injury to DNA of cells.

γ-Irradiated activated spores of *B. cereus* were found to be deactivated after their storage (Gould and Ordal 1968). In the present study activation of akinetes of *S. pascheri* by a lower absorbed dose of γ-rays was not transferred to vegetative cells. Similarly, no activation was observed in akinetes formed from irradiated vegetative cells. The γ-induced effect to any of the three different stages was not transferred to the subsequent stage of a generation of *S. pascheri*. It was reported by Parisi and Antoine (1977) that increased radiation resistance of spores to γ-rays in *Bacillus pumilus* was not transferred to vegetative cell progeny.

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