Effect of Diethyl Sulphate on *Rhizoclonium* Kuetz.

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dES is a well known bifunctional alkylating agents. It has an edge over monofunctional alkylating agents due to its two reactive groups that produce intra and inter sterid cross links in the DNA. Alkylation of DNA may be induced in different ways viz. by alkylation of phosphate groups, breaking the backbone of DNA by alkylating some of the bases giving rise to unstable quarternary nitrogen as a result of alkylation and by depurination of DNA. Alkylation of bases G, A, C and T occurs at 7N, 3N, 7N, 3N and 3N position, respectively.

It has been widely used to obtain a wide spectrum of variants in higher plants (Avadhani and Rao 1968, Choudhory and Dnyansagar 1980, Bose and Bose 1971, Lal 1978). But, there appears to be no literature dealing with the karyological effects of dES on any of the green algae. Therefore, an attempt has been made, in the present communication, to deal with the post-treatment effects of said mutagen on the mitotic events of *Rhizoclonium* Kuetz. with reference to recovery of normal mitotic activities.

Materials and methods

The filaments from synchronous culture of *Rhizoclonium*, were directly treated with 0.05%, 0.1% and 0.15% of dES, for 9 hrs, at pH 7 and temp. $20 \pm 2^\circ\text{C}$ in dark. The treated filaments were thoroughly washed to eliminate every traces of the mutagen used. Portion of filaments from each sample was fixed immediately after treatment and the remaining portion were allowed to grow under control condition (soil extract and Godward's Soln. in equal proportion) with a daily cycle of 14 hrs light and 10 hrs dark.

Karyological effects of the treatment were studied in the fixations made 48 hrs, 72 hrs and 168 hrs after treatment. Microscopic observations of the treated filaments in cultures were also made simultaneously at an interval of one week for a month to observe the survival and morphological variations. The untreated filaments were also grown for control under similar growth conditions and studied likewise.

Acetic acid-ethanol (1:1) was used as fixative. Ironalum-acetocarmine technique (Godward 1948) was employed for cytological preparations. The spectrum and frequency of morphological variants and irregular mitotic figures were calculated in the percentage out of the observations made on 1000 cells.

Results

The treated filaments flourished well in the culture. Apparently no indication of lethality could be marked in any of the treatments. No mitotic figures could be observed in any of the

three concentrations when fixed immediately after treatment. In the fixation made after 48 hrs, mitotic irregularities were observed, the spectrum and frequency of which are summarised in Table 1(A).

In the fixation made after 72 hrs., the spectrum of mitotic irregularities remained the same as observed after 48 hrs but at low frequency. However, the bridge was not observed in 0.1% and change in the orientation of anaphase was completely eliminated (Table 1B). In the fixation made after 168 hrs, no mitotic irregularities were observed except microunclei in 0.15%. But, the deformation of cells accompanied by the change in the diameter and number of nuclei/cell, not observed in preceding fixation were recorded at a frequency mentioned in Table 1(C).

Table 1. Spectrum of abnormalities and their frequency observed as a post-effects of dES treatment on *Rhizoclonium* Kuetz.

Obs. time after treatment	Conc. (%)	No. of nuclei per cell	Diameter of nuclei (μ m)	Lagging (%) Fig. 1	Bridge (%) Fig. 2	Changed orientation of anaphasic axis (%) Figs. 3-4	Micro-nuclei (%) Fig. 5	Deformed cells (%) Fig. 6
(A) 48 hrs.	Control	2 to 4	4.1-8.1	—	—	—	—	—
	0.05	"	"	—	—	—	—	—
	0.1	"	"	2.6	1.5	—	1.5	—
	0.15	"	"	3.5	2.5	1.0	2.0	—
(B) 72 hrs.	Control	A "	"	—	—	—	—	—
	0.05	B "	"	—	—	—	—	—
	0.10	C "	"	1.0	—	—	0.5	—
	0.15	D "	"	2.0	1.0	—	1.0	—
(C) 168 hrs.	Control	"	"	—	—	—	—	—
	0.05	"	5.5-8.3	—	—	—	—	—
	0.10	1 to 5	6-10	—	—	—	—	7
	0.15	1 to 5	6.3-10	—	—	—	0.5	10

Discussion

Alga survived in all the three concentrations i.e. from 0.05 to 0.15% showing thereby that non of them are lethal for the alga under study. In an unpublished report of Labh, 0.15% of dES has been proved lethal for *Chara*. Thus, *Rhizoclonium* appears to be more resistant to dES than *Chara* probably on account of possessing multinucleate segments and thick, stratified cell wall with outermost covering of chitinous layer. All the nuclei in multinucleate segment are not expected to be affected simultaneously and hence, lethality caused by the affected nuclei can easily be compensated through the unaffected ones. However, this generalised conclusion awaits confirmation.

Complete inhibition of mitotic division has been observed as an immediate effect of the treatment with dES. It might be due to the inhibition of mitotic precursor and/or changes in the nutrition level. Increase and decrease in the number of nuclei per cell has simultaneously been observed in the concentrations 0.1% and 0.15%. This might be due to unequal cytokinesis as reported earlier with colchicine in *Nitella missouriensis* (Turner 1970) and in *Nitella flagelliformis* (Sarma and Tripathi 1976). Increase in the diameter of nuclei and deformation of cells have been observed as a post-effect of 0.1% and 0.15%. This may be conceived as a consequence of physiological disturbances caused as a secondary effect of mitotic disorders.

Mitotic irregularities such as laggings, bridges, micronuclei and change in the orientation of anaphasic axis have been observed in the dES treated filaments as reported earlier in higher plants (Chowdhury *et al.* 1971, Bose and Maiti 1971). The highest frequency of aberrations has been noted with 0.15% but this cannot be taken as optimum concentration because of increasing trend of frequency. Further, the upper range of optimum concentration, which

Figs. 1-6. *Rhizoclonium* sp. 1, lagging of chromosomes. $\times 2400$. 2, anaphase showing bridge. $\times 2300$. 3, late anaphase showing changed orientation of axis. $\times 1650$. 4, anaphase showing two affected chromosomes with changed orientation. $\times 2300$. 5, cells showing micronuclei. $\times 1100$. 6, deformed cell. $\times 350$.

is definitely above 0.15% for *Rhizoclonium* seems to be nearer to the higher plants. The spectrum and frequency of observations appears to be dose dependent and inversely proportionate to the laps of time after treatment.

The frequency of mitotic disorders e.g. lagging, bridge, change in the orientation of anaphasic axis and formation of micronuclei appeared first after 48 hrs, declined after 72 hrs and finally eliminated after 168 hrs, except a very few examples of micronuclei. This is in

consistent with the observations of Bhatt *et al.* (1961) for ionizing radiation induced chromosomal aberrations. Prasad and Godward (1974) reported equal rate of elimination of fragments and bridges in *Phalaris canariensis* and lower rate of elimination of micronuclei. In the present investigation, elimination rate of micronuclei appears to be very low compared to fragments as evidenced from the persistence of former even after a month and elimination of the latter only between 72 to 168 hrs after treatment.

Abstract

The filaments of *Rhizoclonium kuetz* were treated with 0.05%, 0.1% and 0.15% of dES for 9 hrs at pH 7 and temp. 20°C in dark. 0.05% was found ineffective while 0.1% and 0.15% induced mitotic disorders like laggings, bridges, micronuclei and changes in the orientation of anaphasic axis. Normal nuclear division was restored between 72 hrs and 168 hrs after treatment, except indication of lethality of 0.15% by the presence of micronuclei even after a month. Deformation of cells, change in the number of nuclei per cell and increase in the diameter of nuclei were seen in 0.1% and 0.15% treated filaments after 168 hrs.

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