Induction of Somatic Mutations in Species of *Allium*

I. Effect of dES on the somatic chromosomes of *Allium cepa*

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Species of the genus *Allium* are cultivated throughout the world for their culinary importance. In the valley of Kashmir *Allium cepa* (onion), *A. sativum* (garlic) and *A. cepa* var. *viviparum* (pran) are consumed in large quantities. The use of *A. cepa* var. *viviparum* is, however, restricted to the muslims only. Both *A. cepa* var. *viviparum* and *A. sativum* are obligate apomicts (Koul and Gohil 1970, 1971) and can not be improved by the conventional hybridization techniques. The present project was undertaken to improve these species through the induction of somatic mutations. Initially, to start with, only three mutagens viz. diethyl sulphate (dES), ethyl methanesulphonate (EMS) and radiations and one polyploidising agent (colchicine) are being tried. Before treating these species with the mutagens on a large scale it was decided to study their effect on the chromosomes of these species. This was planned so as to find out whether these have any deleterious effects on the mitotic cycle and the chromosomes of these species and also to standardize the concentration of mutagen to be used as also the duration of treatment for these three species. The present paper is based on our observations on the effect of dES on the mitotic index and the somatic chromosomes of *Allium cepa*.

Material and methods

Locally available bulbs of onion were placed in small jars with their basal ends dipping in distilled water. After 72 hours, when the roots were 1 inch in length, these were transferred to jars containing dES solution of 20, 25 and 30 mM concentrations and kept there for 1, 2, 3 and 4 hours. dES solutions were prepared in de-ionized water. After each treatment the root tips were allowed to recover in distilled water for 2, 8 and 16 hours. These were then fixed in acetic alcohol (1:3) and changed to 70% ethanol after 24 hours. For all these treatments controls were also maintained for which distilled water was used. All the treatments were carried out at room temperature ($\pm 20^{\circ}\text{C}$).

Squashes were made by Feulgen technique. Root tips were hydrolysed in 1N HCL for ten minutes at 60°C . After staining with Feulgen these were squashed in 1% acetocarmine. Slides were made permanent by passing through acetic acid-n-butyl alcohol series. Six slides of each treatment were scored for data on mitotic index and chromosomal anomalies.

Results

Various concentrations of dES used had different effects on the mitotic cycle and the chromosomes of the root tip cells of *Allium cepa*. With increase in the concentration of mutagen and treatment period there was a progressive increase in mitotic anomalies. These anomalies were maximum when the root tips were kept in 30 mM dES solution for 4 hours. Various anomalies met in the treated tips were, i) extreme condensation of chromosomes, ii) stickiness, iii) chromatin bridges at anaphase and telophase and iv) formation of knob like bodies at the chromosome tips. The mitotic index declined with the increase in concentration and treatment period.

a. *20 mM concentration*: Lowest concentration of dES used (20 mM) had minimum effect on the meristematic cells. In the root tips treated with this concentration the frequency of cells at metaphase was found to be higher than those of the cells at anaphase and telophase. After 1 hour treatment and two hour recovery the frequency of cells at prophase, metaphase and anaphase was equal. However, the percentage of cells at metaphase increased with the increase in treatment period. After 4 hour treatment the number of cells at prophase almost approaches zero. The mitotic index shows a linear decline from the control value of 4.09 (Table 1).

Table 1. Mitotic index values after treatment with dES solution of 20 mM concentration

Duration of treatment	Recovery period		
	2 hours	8 hours	16 hours
1 hour	3.22	3.25	3.28
2 hours	3.21	3.23	3.25
3 hours	3.15	3.19	3.22
4 hours	3.12	3.17	3.22

Table 2. Mitotic index values after treatment with dES solution of 25 mM concentration

Duration of treatment	Recovery period		
	2 hours	8 hours	16 hours
1 hour	3.18	3.24	3.26
2 hours	3.15	3.23	3.24
3 hours	3.13	3.17	3.22
4 hours	3.06	3.13	3.15

b. *25 mM concentration*: Variations from the normal course of mitotic cycle, observed after this treatment, were more or less similar to those made on the root tips treated with 20 mM solution of dES. The frequency of cells at metaphase was higher as compared to the previous treatment and so was the frequency of cells at anaphase and telophase. A noteworthy feature of this treatment was the c-mitotic appearance of the chromosomes at metaphase. In some cells chromosome separation at metaphase was exceptionally good (Fig. 1). The frequency of dividing cells after this treatment, on the whole, was less as a result of which the values for

mitotic index are lower than those calculated after treatment with 20 mM concentration. These values are presented in Table 2.

c. 30 mM concentration: This concentration was found to have toxic effects on mitosis. On one hand mitotic index showed a sharp decline and on the other hand anomalies like, i) separation of chromatids at metaphase (Fig. 2), ii) chromatin bridges at anaphase (Fig. 3) and telophase (Fig. 4) were frequently observed. The frequency of cells at metaphase and prophase was also much lower and was accompanied by an increase in cells at anaphase and telophase. In the root tips treated with this concentration, chromosome ends in some dividing cells had small knob like structures (Fig. 5). These knobs were Feulgen positive. The mitotic index values calculated for this treatment are given in Table 3.

Figs. 1-5. Effect of various concentrations of dES on the chromosomes of *Allium cepa* (for details see text).

Table 3. Mitotic index values after treatment with dES solution of 30 mM concentration

Duration of treatment	Recovery period		
	2 hours	8 hours	16 hours
1 hour	2.79	3.01	3.29
2 hours	2.65	2.99	3.20
3 hours	2.60	2.62	3.14
4 hours	1.52	1.29	2.18

Discussion

Despite the importance of species of *Allium* in cookery and medicine, not much work has been done towards their improvement. Whatever little work is known, has been on *Allium cepa* or common onion. This species being an amphimict can be improved through conventional hybridization techniques. On the other hand species like *A. sativum* and *A. cepa* var. *viviparum* which are sexually sterile pose lot of problems as regards their improvement (Koul and Gohil 1970, 1971 and Koul, Gohil and Langer 1979). However, their propagation solely by vegetative means either through underground cloves or through the bulbils formed in their inflorescences make them good materials for improvement through somatic mutations. In these species somatic mutations, once induced, can be easily preserved and multiplied. Before the treatment of species of *Allium* by the various mutagens for this purpose, *Allium* test has been used to standardize the concentration and duration

of treatment.

Since 1938 when Levan used *Allium* test for the first time, it has been the standard test for studying the effects of various chemicals, pollutants and medicines on the chromosomes (Buiatti and Nutironchi 1969, Fiskesjo 1969, 1979, Kak, Kaul, Puri and Sawhney 1973 and Sabharwal, Gulati and Bhalla 1975).

Despite the fact that dES is a very potent mutagen, to our knowledge, little work seems to have been done to find out its effects on the *Allium* chromosomes. Our observations have shown that like many other chemicals dES also has the ability to alter the mitotic mechanism and induce chromosomal anomalies. Lal (1978) recorded chromosomal anomalies like unequal distributions, precocious separation, bridge and laggard formation during meiosis as a result of dES treatment in *Pennisetum typhoides*. The bridges, however, were found to be the result of stickiness of chromosomes.

dES has been found to be mitotoxic as the mitotic index decreases with increase in the concentration of the mutagen and the treatment period. However, the reversal of this effect after recovery in distilled water indicates that this is not a permanent change. In higher doses it was found to have more deleterious effects. It was found to arrest metaphases (c-mitotic effect with 25 mM concentration), and bring about condensation and stickiness of chromosomes resulting in the formation of chromatin bridges at anaphase and telophase.

The present work has shown that while as 30 mM concentration of dES is very high for use in the induction of mutations as it produces various chromosomal anomalies, concentrations between 20–25 mM might be useful for this purpose.

Summary

The effect of diethyl sulphate (dES), a known mutagen, on the chromosomes of *Allium cepa* has been studied. Three different concentrations of this alkylating agent were tried. While 30 mM concentration has been shown to induce various chromosomal anomalies, 20 mM concentration does not interfere much with the mitotic cycle. 25 mM concentration of this mutagen has c-mitotic effect on the chromosomes of *Allium cepa*. However, all the three concentrations lower the mitotic index.

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