SOME CYTOLOGICAL EFFECTS OF TREFLAN AND MITOMYCIN C ON ROOT TIPS OF *VICIA FABA* L.[1]

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Abstract: Root tips of *Vicia faba* were treated with treflan and mitomycin C separately. The chromosome aberrations were found in roots treated with treflan. The most common types of chromosome aberrations induced by the herbicide were chromosome bridges and acentric fragments. Mitotic indices showed that mitomycin C had a strong effect on the inhibition of mitosis. The effect of the antibiotic was reversible.

INTRODUCTION

A number of investigations (Wilson, 1950, Morrison 1962, Wuu and Grant, 1966) have shown that gene mutation and chromosome aberration can be induced by many chemical agents as well as by irradiation.

Treflan is a yellow-orange crystalline substance of a fluorinated benzene compound. It has been used as a pre-emergence herbicide to control annual weeds in cotton or vegetable fields. Bayer *et al* (1967) have reported that treflan controls weeds primarily by limiting the development of the root system.

Mitomycin C is a violet crystalline antibiotic isolated from *Streptomyces caespitosus* which has been used commonly as a drug in the treatment of cancer because of its effect of the inhibition of DNA synthesis (Merz, 1961).

The present study was conducted to obtain information about the cytological effects of treflan and mitomycin C on root tips of *Vicia faba*.

MATERIALS AND METHODS

The herbicide treflan and the antibiotic mitomycin C were used in this experiment. Seeds of *Vicia faba* were presoaked in tap water overnight, and then germinated in moist sand in plastic flats for eight days. Seedlings with lateral roots of about 3–4 cm long were washed with tap water. The seedlings were transferred and cultivated in the following solutions separately.

(1) Tap water as a check.
(2) Tap water containing mitomycin C in a concentration of 20 p. p. m.
(3) Tap water containing treflan in a concentration of 20 p. p. m.
(4) Tap water containing treflan in a concentration of 200 p. p. m.

The experiment was done in the dark at the temperature of ca. 24°C. These solutions were continuously aerated.

Both control and treflan-treated root tips were collected and fixed at following intervals; 6, 12, 24, 36, 48 and 60 hours. Mitomycin C—treated root tips were fixed at intervals of 2 and 8 hours. From the meristematic cells of the root tips, the

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chromosome aberrations were observed and the mitotic indices were determined. Farmer's fixative and the propiono-carmine squash technique were employed in this experiment.

RESULTS

The data on chromosome aberrations induced by the action of treflan on the root tips of *Vicia faba* are tabulated in Table 1.

Table 1. Frequencies of chromosome aberrations in the root tip cells of *Vicia faba* induced by the action of treflan (200 p. p. m.)

Treatment period (hours)	No. cells studied	Frequencies and types of chromosome aberrations			Total aberrations in %
		Bridges	Fragments	Ball-metaphase	
6	361	1.94	—	—	1.94
12	322	1.55	2.48	—	4.03
24	371	1.62	3.23	1.08	5.93
36	301	3.32	2.33	2.33	7.98
48	294	2.38	1.70	2.04	6.12
60	278	1.44	1.80	2.52	5.76

Only 0.57 percent of chromosome aberration (only fragments) were found in the cells of the root tips of the control. This was much lower than the percentages of the chromosome aberrations found in roots treated with treflan. It can be observed from Table 1, that there is a positive correlation between chromosome aberration percentage and treatment time up to 36 hours. However the percentages of chromosome aberrations decreased if the treatment was continued longer than 36 hours. It is believed that the use of treflan for long periods retards cells from dividing, and that is the reason that the occurrence of chromosome aberrations in root tip cells was reduced. The highest frequency of chromosome aberrations was induced by treatment for 36 hours. Among the chromosome aberrations observed, the commonest ones were chromosome bridges and chromosome fragments. Other type of abnormality such as Ball-metaphase was only observed as a result long periods of treatment.

Chromosome bridges were found in the anaphase or telophase cells in all those treated with treflan. Some of these were single bridges (Fig. 1), while others were two or more bridges. A mitotic bridge is considered as a result of a dicentric chromosome passing to opposite poles at anaphase, and the dicentric chromosome arose from the fusion of two sister chromatids of a broken chromosome (Grant and Harney, 1960).

The acentric fragments (Figs. 2 and 3) were produced in all treflan treated roots except when only treated for 6 hours. The acentric fragment originated in a broken chromosome. The broken ends of the acentric portion are healed and are no longer capable of restitution (Lea, 1955).

Ball-metaphase (Fig. 4) in which the metaphase chromosomes formed a clotting, noted by D'Amato (1948) was also observed in the mitotic cells treated with treflan for longer durations (eg. 24 hr, 36 hr, 48 hr and 60 hr). By shortening the treatment

periods to 6 or 12 hours, mitotic cells did not produce Ball-metaphase aberrations. The percentages of Ball-metaphase induced by treflan increased with an increase in the length of the treatment periods. The maximal occurrence of it was found at the 60 hours' treflan treatment (2.52 percent). According to D'Amato (1948), this phenomenon is due to a poisonous effect. Further development of these Ball-metaphase is rather unlikely. Therefore, it will have little genetic significance.

The chromosomes of some root tip cells which were treated for 60 hours seemed to be polyploids. This being due to the inhitibition or delay of cell wall formation.

Data concerning each phase of mitosis in root meristematic cells of *Vicia faba* cultivated in tap water, in treflan solutions and in mitomycin C solutions are presented in Table 2.

Table 2. Phase of mitosis and mitotic indices in *Vicia faba* root tips in tap water, in treflan solutions and in mitomycin C solutions

Treatment	Concentration (p. p. m.)	Time period (hours)	Phases per 1,000 cells				Mitotic index (‰)
			Prophase	Metaphase	Anaphase	Telophase	
Control	0	0	57.3	16.2	12.8	14.1	100.4
		6	63.4	14.2	14.8	13.7	106.1
		12	50.1	15.3	17.0	15.7	98.0
Treflan	20	6	39.1	16.1	18.0	14.7	87.9
		12	31.0	11.2	12.5	13.3	68.0
Mitomycin C	20	2	29.7	9.8	13.4	10.6	63.5
		8	3.1	1.6	0.5	—	5.2

Mitotic indices were scored from counts made on carmine squash preparations; about 4,000 root meristematic cells were counted for each sample. From the information presented in Table 2. We see that seedling roots of *Vicia faba* cultivated in tap water for 0, 6 and 12 hours showed no significant difference in mitotic activity. Seedling roots of *Vicia faba* cultivated in 20 p. p. m. treflan solution for 6 and 12 hours had lower mitotic indices than the control. However, the mitotic indices in the treflan-treated roots were higher than that in mitomycin C—treated roots. The mitotic index in 2 hours' 20 p. p. m. mitomycin C treatment is 63.5‰. This value is markedly reduced compared with that in controls. When the treatment duration increased from 2 to 8 hours, the mitotic index in the mitomycin C—treated roots fell rapidly to 5.2‰. These results show that mitomycin C—treatment is much more effective in preventing mitosis than the treflan-treatment, and that the degree of inhibition to cell division increased with an increase in the length of the treatment periods. Figures 5 and 6, are photomicrographs of squash preparations of control and mitomycin C—treated root tip. Figure 5, the control, shows a number of mitotic cells distributed normally in the microscopic field. While in Figure 6, which shows mitomycin C—treated root tip squash preparations, No mitotic cells are visible. All of these cells appear to be at interphase. Since mitomycin C has a strong inhibitory effect on mitosis, the root tips treated with this antibiotic did not show any cells with chromosome abnormality.

After 8 hours of cultivation in tap water with mitomycin C at a concentration of 20 p. p. m., the roots of *Vicia faba* were thoroughly rinsed and grown in fresh water free from mitomycin C, and 24 hours later the root tips were fixed for obser-

vation. The number of individual phases of mitosis per thousand cells were as follows; 35.3 (prophase), 18.7 (metaphase), 15.5 (anaphase) and 11.8 (telophase). The mitotic index rose from 5.2 to 81.3. The results indicate that 8 hours of treatment with 20 p. p. m. mitomycin C caused a reversible inhibition to cell division.

DISCUSSION

From this study, we know that the herbicide treflan is capable of inducing chromosome aberrations in the root tip cells. The most common type of chromosome aberrations observed are chromosomes with bridge and acentric fragments. Both of these originated from chromosome breakage. The mechanism of chromosome breakage with treflan appears to be similar to that which has been reported for irradiations and other chimical mutagens (Thoday, 1953). The suggestion has often been made that the radiomimetic effect of chemicals may destroy or disturb the synthesis of certain enzymes which catalyze the numerous biochemical reactions within a living cell, and so upset the cell system thus chromosome breakage is one of the possible effects.

Lea (1955) estimated that only about one out of twenty of the original breaks actually remained to metaphase, at which time the broken ends were healed and no longer capable of uniting with other broken ends to produce structure aberrations. Therefore we believe that many more chromosome breaks are actually produced than are observed, and that their number is reduced by restitution.

The formation of anaphase bridge resulted from a dicentric chromosome passing to opposite poles at anaphase, and the dicentric chromosome arose by the fusion of the two sister chromatids of a broken chromosome (Grant and Harney, 1960). However, the breakage and subsequent reunion of the chromatids is assumed to take place at the time of chromosome duplication under exposure to action of any kind of mutagenic agent (Howard and Pelc, 1952). This indicates that the herbicide treflan acts on chromosomes during interphase, since we know that DNA replication occurs at this stage.

In the present study, it was found that the proper concentration of mitomycin C caused a reversible inhibition to cell division. Therefore, this inhibitory effect might arise from the alteration of gene activity induced by this antibiotic; and probably did not result from genetic reasons, since the inhibition was released and cell division started again in a normal way after the roots were removed and grown in a fresh water without mitomycin C.

Treflan for use in weed control in the fields is generally recommended at 0.75 lb of active ingredient per acre. This dosage produces a solution containing 3,000 p. p. m. of active ingredient which is much higher than that used in this experiment. The dosage of mitomycin C used in medicine is also higher than that used in this experiment.

From a number of studies, it is becoming clear that the antibiotics, herbicides and other pesticides are probably all potential chemical mutagens. The wide spread use of these compounds in agriculture and medicine may produce harmful genetic consequences. Fortunately in most cases, this potential for genetic change is too little to be of much concern. This is partly due to the fact that the mutagenic and lethal thresholds are often close together. Moreover, selection at the level of cellular reproduction is likely to screen out the altered nuclei and will not provide an op-

portunity to perpetuate the altered nuclei. This case was also proved by Wuu and Grant (1966), they reported that the transmission of chromosome abnormalities in pesticide-treated root tip cells of barley was very low and never exceeded 2 percent. However, it should be noted that high dosages of these chemical compounds are effective in inducing genetic changes.

CONCLUSIONS

1. The effect of mitomycin C was stronger than that of treflan in inhibition to root tip mitosis of *Vicia faba*. A contrary result was found in inducing chromosome aberrations.
2. The most common types of chromosome aberrations observed in induced root tip cells by treflan were chromosome bridges and acentric fragments. Both of these originated from chromosome breakages.
3. The percentage of chromosome aberrations induced in root tip cells by treflan ranged from 1.94 percent to 7.98 percent. A positive correlation existed between chromosome aberration frequency and treatment duration up to 36 hours' 200 p. p. m. treflan treatment. However the percentage of chromosome aberrations decreased with an increase in treatment duration from 36 to 60 hours.
4. At 8 hours, the mitotic index in the mitomycin C—treated roots fell rapidly to 5.2‰. This result showed that mitomycin C had a strong effect on the inhibition of mitosis, and that the degree of inhibition to cell division increased with an increase in the length of the treatment periods.
5. After eight hours of cultivation in 20 p. p. m. mitomycin C, the roots of *Vicia faba* were removed to fresh water, and 24 hours later, the mitotic index of these roots rose from 5.2‰ to 81.3‰. This information suggests that the effects of the antibiotic were reversible.
6. The cells with chromosome aberrations are usually screened out, and the transmission of these abnormalities to the next generation is very low, therefore the genetic changes induced by these chemical compounds (treflan and mitomycin C) are too little to be of significance. However high dosages of these chemicals are effective in inducing genetic changes.

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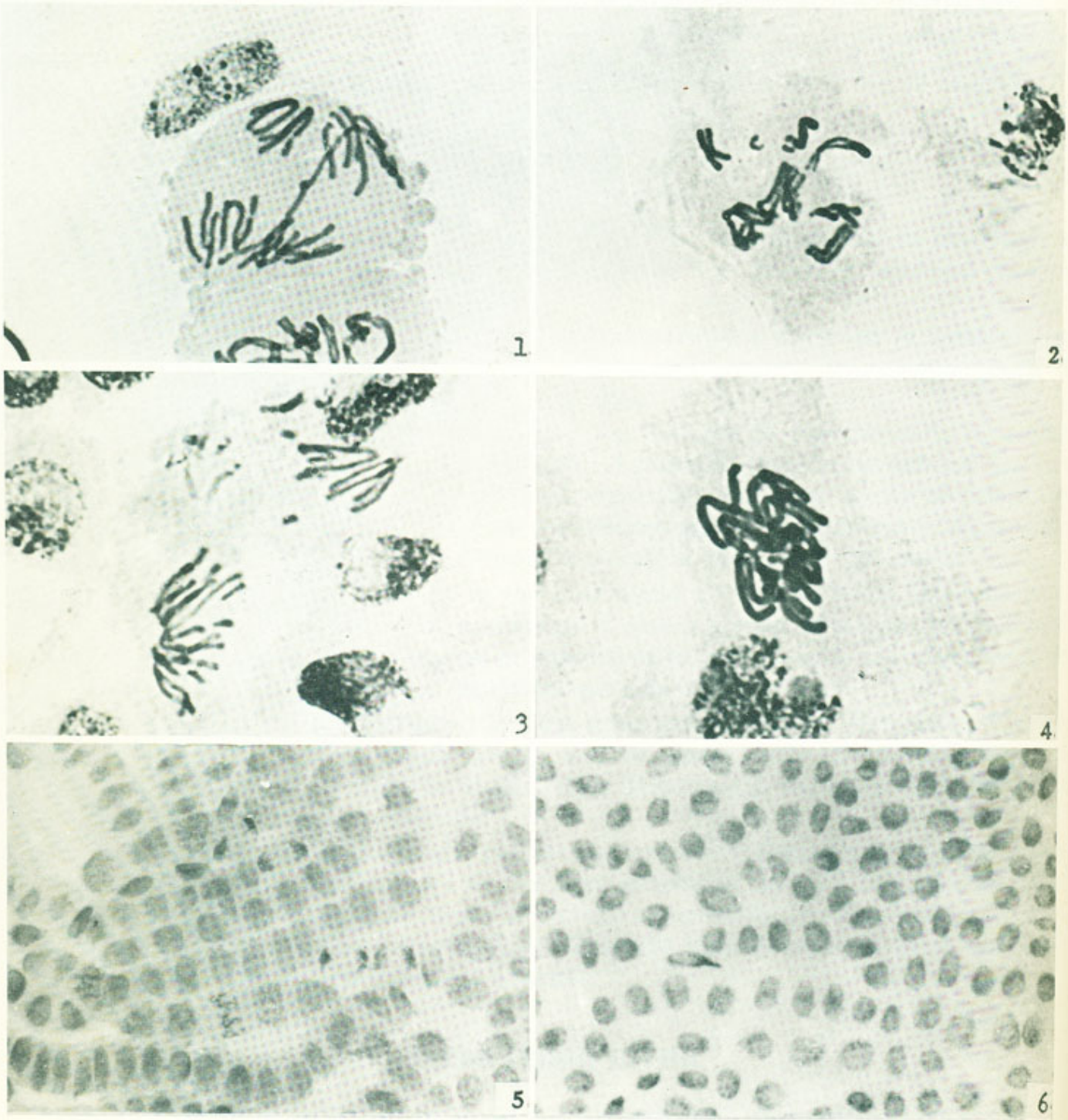

Fig. 1. Anaphase showing a single bridge.
Fig. 2. Metaphase with an acentric fragment.
Fig. 3. Anaphase with acentric fragments.
Fig. 4. Ball-metaphase.
Fig. 5. Control root tip squash preparation.
Fig. 6. Mitomycin C—treated root tip squash preparation.