



EFFECTS INDUCED BY CHROMIUM TRIOXIDE ON ROOT TIP CHROMOSOMES OF *Vicia faba*

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ABSTRACT

The cytogenetic effects of chromium trioxide (CrO_3) in meristematic root tips of *Vicia faba* were evaluated by the analysis of chromosomal alterations in anaphase cells. The CrO_3 concentrations of 0.001, 0.01, 0.05, 0.10, 0.50, 1.0, 1.5, 2.0, 2.5, and 3.0% were applied for 1, 2 and 3 hours without recovery. Longer exposures damaged the tissues. This substance induced chromosomal aberrations and also produced injury in the centromere, leading to chromosomes with centromere inactivated and isochromosomes as well as alterations of the mitotic spindle causing multipolar anaphases and C-metaphases. No relation in concentration-effect was found because the highest frequencies of abnormal anaphases, total aberrations and multipolar anaphases were noted at 0.01% of CrO_3 , which decreased at higher concentrations. This behavior was possibly due to the fact that the absorbance of high concentrations of chromium by the roots, impregnated its cell walls therefore avoiding the entrance of more chromium. When colchicine was applied simultaneously to the treatment, the higher CrO_3 concentration the lower number of C-metaphases in relation to anaphases, thus at low CrO_3 concentrations the colchicine uptake was higher.

RESUMEN

Se evaluó el efecto citogenético del trióxido de cromo (CrO_3) en las células meristemáticas de la raíz de *Vicia faba* mediante el análisis de alteraciones cromosómicas en anafase. Las concentraciones de 0.001, 0.01, 0.05, 0.10, 0.50, 1.0, 1.5, 2.0, 2.5 y 3.0%, fueron aplicadas por 1, 2 y 3 horas sin recuperación, al exponerse a tiempos más largos se produjo deterioro del tejido. Además de la inducción de aberraciones cromosómicas, el CrO_3 también produjo daño al centrómero, manifestado por los cromosomas con el centrómero inactivado y por los isocromosomas, así como alteraciones al huso mitótico expresado por las anafases multipolares y el efecto C-mitótico. En ningún caso se observó relación de concentración-efecto, ya que la frecuencia mayor tanto para anafases anormales como para aberraciones totales y anafases multipolares se observó en 0.01% para posteriormente disminuir. Este comportamiento posiblemente se debió a que el cromo al ser absorbido por las raíces, va impregnando las paredes celulares impidiendo que pueda seguir penetrando. Al aplicar colchicina simultáneamente al tratamiento se nota que a mayor concentración de CrO_3 , menor fue el número de C-metafases encontradas con relación a las anafases, lo que implicó que a las concentraciones bajas de CrO_3 la entrada de colchicina fuera mayor.

INTRODUCTION

Pollution produced by industry in water, soil and air is increasing in every country, damaging organism, sometimes to a very great extent. One of these important contaminants that produces health risks is chromium (see review of Villalobos-Pietrini 1977). The main sources of chromium pollution are chromate-producing factories, metallurgical industries, chrome plating, and the burning of coal (Sullivan 1969).

Plants growing in soils having rocks containing heavy metals, like those treated experimentally with chromium, show disorders such as reduction of leaf size, small necrotic areas and chlorosis (Hunter and Vergnano 1953, Anderson *et al.* 1973).

Some metallic cations, such as chromium trioxide and chromic chloride, diminish DNA synthesis and raise the frequencies of errors when non-complementary deoxynucleotides are incorporated (Sirover and Loeb 1976). The same effect has been observed in bacteria in which the repair mechanisms are also altered (Petrilli and De Flora 1977).

Compounds of hexavalent chromium are irritants and corrosives, possibly due to their capacity as oxidants or to their properties as heavy metal (Baetjer 1956). When chromium trioxide was administered to pregnant hamsters, embryotoxic effects such as cleft palate and fetus resorptions were observed (Gale and Bunch 1979). A dose-response relationship was found when chromium trioxide and potassium dichromate produced chromosomal aberrations in mice (Umeda and Nishimura 1979). In *Vicia faba*, potassium dichromate and calcium chromate induced chromosomal alterations (Gómez-Arroyo and Villalobos-Pietrini 1983). In this work, the effects of chromium trioxide on *Vicia faba* chromosomes are pointed out and are compared with those produced by potassium dichromate and calcium chromate on the same biological material.

MATERIAL AND METHODS

Seeds of *Vicia faba* (Var. major, series C-18-10) were supplied by the Centro de Investigaciones para el Desarrollo Agrícola y Ganadero del Estado de México, México. They were washed with tap water for 2 hours, and soaked for 24 hours. Afterwards they were set between two cotton wool layers in the dark at a constant temperature of $21 \pm 1^\circ\text{C}$. When radicles appeared, the testa were removed to avoid fungal contamination. Treatments were made to primary roots sized 3-4 cm.

Concentrations of 0.001, 0.01, 0.05, 0.10, 0.50, 1.0, 1.5, 2.0, 2.5 and 3.0% of chromium trioxide (Merck) were applied for 1, 2 and 3 hours without recovery. Concentrations and exposure times were selected from preliminary experiments.

The solutions of chromium trioxide were put in containers covered with aluminium paper with holes which permitted the roots to be exposed. Each treatment was applied to 5 seedling roots. The experiments were in the dark. Each treatment time had a control in which roots were submerged in distilled water the same handling as exposed plants.

About 2 mm of the root tips were cut and subjected to the acetorcein technique (Villalobos-Pietrini 1965). Slides were made permanent with Conger and Fairchild's method (1953).

The damage produced by chromium trioxide was scored as chromosomal aberrations in anaphase cells. For each treatment time, 10 slides were analyzed. That is 30 for each concentration.

Due to the fact that the increase of concentrations produced blackness in the roots, the treatments with the various concentrations of CrO_3 and colchicine 0.05% were not longer than 3 hours. The scope of that was to detect the penetration of the colchicine by means of the C-metaphases induced. The controls were exposed only to colchicine for the same 3 hours. The technique to make the slides permanent was described above.

RESULTS

The effects of chromium trioxide on meristematic root tip cells of *Vicia faba* were evaluated by the analysis of chromosomal aberrations in anaphase as fragments and bridges with and without fragments and other alterations like chromosomes with inactivated centromere, isochromosomes and multipolar anaphases.

In all concentrations, longer treatments harden the tissues in such a way as to make squash procedure very difficult and even when root tip squashes were obtained it was not possible to examine the cells under the microscope because a monolayer was not obtained. The tissue also contracted when the slides were dehydrated and the coverslip unfasten.

Table I shows the results obtained grouping the data of concentrations of CrO_3 applied for 1, 2 and 3 hours. The alterations were not consistent with the different concentration used. The higher frequencies were obtained with 0.01%; decreasing when chromium concentration increased, however the number of anaphases did not diminished.

TABLE I. ALTERATIONS (-CONTROL) INDUCED BY CHROMIUM TRIOXIDE (CrO_3) DURING 1, 2 AND 3 HOURS OF TREATMENT ON *Vicia faba* CHROMOSOMES

Concentration %	Total anaphases	% Abnormal anaphases	% Total aberrations	% Chromosomes with inactivated centromere	% Iso- chromosomes	% Multipolar anaphases
0.001	8054	1.82	1.27	1.21	0.31	0.40
0.01	4492	25.40	45.81	11.01	0.31	6.55
0.05	2456	15.28	19.98	12.26	0.48	0.96
0.10	1905	17.06	16.02	3.40	2.58	0.13
0.50	5656	8.57	11.98	2.35	1.23	0.05
1.00	9072	8.16	12.46	1.00	0.37	0.03
1.50	8022	1.78	3.50	0.08	0.10	0.07
2.00	6181	4.57	5.00	1.02	0.72	0
2.50	7272	2.56	3.63	0.09	0.36	0
3.00	4941	4.60	2.71	3.61	0.29	0.04

When the treatments of CrO_3 were combined with colchicine (0.05%), the number of C-metaphases with CrO_3 0.01% were similar to the control; meanwhile with the higher concentrations, it diminished progressively to the point of its disappearance.

DISCUSSION

Chromosomal alteration induced by physical and chemical agents can be analyzed in metaphase and/or anaphase periods. For this work the latter was chosen because of the interest in scoring effects in the centromeric region of the chromosomes as is the case of the chromosomes with inactivated centromere and the isochromosomes (Gómez-Arroyo and Villalobos-Pietrini 1983, Gómez-Arroyo *et al.* 1985, 1986) and the multipolar anaphases produced by spindle disturbances (Gómez-Arroyo *et al.* 1986).

The chromosomal aberrations described by Gómez-Arroyo and Villalobos-Pietrini (1983) with short treatments (one, two and three hours) of potassium dichromate and calcium chromate in *Vicia faba* were also observed in this work with short treatments of chromium trioxide.

CrO_3 and CrCl_2 decreased the fidelity of DNA synthesis *in vitro*, increasing the error frequency of deoxynucleotide incorporation (Sirover and Loeb 1976). Calcium chromate induced errors in recovery mechanisms in *Salmonella* (Petrilli and De Flora 1982). On the other hand it was shown that the concentration of metals with carcinogenic and mutagenic activity, decreased DNA polymerase activity and increased errors that incorporate deoxynucleotides (Miyaki *et al.* 1977). But dichromate ion represents a special case because high concentration inhibited DNA polymerase without altering deoxynucleotide incorporation and has also been described as carcinogenic and mutagenic (Miyaki *et al.* 1977). All concentrations of applied CrO_3 produced centromere alterations resulting in chromosomes with inactivated centromeres and isochromosomes. The latter were due to the transverse division of the centromere. The same behavior was noted by Gómez-Arroyo and Villalobos-Pietrini (1983) with potassium dichromate and calcium chromate. In general the damage to non-centromeric portions of the chromosomes was always higher than to centromere region.

One of the consequences of spindle disturbances is multipolar anaphases (Gómez-Arroyo *et al.* 1986). This was observed in all concentrations applied with the exception of 2.0 and 2.5%. The integration of more than two groups of polarized chromosomes results in the reduction of the chromosomal number in each one (Levan 1938).

Roots treated with 0.01 and 0.05% chromium trioxide showed C-mitotic effects, similar results to those obtained by Levan (1945) in *Allium cepa*. C-metaphases were produced by the inactivation of the mitotic apparatus concomitant to the delay of the centromere division (Levan 1938). Heavy metals had great affinity to the sulphhydryl groups (Bacq 1946), inducing great changes in the process of cell interchange (Oehlkers 1949). The binding of chromium to sulphhydryl groups changed the spindle formation in such a way that the chromosomes did not reach the equatorial plate thus producing C-metaphases, a similar mechanism to that proposed by Carbajal (1980) to explain the cadmium C-mitotic effect on *Eichhornia crassipes* roots (Rosas *et al.* 1984).

It has been observed that chromium produces coagulability of cytoplasmic proteins in plants (Mukherji and Kumar 1978), CrO_3 improves the wathering resistance of wood to degradation by UV irradiation, it also improves the durability of stains and paints (Feist and Ellis 1978). The bleaching of cells was also observed, because chromic acid interferes with acetic staining (Darlington and La Cour 1962), so distilled water was used in the squash procedure instead of acetic acid.

Chromium also reacted with proteins (Grogan and Oppenheimer 1955) binding the carboxyl groups of the peptide chains and forming complexes with proteins (Gustavson 1958). Therefore, when chromium bonds to tubulin molecules, it changed their structure and produced C-metaphases. There was no concentration response relationship of multipolar anaphases or C-metaphases, since lower concentrations showed the greatest frequencies probably due to the fact that higher concentrations produced drastic cytotoxic effects leading to cell death, thereby preventing the expression of the damage.

Although hexavalent chromium compounds were mutagenic and toxic, depending on the concentration, in studies run on *Vicia faba* treated with chromium, Gläss (1956) found that the highest concentration did not produce the highest frequency of aberrations which was in agreement with the effects of CrO_3 in this work. However, it was found that the higher concentration of potassium dichromate the higher frequency of aberrations in *Vicia faba* root tips (Gómez-Arroyo and Villalobos-Pietrini 1983).

This behavior stimulated the decision to use a marker which permitted the entrance of CrO_3 to the cells to be checked. Because of this, colchicine was applied simultaneously to CrO_3 treatments, scoring C-metaphases for evaluation. The highest number of C-metaphases was produced by the low concentrations (similar to control). That means, when higher concentrations of CrO_3 were taken in, the entrance of CrO_3 started being blocked, as well as the colchicine, giving as a result low frequencies of chromosomal alteration on one hand and low C-metaphases compared with the number of anaphases on the other.

The effectiveness of some chromium compounds in producing sister chromatid exchanges in cultured human lymphocytes was as follows: $\text{CaCrO}_4 > \text{CrO}_3 > \text{K}_2\text{Cr}_2\text{O}_7$ (Gómez-Arroyo *et al.* 1981). Other descriptions show that PbCrO_4 produced sister chromatid exchanges and chromosomal aberrations in cultured human lymphocytes (Douglas *et al.* 1980).

The production of chromosomal aberrations by chromium may be due to its interaction with nucleic acids and nucleoproteins, affecting their physico-chemical properties (Herrmann and Speck 1954, Fuwa *et al.* 1960) and by the iron and other metals substituted in essential compounds (Hewith 1953, DeKock 1956) or in nucleic acids (Sirover and Loeb 1976, Petrilli and De Flora 1977).

The carcinogenic activity of chromium is allotted to epoxyaldehydes derived from the lipidic tissues, hydrolized by lipases, liberated from the liposomes when cells were damaged by irritants and oxidant compounds as the hexavalent chromium (Schoental 1975).

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