Journal of Zhejiang University SCIENCE
ISSN 1009-3095
http://www.zju.edu.cn/jzus
E-mail: jzus@zju.edu.cn

# Mutagenic effects of chromium trioxide on root tip cells of *Vicia faba*[*]

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Received Feb. 19, 2004; revision accepted July 1, 2004

**Abstract:** In this study on the mutagenic effects of different concentrations of chromium trioxide ($CrO_3$) on *Vicia faba* root tip, micronucleus assay and chromosome aberration assay were used to determine the mitotic indexes, micronucleus rate and chromosome aberration rate of *Vicia faba* root tip cells. The results showed that the effects of $CrO_3$ concentration on the mitotic indexes were complicated. $CrO_3$ increases the micronucleus rate of *Vicia faba* root tip cells. It was found that within certain range of $CrO_3$ concentration the micronucleus rate increased systematically with increased concentration of $CrO_3$, but that the micronucleus rate decreased at higher level of $CrO_3$ and that $CrO_3$ also caused various types of chromosome aberration at a rate which increased systematically with increased concentration of $CrO_3$. We concluded that $CrO_3$ has significant mutagenic effect on *Vicia faba* root tip cells.

**Key words:** Chromium trioxide ($CrO_3$), *Vicia faba*, Mitotic index, Micronucleus rate, Rate of chromosome aberration
**doi:**10.1631/jzus.2004.1570 **Document code:** A **CLC number:** Q943

## INTRODUCTION

Chromium trioxide, as an analytical reagent and oxidant, is widely applied in chromate manufacture, electroplating, printing, tanning and fabric mordant dyeing, and many other industries. The environmental contamination of chromium is increasingly serious nowadays. The harmful effects of chromium compounds (especially hexavalent chromium compounds) on human health had been reported in China and abroad (Liu *et al*., 1991; Li *et al*., 1997), and soluble hexavalent chromium compounds are considered as carcinogens to human lung (Sorahan *et al*., 1998). Previous studies showed that potassium dichromate and sodium dichromate could induce significant increase of micronucleus rate for bone marrow polychromatophilic erythrocyte (PCE) in rodents (Balansky *et al*., 2000). But reports on the effect of hexavalent chromium compounds on plant cells have not been found by the authors so far. In recent years, the micronucleus assay and chromosome aberration assay of *Vicbia faba* root tip had been successfully applied in detection of environmental mutagens and studies of mutagenesis (Dai *et al*., 1995). In this work, we studied the effect of different concentration of chromium trioxide on the mitotic index, micronucleus rate and chromosome aberration rate in *Vicia faba* root tip cells, with the aim of gaining reliable experimental data and theoretical criteria to elucidate the aberration effects caused by chromium compound.

[*] Project supported by Wenzhou Technology Bureau (No. S2002A015) and Wenzhou "551" Talented People Fund of China

## MATERIALS AND METHODS

**Materials**

*Vicia faba* L. seeds (collected from Dongtou of Zhejiang Province, an island county in the East China Sea), and chromium trioxide (produced by Pujing Chemical Engineering Factory, Shanghai, at concentrations of 6.25 mg/L, 12.50 mg/L, 25.00 mg/L, 37.50 mg/L and 50.00 mg/L in distilled water).

**Methods**

Plump and even sized *Vicia faba* seeds were soaked in distilled water for 1 day, then cultured at 23 °C in incubator. When the roots grew to 1–2 cm long, they were treated with distilled water, and 6.25 mg/L, 12.50 mg/L, 25.00 mg/L, 37.50 mg/L and 50.00 mg/L of chromium trioxide solution for 4 h respectively (Table 1); then cultured again for 24 h. The excised root tips were fixed in Carnoy's fluid (anhydrous alcohol:glacial acetic acid=3:1 v/v) for 24 h, and kept in 70% alcohol in refrigerator at 4 °C. These samples were sectioned routinely and stained with modified carbol fuchsin. The cell mitotic index, micronucleus permillage and chromosome aberration percentage were examined and counted on squashes microscopically, and the aberrant cells were microphotographed (Qian, 1998). The experimental grouping and treatment is presented in Table 1 in detail.

Each treatment as described above was repeated at least three times; analysis of variance used SPSS software (SPSS, 10.0 version).

## RESULTS

**The mitotic indexes as affected by chromium trioxide**

As shown in Table 2, the mitotic indexes of Groups C and F were very significantly different ($P<0.001$) from the index of Group A (control). Group E's mitotic index was higher than that of the control ($P<0.05$); while the differences between Groups B, D and A were not so significant. These results revealed that the effects of chromium trioxide on the mitotic index of *Vicia faba* root tip cells depend on their concentrations.

**The micronucleus rates of *Vicia faba* root tip cells**

As revealed in Table 3, chromium trioxide can increase the micronucleus rates of *Vicia faba* root tip

**Table 1 Grouping and treatment of the *Vicia faba* root tips**

| Group | Number of root tip ($n$) | Concentration of $CrO_3$ (mg/L) |
|---|---|---|
| A (control) | 12 | 0 |
| B | 12 | 6.25 |
| C | 12 | 12.50 |
| D | 12 | 25.00 |
| E | 12 | 37.50 |
| F | 12 | 50.00 |

**Table 2 The effects of chromium trioxide on mitotic index of *Vicia faba* root tip cells**

| Group | Number of root tip ($n$) | Cell number observed in each root tip | Mitotic index for each root tip % ($x$) | Mitotic index % ($\bar{X} \pm s$) |
|---|---|---|---|---|
| A (control) | 12 | 1000 | 3.0 5.0 3.3 3.6 4.0 3.1<br>3.2 3.5 3.8 4.1 4.5 4.3 | 3.78±0.62 |
| B | 12 | 1000 | 4.1 3.9 3.6 4.2 4.3 3.8<br>3.9 4.5 4.4 4.1 4.5 3.7 | 4.08±0.31 |
| C | 12 | 1000 | 4.8 4.8 4.9 5.0 4.7 5.2<br>5.5 4.9 5.0 5.3 5.1 5.0 | 5.02±0.23*** |
| D | 12 | 1000 | 4.5 3.3 3.8 3.1 3.4 3.6<br>3.5 3.7 3.4 3.9 4.0 3.2 | 3.62±0.39 |
| E | 12 | 1000 | 4.6 4.7 4.2 4.5 4.1 3.9<br>3.8 3.8 3.7 4.4 4.2 4.3 | 4.18±0.33* |
| F | 12 | 1000 | 5.8 6.4 4.1 4.3 5.3 5.2<br>5.0 4.8 5.6 5.3 5.4 4.9 | 5.18±0.63*** |

Note: the other groups compared with Group A, *$P<0.05$, ***$P<0.001$

cells. Within certain range of the chromium trioxide concentration, the micronucleus rate increased with chromium trioxide concentration, culminating at (8.75±1.22)‰ in Group C. Thereafter it decreased with chromium trioxide concentration, especially in Group D.

**The effects of chromium trioxide on the chromosome aberration rate of *Vicia faba* root tip cells**

Table 4 data show that chromium trioxide can strongly induce the chromosome aberration of *Vicia faba* root tip cells; and that there is obvious dose-effect relationship between the aberration rate and chromium trioxide. Within the experimental range of concentrations, the chromosome aberration rate increased with increase of concentration, and the differences among groups were very obvious ($P$<0.001).

**The effect of chromium trioxide on the mitosis of meristem cells in *Vicia faba* root tip**

Microscopic examination of the squashes of *Vicia faba* root tip meristem cells showed that the abnormal phenomena occur through their prophase, metaphase, anaphase and telophase in the mitotic cycle.

**Table 3 The effect of chromium trioxide on micronucleus rate of *Vicia faba* root tip cells**

| Group | Number of root tip ($n$) | Number of interphase cell observed in each root tip | Number of cell with micronucleus per root tip | Mironucleus aberration rate ‰ ($\bar{X} \pm s$) |
|---|---|---|---|---|
| A (control) | 12 | 1000 | 3 2 2 2 1 0<br>1 2 2 3 0 2 | 1.67±0.98 |
| B | 12 | 1000 | 6 7 5 4 6 5<br>5 6 7 7 6 8 | 6.08±1.16*** |
| C | 12 | 1000 | 8 7 9 10 8 9<br>10 9 11 7 8 9 | 8.75±1.22*** |
| D | 12 | 1000 | 2 2 3 2 3 4<br>3 2 2 3 4 2 | 2.67±0.78* |
| E | 12 | 1000 | 3 4 3 2 3 2<br>3 4 3 3 3 4 | 3.08±0.67** |
| F | 12 | 1000 | 1 4 3 5 3 3<br>4 5 2 2 2 3 | 3.17±1.11** |

Note: the other groups compared with Group A, *$P$<0.05, **$P$<0.01, ***$P$<0.001

**Table 4 The effects of chromium trioxide on chromosome aberration rate of *Vicia faba* root tip cells**

| Group | Number of root tips ($n$) | Number of miosis cell observed in each root tip | Number of cells with chromosome aberration | chromosome aberration rate % ($\bar{X} \pm s$) |
|---|---|---|---|---|
| A (control) | 12 | 400 | 8 5 8 7 6 6<br>7 8 9 7 8 6 | 1.77±0.29 |
| B | 12 | 400 | 32 35 36 40 37 33<br>38 39 34 35 41 38 | 9.13±0.70 |
| C | 12 | 400 | 72 64 87 89 70 78<br>81 70 85 74 77 69 | 19.08±1.97*** |
| D | 12 | 400 | 95 99 107 99 97 100<br>110 90 95 98 101 98 | 24.77±1.32*** |
| E | 12 | 400 | 101 105 110 99 103 108<br>109 105 98 111 102 106 | 26.19±1.07 |
| F | 12 | 400 | 116 107 126 142 125 121<br>130 125 117 118 128 118 | 30.69±2.19*** |

Note: the other groups compared with Group A, ***$P$<0.001

Prophase: The abnormal phenomena in prophase were mainly the occurrence of micronuclei, and fragments (Figs.1a and 1b). The micronuclei and the fragments were the products of the damaged or abnormally acting chromosomes during the last mitotic divisions respectively.

Metaphase: The abnormal phenomena in metaphase were mainly in four aspects. Firstly, the occurrence of micronuclei (Fig.1c). Secondly, the appearance of chromosome fragmentation (Fig.1d). Thirdly, individual chromosomes could not reach the equatorial planes due to their abnormal activities (Fig.1e). Fourthly, the chromosomes arrangement in groups on equatorial planes resulted from the abnormal activities in chromosomes (Figs.1f, 1g and 1h) and multi-polar phenomena in these kinds of cells in anaphase will appear there.

Anaphase: In comparison with normal mitotic division, the serious unsynchronized movements to two poles among the chromosome in anaphase leads to the lag in some of the chromosomes, the formation of breakage-fusion bridges and fragments, as well as the unequal multi-polar movement and distribution.

Chromosomal lag phenomena: Most of the chromosomes moved to both poles normally, only individual chromosomes or chromosomal fragments remained between two poles (Fig.1i). This reflects that individual chromosomes had moving speed and process differing from those of the others.

The chromosomal bridge: The chromosomal bridge, one of the main features of abnormal cell division and chromosomal aberration, results from the formation of dicentric and acentric chromosomal fragments from refusion. This accords with our observation that the formation of chromosomal bridges is accompanied by the occurrence of the chromosomal fragments (Fig.1j).

The multi-polar distribution of chromosomes: In the anaphase of a normal mitosis, the chromosomes move equally to the opposite two poles with the traction of the spindle fibers. However abnormal four-pole unequal distribution was seen in the *Vicia faba* root tip cells which had been treated with chromium trioxide. Furthermore, the individual chromosomes were found to be drifting among the poles in multi-polar-distribution (Fig.1k).

Telophase: The abnormalities in telophase mainly include the micronuclei, chromosomal fragments, lagging chromosomes, the chromosomal bridges, etc.

The formation of micronuclei: Fig.1l shows that the micronuclei probably formed in the last mitotic division resulting from the unsychronized uncoiling in chromosomes. While Fig.1m reveals micronuclei formed from chromosomal fragments, individual chromosome, or a few chromosomes, which were discarded out of the major nuclei in telophase due to the damage or abnormal activities of chromosomes.

Chromosomal loops: The damage or abnormal activities of individual chromosome lead to chromosomal loops. It is generally believed that the loop-like structures form at the adhesive ends of the damaged telomeres (Fig.1n). In addition, the chromosomal fragments and chromosomal lag also occur in anaphase (Figs.1o and 1p).

Chromosomal bridges: The chromosomal bridges produced in telophase are two kinds, mono-bridges or bi-bridges (Figs.1q, 1r and 1s).

Unsychronized uncoiling in chromosomes: The telophase is actually a rebuilding process of daughter nuclei. The chromosomes begin to uncoil as soon as they reach both poles. Due to the abnormal activities in individual chromosome, unsynchronized uncoiling occurs, in that most of the chromosomes have been uncoiled and form chromatins, while a few still exist in the form of chromosomes (Fig.1t).

## DISCUSSION

In the industrialized society, the contamination of heavy metals has come more and more serious because of the development and exploitation of the mineral resources. The harmful effects of chromium compounds (especially hexavalent chromium compounds) on human health, such as the erosive action of chromic acid mist on noses, throats, pharynxes and skins, as well as the increased in-

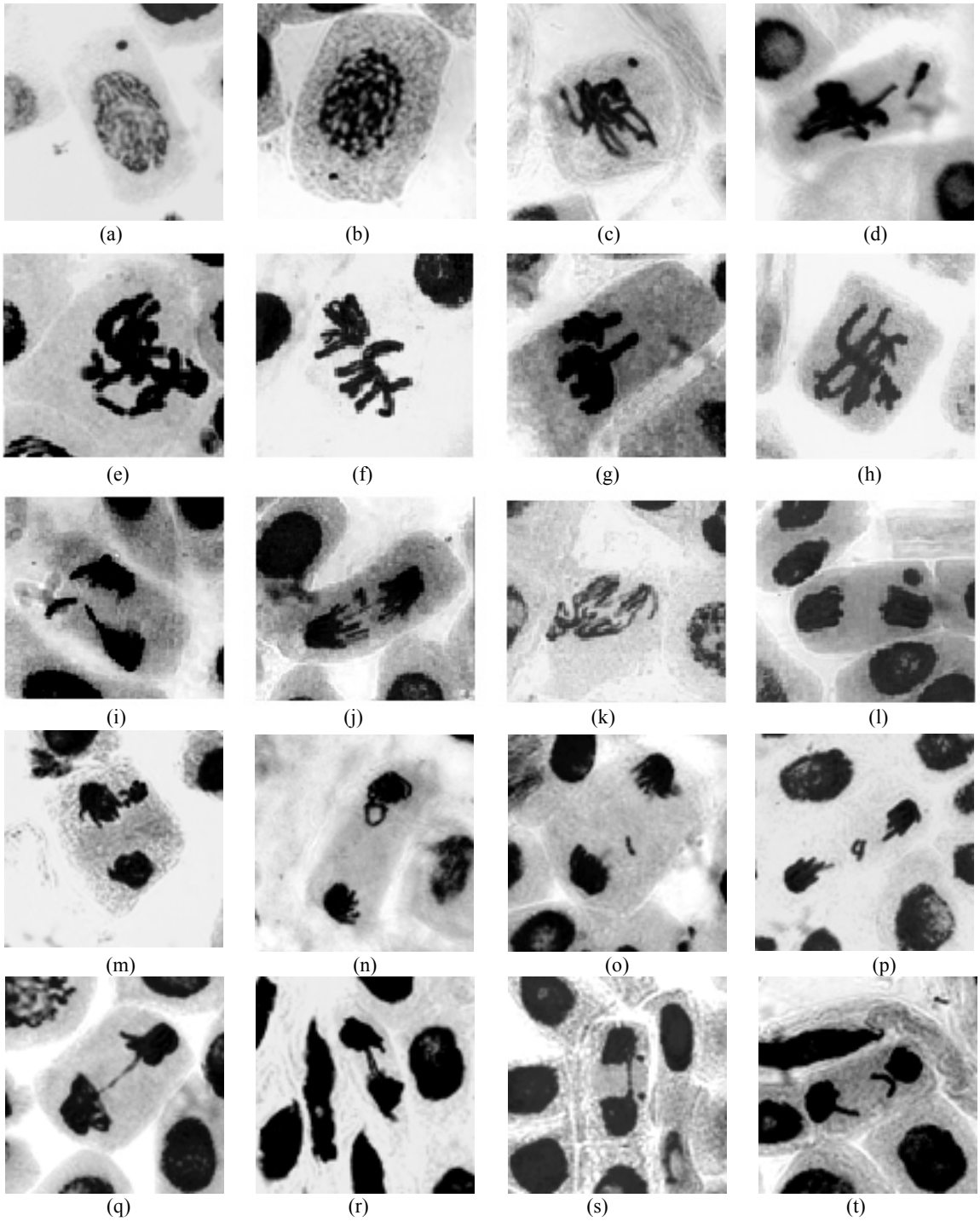


**Fig.1 The effects of $CrO_3$ on mitosis of *Vicia faba* root cells**

(a): micronucleus in prophase; (b): fragment in prophase; (c): micronucleus in metaphase; (d): lagging chromosome in metaphase; (e): chromosome fail to congress on equatorial plane; (f), (g), (h): chromosome was divided into two parts on equatorial plane in metaphase; (i): laging chromosome in anaphase; (j): chromosome bridge and fragment in anaphase; (k): chromosome reach to four poles; (l): micronucleus in telophase; (m): micronucleus was formed in telophase; (n): chromosome loop in telophase; (o): fragment in telophase; (p): lagging chromosome in telophase; (q): one chromosome bridge in telophase; (r): two chromosome bridge in telophase; (s): one chromosome bridge and two micronuclei; (t): chromosome uncoiling abnormally in telophase

cidence of lung cancer caused by chromium salts have attracted more and more attention (Liu *et al*., 1991; Sorahan *et al*., 1998; Sjogren *et al*., 1994). The highly bioactive hexavalent chromium compounds can easily get through cell membrane, and generate some active oxides, which can combine with the intracellular DNA, and lead to the unreliable intercrossing connection, and duplication in DNA, and ultimately result in chromosomal aberration and tumourigenesis (Li *et al*., 1995). The hexavalent chromium compound also has cytotoxicity and can even lead to DNA damage (Edward and Karen, 1994).

This experiment revealed that the effects of $CrO_3$ on mitotic index of *Vicia faba* root tip meristem cells depend on its concentrations. For instance, among five concentration groups, Groups C and F can increase the mitotic index significantly. $CrO_3$ can also induce increases in micronucleus rates, the highest of which was caused by $CrO_3$ concentration of 12.5 mg/L. Relevant data indicated that the micronuclei forms in two ways: One is, the chromosomal fragments formed in the last $G_2$ could not act in phase with normal chromosomes, and are rejected to the outside of the nuclei in interphase. The other is the occurrence of various forms of lagged chromosomes, non-equatorial plane aggregated chromosomes, and the chromosomal grouping (Li, 1997). Within the concentration range in this experiment, the micronucleus rate increased with higher concentration of $CrO_3$. The reason is possibly that $CrO_3$ does more harm to cells and leads to increasing chromosomal aberration as the concentration of $CrO_3$ goes up. The micronucleus rate reaches the peak stage [(8.75±1.22)‰] when the $CrO_3$ concentration is at the level of 12.5 mg/L, which is significantly higher than that of the control ($P$<0.001); but when the concentration of $CrO_3$ continues to increase, the micronucleus rate goes down on the contrary. The reasons responsible for the above facts seem to be that higher $CrO_3$ concentration not only leads to the production of chromosomal aberration, but also effectively inhibits the polymerization of spindle fiber tubulin in cells. As a result, the cells may remain in the mitotic period and therefore makes the micronucleus rate in interphase descend. Accordingly, the micronucleus rates of Groups D, E and F do not accord with their chromosomal aberration rates.

The present experiment also showed that different concentrations of $CrO_3$ could lead to various forms of chromosomal aberration; and that the aberrant rate goes up with the concentration. The chromosomal aberration might be induced by the following ways: Firstly, chemical compounds directly affect DNA and lead to chromosomal aberration. Secondly, chemical compounds could disturb the synthesis of DNA and protein, or the translation of RNA, so that no materials relating to the chromosomal movement could be formed, and the chromosomal aberration occurred eventually. Thirdly, chemical compounds can prevent the re-establishment of the chromosome under normal conditions through interfering with the normal repairing of some damages to the new refusions, such as the rearrangement of chromosomal bridges, loops and fragments. The reasons responsible for the lagging of chromosomes or failure to reach the equatorial plane probably lay in the formation of spindle fiber and the destruction of its function by the $CrO_3$, or the movement regulations of the chromosomes per second are interfered with so that the chromosomes cannot reach the equatorial plane on time. Generally speaking, $CrO_3$ also leads to the polarization. However, the structure of cell division and the determination of its polarization are still unknown. The commonly agreed opinion is that they are controlled by different mechanisms in different kinds of cells (Xing and Liang, 1997), and that the structure of the dividing poles in *Vicia faba* may belong to “thepolar plate”. The occurrence of many chromosomal fragments and chromosomal bridges observed in this experiment indicates that $CrO_3$ may more or less affect the structure and conformation of DNA, the main component of the chromosomes in *Vicia faba* rot tip cells. The effect of $CrO_3$ on mitotic division in plant cells has been fully confirmed, whereas the effect on sexual cell i.e. meiosis in higher plants, and the detailed mechanism of the aberrant effect of $CrO_3$ requires

more research in the future.

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