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SISTER CHROMATID EXCHANGES INDUCED BY THINNER IN *Vicia faba*

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ABSTRACT

Root meristem cells of *Vicia faba* were used as the test system for the detection of sister chromatid exchanges (SCEs) through the fluorescence Plus Giemsa technique. The results indicate that thinner composed of: 52.0% toluene, 25.5% n-hexane, 12.5% ethanol, 6.0% ethly acetate, 2.0% isopropanol, 1.0% benzene and 1.0% n-heptante applied at concentration of 0.003, 0.006, and 0.012% induced a positive response in the frequency of SCEs.

RESUMEN

Se emplearon células meristemáticas de la raíz de *Vicia faba* para la detección de intercambios de cromátidas hermanas (ICH) mediante la técnica de fluorescencia mas Giemsa. Los resultados indicaron que el tíner compuesto de: tolueno (52.0%), n-hexano (25.5%), etanol (12.5%), acetato de etilo (6.0%), isopropanol (2.0%), benceno (1.0%) y n-heptano (1.0%), aplicado en concentraciones de 0.003, 0.006 y 0.012% indujo respuesta positiva en la frecuencia de ICH.

INTRODUCTION

The use of higher plants as test systems for screening and monitoring environmental mutagens is widely know. Different genetic and chromosomal alterations can be analyzed in higher plants, and this fact together with other advatages, such as their ease in handling and their relatively low cost, make them valuable biological test assay that could be incorporated into the tier testing system (Grant *et al.* 1981).

Vicia faba has long been used in numerous radiobiological and cytological studies. Until now more than 80 different agents have been tested in this plant assay system using chromosomal aberrations as indicators of genetic damage (Ma 1982).

Among the chemical agents which have been studied in *Vicia faba,* are industrial solvents which are notable potent toxic substances. Thinners are industrial products

that result from a balanced mixture of solvents. They can be used as ingredients of paints and varnishes(Gutiérrez-Flores 1975).

Toxicological investigations with thinners have shown that acute exposure to such substances affects the central nervous system causing hypermobility and hallucinating conduct (Guzmán-Flores 1975). It has also been observed that thinners produce anemia and hereditary defects in blood cells (Ferrara-Castro 1976).

In the root-tip meristematic cells of *Vicia faba,* thinner has proven to be an inducer of chromosomal aberrations (Gómez-Arroyo 1980). Another cytogenetic technique for the study of low chemical doses is the sister chromatid exchange (SCE) assay system. A high number of sister chromatid exchanges can be induced per chromosome by agents known to produce chromosomal aberrations; therefore, Wolff (1977) has proposed that the quantification of only 20 cells can give statistically significant results.

Even though most of the investigations directed to analyze the frequency of SCEs have been performed using animal cells (Latt 1974, Carrano and Wolff 1975), there have been some studies carried out in higher plants such as *Vicia faba* (Kihlman and Kronborg 1975, Scheid 1976), *Allium cepa* (Schvartzman and Cortés 1977), *Tradescantia paludosa* (Grant and Goldstein 1983), *Secale cereale* (Friebe 1978) and *Hordeum vulgare* (Schubert *et al.* 1980).

Before analyzing SCEs in plant cells, there are some obstacles which must be overcome. One of these is the low incorporation capacity that roots have for 5-bromodeoxyuridine (BrdUrd), which results in poor differential staining. Another problem is the resistance offered by cell walls that have to be squashed, and finally the high content of RNA that causes an excessive staining of the cytoplasm (Kihlman and Andersson 1982).

The Fluorescence Plus Giemsa technique (FPG) which has been adapted to plants has overcome each of the above obstacles. Low incorporation of BrdUrd is increased by the addition of 5-fluorodeoxyuridine (FrdUrd) (Haut and Taylor 1967), and cell wall resistance to squashing can be solved by using enzymes and acids that soften tissue (Kihlman and Andersson 1982). In spite of the fact that these modifications result in more elaborate techniques, there are some aspects of SCEs that can be studied in these sytems in great detail. This is partly due to the large chromosomes possessed by some plants combined with their low chromosome number (*Vicia faba* and *Tradescantia* 2n = 12, *Secale cereale* 2n = 14, *Allium cepa* 2n = 16). All these characteristics make higher plant systems excellent biological resource material for analyses of environmental agents (Grant *et al.* 1981). One of these assays using SCEs, can be used to determine chromosome exchanges with great accuracy.

Since it has been shown that thinner induces chromosomal aberrations in *Vicia faba,* this study was planned to use this same organism, and to determine the effect of very low concentrations of thinner on meristematic root tip cells using the sister chromatid exchange assay.

MATERIAL AND METHODS

Seeds of *Vicia faba* (var. minor) were set to germinate between two wet cotton layers, at 21°C in the dark. They were previously washed in tap water for two hours, and soaked for 24 hours to accelerate germination. When radicles emerged

(between the 4th and 5th day), the testa was removed to avoid fungus or bacterial infection. Those radicles with a length of 2-3 cm were selected and exposed for one cell cycle time to the following mixture: 100 μM of 5-bromodeoxyridine (BrdUrd), 0.1 μM of 5-fluorodeoxyuridine (FdUrd) and 5 μM of uridine (Urd).

The root tips were then treated for one hour with 0.003, 0.006 and 0.012% thinner. The thinner components consisted of: toluene (52.0%), n-hexane (25.5%), ethanol (12.5%), ethyl acetate (6.0%), isopropanol (2.0%), benzene (1.0%) and n-heptane (1.0%).

The roots are grown during the second round of DNA replication in the presence of 100 μM of thymidine (dThd) and 5 μM of Urd.

The roots are exposed to 0.05% colchicine the last 3 hours before fixation overnight in cold methanol-glacial acetic acid (3:1). During the treatments, the roots were not exposed to light and the temperature was 23 $\pm$ 1°C.

Root meristem regions were squashed in pectinase on slides previously coated with a 10:1 mixture of gelatin and chrome alum (chromium potassium sulfate).

Cover slips were removed and the slides rinsed in 0.5 $\times$ SSC and stained with a "33258 Hoescht" solution, prepared by adding 1 mg of fluorochrome to 1 ml of ethanol; then 0.1 ml of this solution was dissolved in 100 ml of SSC.

Differential contrast was improved by exposing the slides to ultraviolet light (Westinghouse type 5B-30, 250 v), 10 cm from the source for 30 minutes. Following irradiation, the slides were incubated for 60 minutes at 55°C in 0.5 $\times$ SSC. Staining was carried out by using a solution containing 3% Giemsa in tap water for 7-9 min. Finally, preparations were rinsed in distilled water, air dried, and dehydrated with xylene. Canada balsam was used as a mounting medium.

For each concentration of the thinner tested, 250 subacrocentric chromosomes (S) and 50 metacentric chromosomes (M) were scored. These chromosome numbers are equivalent to 25 metaphases. Slides were double blinded before scoring.

RESULTS

Each experiment was carried out in duplicate and a Student's "t" analyses was used to test for statistical significance between the two experiments. No statistical difference between the two experiments was found.

The results showed that the concentrations of thinner tested (0.003, 0.006 and 0.012%) induced an increment in the frequency of SCEs (Fig. 1).

The mean values obtained (Table 1) increased as concentration increased. In every case the values proved to be statistically different to the control ($p < 0.001$).

DISCUSSION AND CONCLUSIONS

Vicia faba has proved to be a sensitive and reliable system for the detection of SCEs induced by various chemical agents. One aspect of induced SCEs that has only been studied in plant cells is the existence of dot SCEs. Kihlman (1975) reported this phenomenon in *Vicia faba* and later Schvartzman and Cortés (1977) in *Allium cepa*.

The presence of dot or minute SCEs in this work in clearly consistent with the

Fig. 1. Sister chromatid exchanges frequencies induced by thinner.

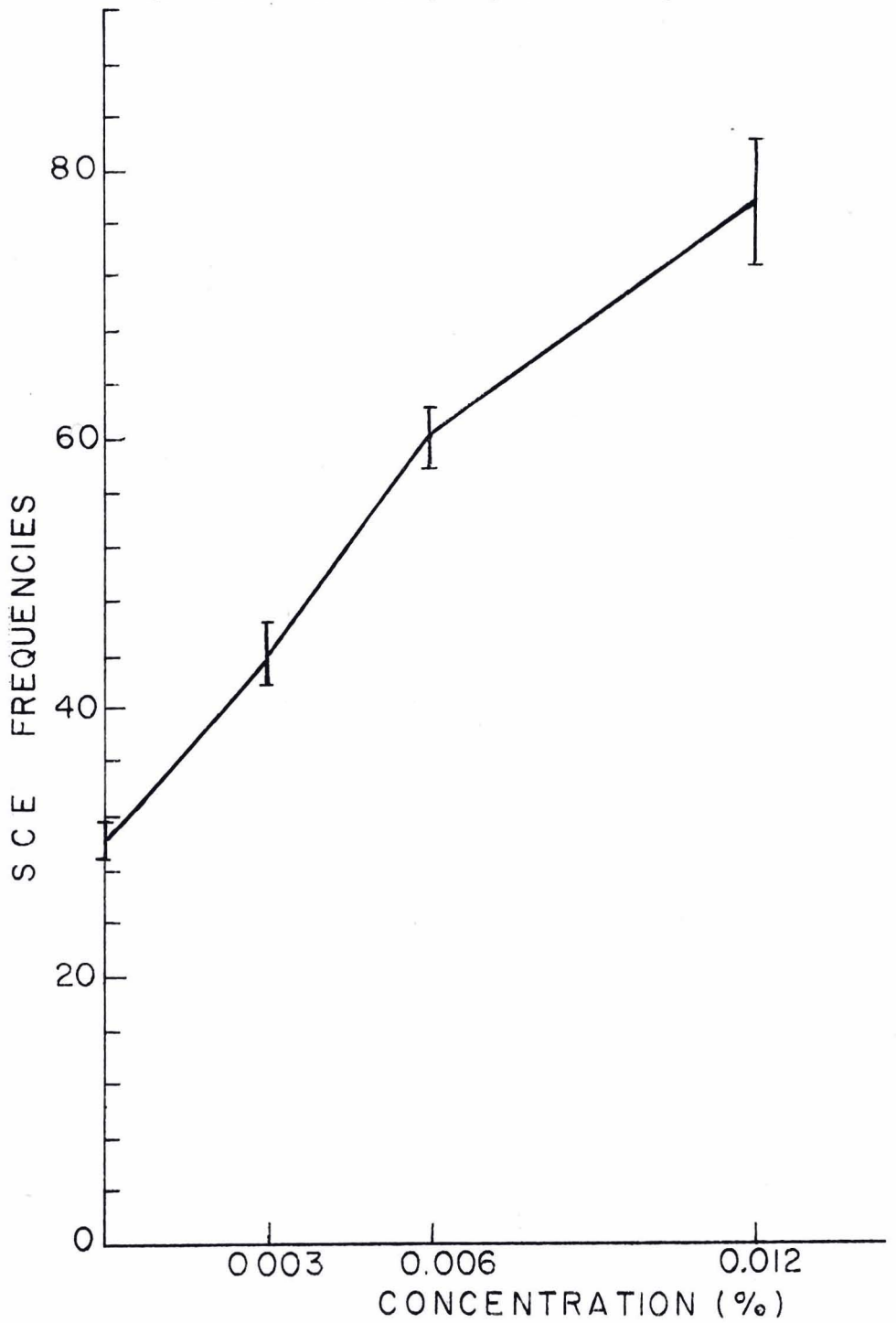

TABLE I. SISTER CHROMATID EXCHANGES INDUCED BY THINNER IN *Vicia faba*

Concentration (%)	*SCE* $\bar{X} \pm S.E.$	"*t*" *value*
Control	30.50 ± 0.8026	
0.003	43.96 ± 1.4818	5.8921 *
0.006	60.50 ± 1.5446	12.7812 *
0.012	77.46 ± 2.9652	12.4632 *

* $P < 0.001$.

above mentioned findings. Dot SCEs were observed in control and treated cells, and their size varied from a minute point to a narrow line that covered the width of a chromatid.

The fact that minute SCEs have only been reported in plant cells could be partly due to the large size of the chromosomes which occur in plants and which make SCEs easy to identify.

The frequency of SCEs seems to be related to the number of rounds of DNA synthesis to which the cells are exposed to via the base analogue 5-BrdUrd (Schvartzman *et al.* 1979). Therefore, Kihlman and Andersson (1982) have proposed that chromosomes which have been exposed to BrdUrd for two rounds of replication (TB-BB) will show a higher frecuency of SCEs than those exposed for only one round of replication (TT-TB). In the latter case, the mean frequency of SCEs would be 23 per metaphase. The results obtained in the present work showed a higher "spontaneous" mean value of SCEs in chromosomes with a TT-TB constitution, as these values went from 30 to 32 SCEs per metaphase. The discrepancies in these studies could be the result of the authors using different varieties of *Vicia faba.*

The frequency of SCEs is also related to the DNA content. According to Geard and Peacock (1969), the mean number of SCEs in the subacrocentric chromosomes (S) of *Vicia faba* is 1.64 ± 0.04, while in the metacentric chromosomes (M) of *Vicia faba* it is 4.05 ± 0.16 (taking into account that the content of DNA is 44 picograms per cell, Baetcke *et al.* 1967). The spontaneous numbers of SCEs found in this work is consistent with the reports of Geard and Peacock (1969), in which a mean spontaneous frequency of five was found in the M chromosomes, while a smaller frequency was observed for the S chromosomes.

Until now, a large number of substances have been reported to induce SCEs in plant and animal systems. Most are classified as S-dependent agents since their effect on chromosomes is only expressed as an aberration after DNA synthesis has occurred. Some of the most well-known S-dependent agents are the alkylating agents (Bender *et al.* 1973). However, two S-independent agents have also been reported to be efficient inducers of SCEs: the antibiotic streptonigrin and long-wave U. V. According to Kihlman and Andersson (1982) this fact suggests that their effect is only partially S-independent.

The thinner used in this work has been reported to produce chromosomal aberrations in an S-independent manner (Gómez-Arroyo *et al.* in press), and therefore, thinner could also be included among those S-independent agents that are able to induce SCEs. Various types of base damage rather than strand breaks seems to be the result of DNA lesions induced by these agents so that the formation of SCEs occurs later.

The high number of SCEs induced by different concentrations of thinner in *Vicia faba* means that this biological material is extremely sensitive and useful in the detection of the demage caused by chemical agents on DNA. From this data it can also be concluded that the thinner used must be considered a highly toxic agent with mutagenic activity since genetic damage was induced at very low concentrations.

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