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Effects of auxin and cytokinin on induction of sister chromatid exchanges in cultured cells of wheat (*Triticum aestivum* L.)

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Summary. In order to know the mutagenic effects of synthetic auxins (NAA, 2,4-D, and 2,4,5-T) and a cytokinin (kinetin) in vitro, sister chromatid exchanges (SCEs) were analyzed in cultured cells of a hexaploid wheat (*Triticum aestivum* L.). In the MS medium supplemented with 2.0 mg/l 2,4-D, the mean number of SCEs per cell was 15.2, and per pg of DNA, 0.42. No significant effect was found in the treatments of NAA or 2,4-D at concentrations of 0.5–10.0 mg/l, whereas more than 2.0 mg/l of 2,4,5-T induced dramatic increases of SCEs. Kinetin itself had no significant effect on SCE induction, but there was a tendency that SCEs induced by 2,4,5-T were suppressed by kinetin.

Key words: *Triticum aestivum* – SCEs – Auxin – Cytokinin – Tissue culture

Introduction

Genetic changes such as chromosomal aberrations and gene mutations are known to occur during in vitro cultures of cells and tissues in a wide range of plant species (D'Amato 1977; Bayliss 1980). However, the mechanism for induction of the variations is still unknown. It is suggested that the variations originate from culture medium and conditions, as well as polyploidy and genetic constitution of the initial explants. Among the components in the medium, synthetic auxins such as 2,4-D have been proposed to be the most possible mutagen, but their mutagenic effect is not clearly shown yet (Bayliss 1980). Sister chromatid exchanges (SCEs) have been proved to be a sensitive indicator for evaluating the mutagenic effect of chemical substances (Latt 1974; Perry and Evans 1975). In this study, therefore, the effects of three synthetic auxins and a cytokinin on induction of SCEs were studied in cultured cells of a hexaploid wheat.

Materials and methods

Calli were induced from immature embryos of *Triticum aestivum* cv Chinese Spring and maintained on MS (Murashige and Skoog 1962) medium supplemented with 2.0 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid), according to the method by Sears and Deckard (1982). For SCE analysis, 3-month-old calli were suspended in 100-ml Erlenmyer flasks containing 30 ml of liquid MS media supplemented with NAA (α-naphtylacetic acid), 2,4-D or 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), at the concentration of 0–10.0 mg/l, and/or kinetin at 0.1–1.0 mg/l. BudR (5-bromodeoxyuridine) was simultaneously added to the media at the final concentration of 50 μ*M*. These cultures were agitated on a gyratory shaker at 100 rpm at 25° ± 2 °C in the dark. After two cycles of cell division (65–72 h), cultured cells were fixed in acetic alcohol (3:1 ethanol:acetic acid), and stored at −20 °C until slide preparations were made.

Chromosome preparations were made according to the air-dry technique by Murata (1983) with minor modifications. The fixed cells were rinsed with distilled water three times at 10 min intervals by centrifugation (200 × g). The supernatant was replaced by the enzyme solution, which consisted of 2% (w/v) cellulase Onozuka RS (Kinki Yakult) and 20% (v/v) pectinase (Sigma) (pH 4.5–4.8), and placed in a water bath at 30 °C for 1.5–2 h. Cells were well-suspended with a Pasteur pipette and filtered through 70 μm nylon mesh. The enzyme soloution was removed by centrifugation and the cell pellet was rinsed with distilled water, and fixed with the fresh acetic alcohol. Following two changes of the fixative, the cell suspension was dropped onto cold and wet slides, and flame-dried.

The slides were stained first with 0.8% (w/v) acetocarmine solution, and photographs of metaphase cells with good spread of chromosomes were taken. For differential staining of chromatids, the same slides were stained with 2% (v/v) Giemsa solution diluted with 0.3 *M* Na_2HPO4 (pH 10.4) for 15 min (Alves and Jonasson 1978), rinsed with distilled water, and air-dried. SCEs were scored in 20 cells per treatment.

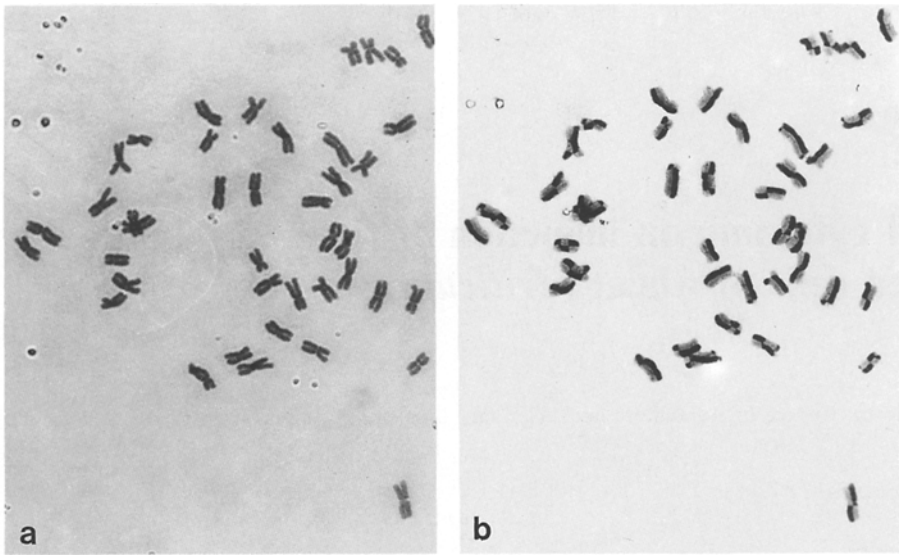

Fig. 1 a and b. Metaphase chromosomes of a wheat cultured cell stained with acetocarmine (**a**), and with alkali Giemsa solution showing differential sister chromatid exchanges (**b**)

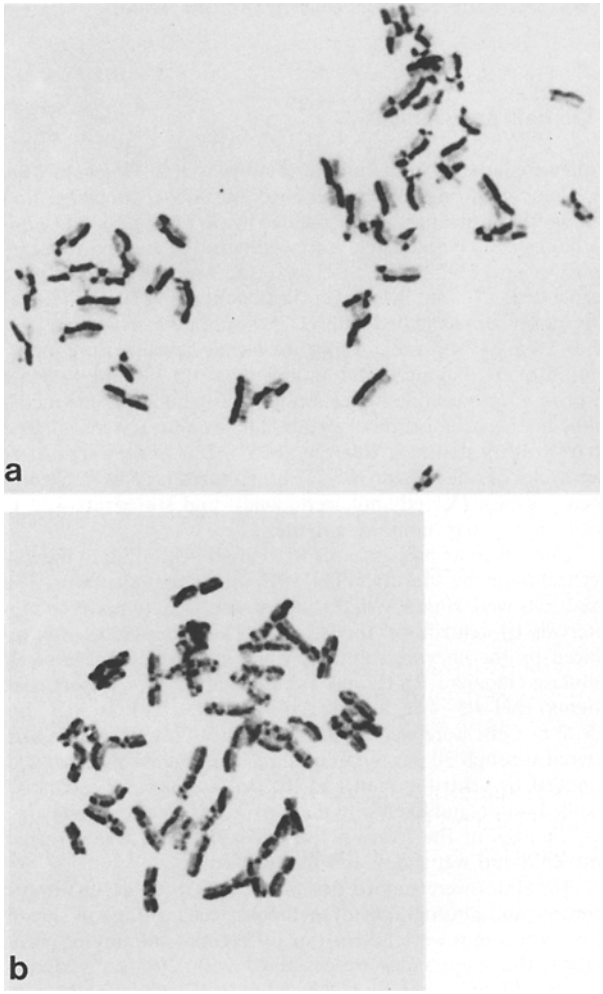

Fig. 2 a and b. Metaphase cells showing sister chromatid exchanges induced by 2,4,5-T

Table 1. Effects of NAA, 2,4-D and 2,4,5-T on induction of SCEs in cultured cells of wheat

Auxin	Mean of SCEs/cell (range)				
	Concentration (mg/l)				
	0	0.5	2.0	5.0	10.0
NAA	19.6a[a] (12–30)	17.4a (14–21)	16.6a (14–20)	19.3a (15–28)	22.9a (16–40)
2,4-D	–	17.3a (12–25)	15.2a (13–18)	18.2a (13–26)	28.0a (13–42)
2,4,5-T	–	15.4a (12–21)	67.0b (44–87)	116.0c[b] (111–121)	N.C.[c]

[a] Entries having the same letter are not significantly different at the 5% level, according to the Tukey's test
[b] Mean of SCEs in two cells
[c] No cells showing sister chromatid differentiation

Results

Good differentiation of chromatids were obtained by staining with alkali Giemsa solution, following stain with acetocarmine (Fig. 1). This technique is simple, requiring considerably fewer procedural steps than the FPG (fluorescence plus Giemsa) technique usually used for SCE analysis. Furthermore, comparisons with karyotypes stained with acetocarmine could lead us to strict analysis of SCEs, because centromeres or twisted parts of chromosomes were easily detected.

Calli were induced on MS medium supplemented with 2.0 mg/l 2,4-D, and had been maintained on the same medium for 3 months. Therefore, those cells had

Table 2. Effect of kinetin and its interaction with 2,4,5-T on induction of SCEs in cultured cells of wheat

Conc. of 2,4,5-T (mg/l)	Mean of SCEs/cell (range)			
	Concentration of kinetin (mg/l)			
	0	0.1	0.5	1.0
0	19.6a[a] (12–30)	20.3ab (15–24)	20.8ab (13–34)	21.8ab (13–45)
2.0	67.0c (45–87)	46.9bc (34–63)	46.6abc (31–56)	43.8abc (28–69)

[a] Entries having the same letter are not significantly different at the 5% level, according to the Tukey's test

been habituated to the concentration of 2,4-D. In that medium, a mean number of SCEs per cell was 15.2, which was the lowest among the treatment in this experiment (Table 1).

The effect on induction of SCEs was different among three kinds of auxin; NAA, 2,4-D, and 2,4,5-T (Table 1). No significant effect was found in the treatments of NAA or 2,4-D at concentrations of 0.5–10.0 mg/l, although 10.0 mg/l of 2,4-D caused a slight increase of SCEs. At a concentration of 0.5 mg/l, 2,4,5-T had no significant effect. However, the concentrations of 2.0 and 5.0 mg/l induced dramatic increases of SCEs (Fig. 2). Those SCEs per cell were 67.0 and 116.0 on the average, respectively. Since inhibitory effects to cell divisions appeared at more than 5.0 mg/l of 2,4,5-T, SCEs were scored only in two cells at 5.0 mg/l. No cells with sister chromatid differentiation were observed at the concentration of 10.0 mg/l. These inhibitory effects were also supported by the observation of slow callus growth in the media supplemented with more than 5 mg/l 2,4-D or 2,4,5-T (no data presented).

No significant increase was found in the treatment with kinetin itself at the concentration of up to 1.0 mg/l (Table 2). No cell divisions were observed in the medium supplemented with more than 2.0 mg/l kinetin. In the medium with 2.0 mg/l 2,4,5-T, however, the supplements of kinetin at 0.1–1.0 mg/l decreased the number of SCEs, although the differences were not significant. It was noticed that the numbers of SCEs per cell were considerably variable within a treatment, particularly when supplemented with 2,4,5-T. This probably caused the different responses to the hormones among the cells, depending on their location in callus tissue.

Discussion

The number of SCEs per cell (15.2) observed in the callus-inducing medium with 2.0 mg/l 2,4-D is relatively high compared to those (6.9–14) observed in human lymphocytes in vitro (Kihlman and Kronborg 1975). However, wheat cells used here were hexaploid ($2n=6x=42$), having a considerably large amount of DNA: 36.2 pg per 2C cell (Bennet 1972). Therefore, a mean number of SCEs per pg of DNA calculated is 0.42, the value of which is lower than those (0.9–1.9) in human lymphocytes in vitro, but comparable to 0.42/pg in *Vicia faba* and 0.3/pg in chicken in vivo (Kihlman and Kronborg 1975).

Since no SCE analysis has been made in wheat in vivo, the effect of cell and tissue cultures on SCE induction could not be estimated. Recently, Dolezel and Novak (1986) reported that there was a significant difference between SCEs observed in root meristems and in callus tissues of *Allium sativum* (6.4 versus 7.1–7.8). However, their comparison between in vitro and in vivo was made only for SCEs per chromosome, since no complete metaphase cells with chromatid differentiation were found. In the present study with wheat cultured cells, the numbers of SCEs per chromosome were 0.36 (15.2/42), being much smaller than Dolezel and Novak's (1986) result. This difference would be caused mainly by the differences of materials used (e.g., species, tissue, etc.) and also the culture condition.

Cytological and genetical effects to plants of herbicidal auxin hormones such as 2,4-D and 2,4,5-T have been studied since the 1940s. Croker (1953) reported that contents as low as 25 mg/l of 2,4-D and 2,4,5-T induce cytological aberrations in root tips of onion. In plant tissue cultures, however, herbicidal hormone – particularly 2,4-D – has been used for a long time as an auxin source, one of the most important factors. The concentrations used were much less (<10 mg/l) than those inducing cytological abnormalities in intact plants. The result of this study showed that NAA or 2,4-D has almost no significant effect on induction of SCEs in vitro, but 2,4,5-T significantly induces SCEs. This suggests that 2,4,5-T is much more mutagenic than NAA and 2,4-D. Similar effects were also detected in the treatment for 2 weeks with 2,4,5-T (Murata 1985).

Nevertheless, Dudits et al. (1975) indicated that 2,4,5-T is a better auxin source with respect to callus proliferation in wheat than 2,4-D or IAA. Lazar et al. (1983) also reported that 2,4,5-T at 0.5–1.0 mg/l concentrations was most promotive for wheat callus proliferation, compared to 2,4-D and picloram, particularly in combination with 0.1 mg/l kinetin. However, they found that more abnormal plantlets appeared from the calli cultured at high concentration of 2,4,5-T than at low concentration (0.01 mg/l). This might also support the mutagenic effects of 2,4,5-T.

No additive SCE induction was found in the medium with kinetin itself. However, it is suggested that kinetin might have an effect suppressing the SCEs induced by

2,4,5-T. Therefore, it would be important to use 2,4,5-T in combination with kinetin, although wheat tissues are known to require no cytokinin for callus proliferation. Further studies in different species and/or tissues are needed to elucidate the mutagenic effects of auxins and their interaction with cytokinins in vitro.

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