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Colchicine-like effect of propyzamide on the root meristematic cells of *Vicia faba* L.

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A herbicide, propyzamide inhibited mitosis of broad bean (*Vicia faba* L.) root tip cells. Mitotic indices and photomicrographs showed that propyzamide affected mitosis in a very similar manner to colchicine. Mitotic cells accumulated at metaphase and neither nuclear nor cell division figure was observed. Root tip cells became polyploids when roots were treated with propyzamide solution for longer period than one cell cycle. However, some differences of action were recognized between propyzamide and colchicine. The effect of propyzamide was removed rapidly compared with that of colchicine. After transferring the roots from propyzamide solution to water, the root tip cells recovered their normal mitoses only after 5 hr.

N-(1, 1-Dimethyl propynyl)-3, 5, dichlorobenzamide (propyzamide) is one of herbicides and marketed under the name "Kerb". The structural formula of propyzamide is shown in Fig. 1.

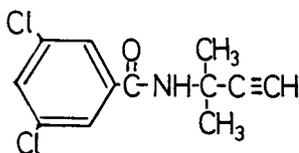


Fig. 1. The structural formula of propyzamide.

According to the explanatory note for the agricultural use, "Kerb" is used for selective removal of weeds, particularly of graminaceous plants in lawn. It is considered that the mechanism of its action as herbicide is concerned with the inhibition of cell division because abnormal nuclei were observed in root tip cells treated with propyzamide (CALRLSON *et al.*, 1972; SUZUKI 1976). However, detailed cytological study on the effect of this herbicide has not been carried out. In the present paper, we report preliminarily the effects of propyzamide on the cell division and chromosome behavior of *Vicia faba* root meristematic cells.

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Materials and Methods

Seeds of *Vicia faba* were soaked in tap water for two days, then planted in moist vermiculite and grown in the dark for 4 days at 20 C. Experiments were carried out with about 3 to 6 cm long primary roots. After washing the seedlings in tap water, the roots were immersed into well-aerated 0.1 g/l "Kerb" solution. In the preliminary experiment, the lowest concentration of "Kerb" to induce abnormal mitoses was 0.001 g/l. In the present study, we used 0.1 g/l "Kerb" concentration to examine its action on cells more reliably. Since "Kerb" contains 50% of effective ingredient propyzamide, we considered that 0.1 g/l "Kerb" solution is equal to 0.05% (w/v) propyzamide solution.

Two kinds of experiments were carried out. In the first experiment, roots were treated with 0.1 g/l "Kerb" solution for various time length (2-96 hr) and fixed immediately after the treatment. In the second experiment, roots were treated with 0.1 g/l "Kerb" solution for 2 hr and transferred into tap water for recovery and fixed after various durations. Fixation was carried out in alcohol-acetic acid 3:1 and preparations were made by Feulgen's squash method.

Besides, the colchicine treatments were performed in order to compare the effect of propyzamide with that of colchicine. The concentration of colchicine was 0.05% (w/v), which is usually used for the accumulation of metaphase cells in root meristems. The procedure of colchicine treatment was the same as that of propyzamide.

Mitotic indices (percentage of cells in mitoses) were determined by scoring at least three slides per fixation time with 700 cells from each slide.

Results and Discussion

The treatment with 0.1 g/l "Kerb" solution clearly inhibited cell divisions of *Vicia faba* root tip cells (Plate I, A, B). Mitoses became the state of so-called "c-mitosis" as is seen by the colchicine treatment. Anaphase and telophase cells were scarcely observed and the proportion of metaphases to mitoses increased. Metaphase chromosomes were contracted more tightly than those in normal metaphases and scattered throughout the cytoplasm. Many cells became polyploids when roots were treated with propyzamide for a longer period than one cell cycle (Plate I, C). This polyploidization of the cells seemed to continue as long as propyzamide treatment was kept on. The treatment for 4 days often produced huge cells containing about one hundred chromosomes.

Mitotic indices began to increase after the initiation of the treatment with propyzamide (Fig. 2). After 12 hr of treatment, mitotic figures attained about twice level as high as that of control. This increase of mitotic cells was mainly due to the accumulation of metaphase cells and the proportion of prophase to total cells did not change significantly. Mitotic indices also increased by the colchicine treatment and the outlines of the change of mitotic indices of two chemicals fairly resembled to each other, suggesting that the cytological effect of propyzamide is very similar to colchicine.

However, some differences were recognized between two chemicals in the viewpoint how many hours were required to recover their normal cell divisions in the course of recovery period (Fig. 3). Mitotic indices of cells treated with colchicine maintained higher level after the transference into colchicine-free water. The mitoses were c-mitotic even up to 28 hr recovery period and normal cell divisions were observed barely after 52 hr. In the colchicine treatment it was required about 2 days to recover normal cell divisions only by the 2 hr treatment. In the case of propyzamide, however,

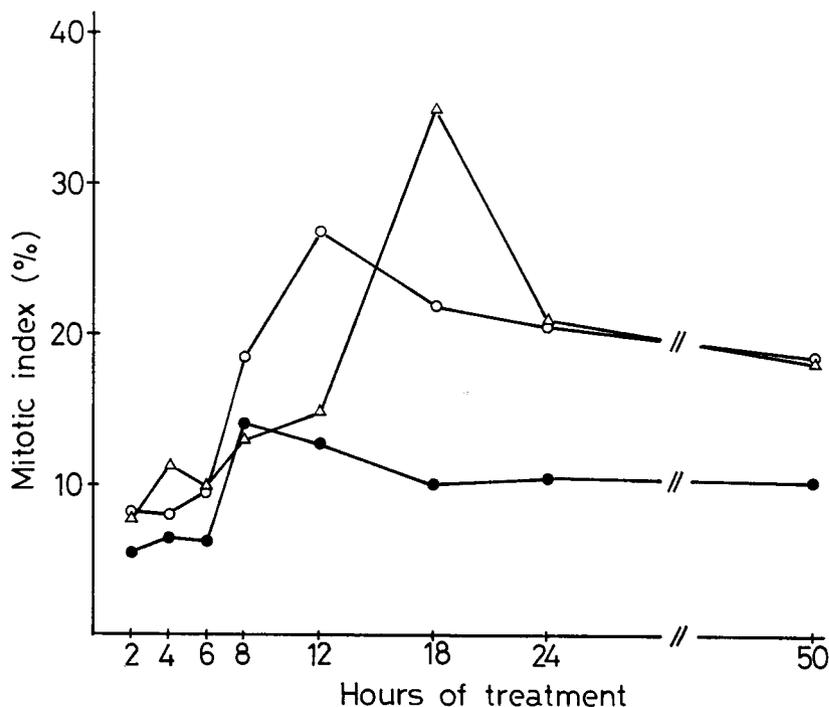


Fig. 2. The change of mitotic indices obtained in *Vicia faba* root tips after the initiation of the treatments. (—●—) control; (—○—) 0.1 g/l "Kerb" solution; (—△—) 0.05% colchicine.

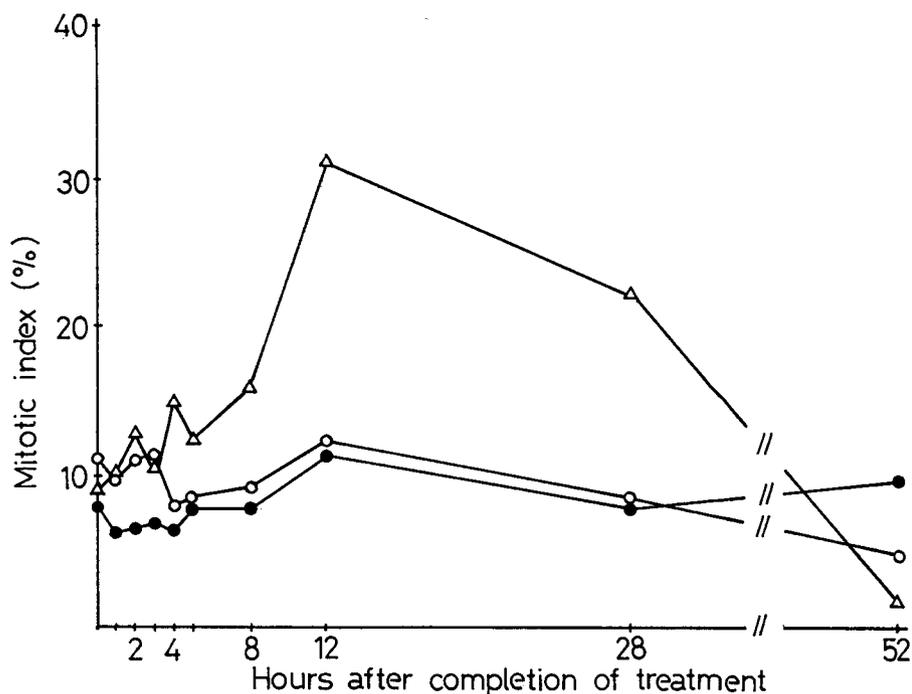


Fig. 3. The change of mitotic indices obtained in *Vicia faba* root tips after the completion of the treatments for 2 hr. (—●—) control; (—○—) 0.1 g/l "Kerb" solution; (—△—) 0.05% colchicine. The mitotic indices of colchicine and propyzamide for 52 hr recovery period were lower than control because multinucleate cells increased in the root tips and some of these cells could not enter upon mitoses.

mitotic index was reduced to control level 4 hr after the completion of the treatment. Mitoses were c-mitotic only until 2 hr recovery period and they resumed their normal divisions after 5 hr.

During recovery periods, when mitoses returned from c-mitoses to normal cell divisions, the movement of chromatids toward poles was disturbed considerably both in propyzamide and colchicine treatment. Mitoses with multipoles and lagging chromosomes appeared frequently (Plate I. D). The duration of this abnormality was shorter in the propyzamide treatment than in the colchicine treatment. In the case of propyzamide treatment for 2 hr, such aberrant cell divisions confined themselves to 3 to 4 hr recovery period and thereafter such aberrant anaphase and telophase were not observed (Fig. 4). In the colchicine treatment for 2 hr, on the contrary, abnormal division figures appeared 28 hr after the completion of the treatment and even after 52 hr recovery period, when most cells regained normal cell divi-

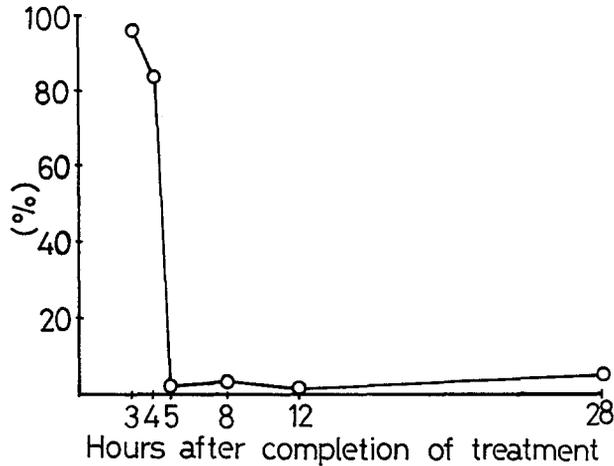


Fig. 4. Percentage of telophases with multi-poles and/or lagging chromosomes obtained in *Vicia faba* root tips after the completion of 0.1 g/l "Kerb" treatment for 2 hr. Cell divisions were completely arrested at metaphase until 2 hr recovery period so that no telophase was observed. At least 100 telophase cells were analyzed at each recovery time.

sions, cells with multipoles and/or lagging chromosomes were still observed.

Time required to restore normal cell divisions after the completion of propyzamide treatment were affected by the length of the treatment. When root tips were treated with propyzamide for 4 days and transferred into water, not all cells restored normal cell divisions so fast as in the 2 hr treatment. Most mitoses remained in the state of c-mitoses 5 hr after the completion of the treatment. But some mitoses existed which regained normal cell divisions in early recovery periods although the cells had become polyploids.

Chromosome stickiness, chromosomal-bridge, and chromosome fragmen-

TABLE 1. Number of metaphase cells with chromosomal aberrations after the treatment with 0.1 g/l "Kerb" solution

Duration of treatment (hr)	Number of metaphase cells analyzed	Number of metaphase cells with chromosomal aberrations	Type of aberration
2	106	0	—
6	105	0	—
18	108	0	—
24	82	1	Isochromatid break

tation were scarcely observed (Table 1), indicating that propyzamide is not provided with distinct chromosome breaking effect.

Many chemicals are known to induce colchicine-like effect on plant and mammalian cells (DOXEY, 1949; AMER, 1965; SAWAMURA, 1965; MANN and STOREY, 1966; LINGOWSKI and SCATT, 1972; SAWADA and ISHIDATE 1978) and these chemicals are generally called "c-mitotic" agents. According to BOWEN and WILSON (1954), the term "c-mitosis" must be used carefully because "this term, by extension, has been used in reference to superficially similar cytological effects induced by a number of non-polyploidizing agents". Propyzamide has the effect of polyploidization and accumulates mitoses at metaphase. It is ascertained here that propyzamide is true c-mitotic agent and the mode of its action is very similar to colchicine.

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Plate I.

- A. An arrested metaphase cell of *Vicia faba* root tip treated with 0.1 g/l "Kerb" solution for 2 hr. Contracted chromosomes are scattered throughout the cytoplasm.
- B. *Vicia faba* root tip cells treated with 0.1 g/l "Kerb" solution for 2 hr. Mitoses have become c-mitoses.
- C. An arrested metaphase cell of *Vicia* root tip treated with 0.1 g/l "Kerb" solution for 96 hr. This cell has become octaploid containing 48 chromosomes.
- D. An abnormal anaphase cell of *Vicia faba* root tip treated with 0.1 g/l "Kerb" solution for 98 hr and fixed 3 hr after the completion of the treatment. The segregation of chromosomes is considerably disturbed.

