AN ALTERNATIVE PRETREATMENT METHOD FOR MITOTIC CHROMOSOME OBSERVATION IN POTATOES

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Abstract

An alternative pretreatment method for mitotic chromosomes in potato root tips is described. Root tips were treated with a synthetic pyrethroid, a permethrine, {3-Phenoxybenzil (±) -*Cis, trans*, 3-(2,2 dichlorovinyl) -2,2-dimethyl ciclopropane carboxylate}. with concentrations of 10ppm, 100ppm, 200ppm, and 500ppm. Cold water and 0.02M 8-hydroxyquinoline were used for comparison at 4 C. Mitotic index was obtained for all treatments by using a standard squash method, and pretreatment with 100ppm of the pyrethroid demonstrated a large number of mitotic cells and well condensed chromosomes for a comprehensive observation in mitotic cells.

Compendio

Se presenta una tecnica alternativa para tratar chromosomas mitoticos como pretratamiento en raices de papas. Raices se trataron con pyrethroides syntheticos, una permethrina, (carboxilato ciclopropano-3- Fenoxibencilo (±) *Cis trans*-2,2-dimetilo 3-(2,2-diclorovinila) en concentracion de 10ppm, 100ppm, 200ppm, y 500ppm. Agua helada y 8-hydroxyquinolina 0.02M se emplearon como controles para comparar los diferentes tratamientos con pyrethroides a una temperatura de 4C. El indice mitotico se determinó por el method microscopico establecído para preparar muestras de puntas de raices. Con el pretratamiento con 100ppm de pyrethroide a 100ppm se encontró un gran numero de celulas mitoticas y chromosomas bien observables.

Introduction

Chromosome counts in mitotic cells are tedious on potatoes, while identifying ploidy level and chromosome number are essential for routine ploidy manipulation in germplasm enhancement (6). There is no universal protocol which would constantly provide reasonable number of mitotic cells for chromosome observation. One of the major reasons is that sam-

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pling conditions drastically affect the status of mitosis in root tips, while the techniques in cytology also influence the reliability of observations (2).

It is known that some pretreatment methods such as colchicine, 8-hydroxyquinoline, α-bromonaphtalene, and ice-chilled water are effective to prolong metaphase and to condense chromosomes for microscopic observation of mitosis. Such pretreatment methods influence the mitotic index and size of chromosomes.

A group of insecticides which belongs to pyrethroids affects karyokinetic spindle formation in plant mitotic cells, and has been shown to affect the mitotic index, particularly at low temperatures (3). We have tested a new pretreatment method using a synthetic pyrethroid to increase mitotic indices for more comprehensive chromosomal characterizations such as chromosome counting and karyotyping in mitosis in potato root tips.

Materials and Methods

A commercial pyrethroid product, a permethrine, Ambush 50EC {3-Phenoxybenzil (±) -*Cis*, *trans*, 3-(2,2 dichlorovinyl) -2,2-dimethyl ciclopropane carboxylate}, {Imperial Chemical Industries (ICI), UK} was employed for pretreatment. Concentrations of 10 ppm, 100 ppm, 200 ppm and 500ppm which were diluted with distilled water, were used for pretreatment at 4 C with the duration of 24 hours at pH 5.8, adjusted with 1N HCl. These treatment conditions were determined based on Klein (3). Cold water at 4 C for 24 hours and 0.02 M 8-hydroxyquinoline at 4 C with 5 hours treatment were used for comparison. Two diploid breeding lines, IvP 35 and 85.37.38, were used for the comparison of concentrations and treatments (Table 1). Two tetraploid cultivars, Serrana.INTA and LT-8.CIP, were also tested with the optimized pretreatment method (Table 2).

Plant materials were grown under ca. 16 hour day light condition with maximum 25 C day and minimum 10 C night temperatures in a small growth chamber. Small tubers were planted in compost soil, and soil was kept humid but not wet to urge enough rooting. Young plants (5 to 10 cm height) with vigorous root development were used for sampling. Roots from the same sampling date were used for the comparison of different treatments, and the samplings were made three times at different dates with a week interval.

After pretreatment, the samples were fixed in farmer solution (3 parts absolute alcohol and 1 part glacial acetic acid for 24 hours) followed by a standard root tip squash method which is used elsewhere. Lacto-propionic orcein was used for chromosome staining. A common light microscope was used for the observation with magnification ranging from x 200 to x 1000. Ten roots per each treatment were used for observation, with at least two roots from each sampling date over total of three samplings.

TABLE 1.—*Mitotic indices with different pretreatment methods for root tips of two diploid potatoes, IvP 35 and 85.37.38.*

Pretreatment[1]		Mitotic Index (# of cells)[2]					
		I[3]	P	M	A	T	%metaphase
Ice-chilled water	(24 hours)	990	1	7	0	2	0.7
Pyrethroid 10ppm	(24 hours)	977	0	23	0	0	2.4
Pyrethroid 100ppm	(24 hours)	932	0	68	0	0	6.8
Pyrethroid 200ppm	(24 hours)	938	1	58	1	2	5.8
Pyrethroid 500ppm	(24 hours)	983	0	17	0	0	1.7
8-hydroxyquinoline	(0.02M, 5 hours)	923	14	63	0	0	6.3

[1]All treatments were made at 4 C.

[2]The results of two potato lines were combined:

$r_{(\text{IvP 35/85.37.38 mitotic index, d.f.=3})} = 0.999^{**}$, significant at the 1% level.

[3]Abbreviations for interphase, prophase, metaphase, anaphase, and telophase at mitosis, respectively.

TABLE 2.—*Effect of a pyrethroid with an optimized pretreatment condition (4C, 24 hour treatment) on mitotic index in potato root tips on two tetraploid cultivars Serrana.INTA and LT-8.CIP.*

Pretreatment	Mitotic Index[1]					
	I[2]	P	M	A	T	%metaphase
Cold water	940	3	5	0	5	0.5
Pyrethroid 100ppm	923	0	61	1	0	6.2

[1]The results on two cultivars were combined:

$r_{(\text{Serrana/LT-8 mitotic index, d.f.=3})} = 0.999^{**}$, significant at the 1% level.

[2]Abbreviations for interphase, prophase, metaphase, anaphase, telophase at mitosis, respectively.

Results and Discussion

The data of two diploid breeding lines were combined since there was no major difference in the frequency of mitotic phases ($r = 0.999^{**}$) (Table 1). It was observed that pyrethroid treatment was more consistently effective than cold water treatment (Table 1). Although the pyrethroid treatment has the same level of metaphase frequency at the concentration of 100ppm compared with 8-hydroxyquinoline, it should be noted that mitotic cells with the pyrethroid treatment provided better visibility of chromosomes than 8-hydroxyquinoline treatment. The observed effects of the pyrethroids were in the following: 1) mean cell size was greater, 2) chro-

mosomes spread well (Fig. 1 and Fig. 2), and 3) size and shape of chromosomes could be influenced by the concentration of the pyrethroid (Fig. 2). Furthermore, some irregularities in mitosis were observed: 1) chromosome associations at metaphase, 2) unsynchronized chromosome separation at anaphase, 3) restitution nuclei, and 4) multinucleate cells. In summary, pyrethroid treatment of potato chromosomes disturbs the function of karyokinetic spindles causing C-mitotic changes, as was reported for *Allium* spp. by Klein (3). An optimized pretreatment condition was tested and presented in Table 2 using tetraploid cultivars (Fig. 2).

Observing potato chromosomes by standard microscopic methods is still popular and important for routine work in ploidy manipulation, especially for a research program with a low budget, permitting accurate identification of chromosome number and karyotyping. Combined with new techniques for the determination of DNA content and ploidy level such as flow cytometry (1), improved methods of cytological characterization will lead to a better understanding and utilization of genetic resources.

High mitotic indices are essential for karyotyping and physical gene localization by c-banding and *in situ* hybridization (4, 5). Furthermore, reasonable chromosome identification in terms of shape and size is also

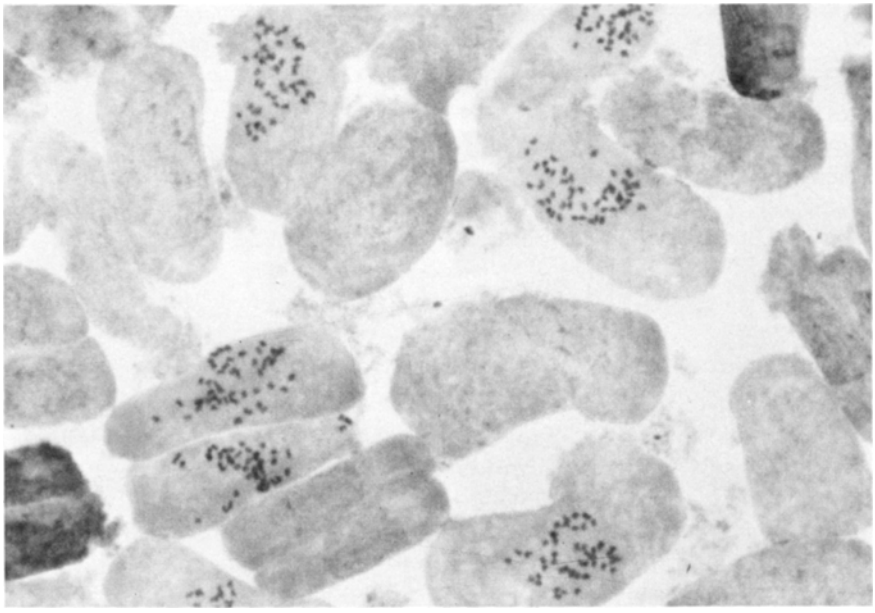

FIG. 1. A high frequency of metaphase in a microscopic view in a 4x potato clone, Serrana.INTA. The concentration of pyrethroid was 200ppm. Several cells just in a view could be used for chromosome counting.
The photograph was taken at the magnification of x400.

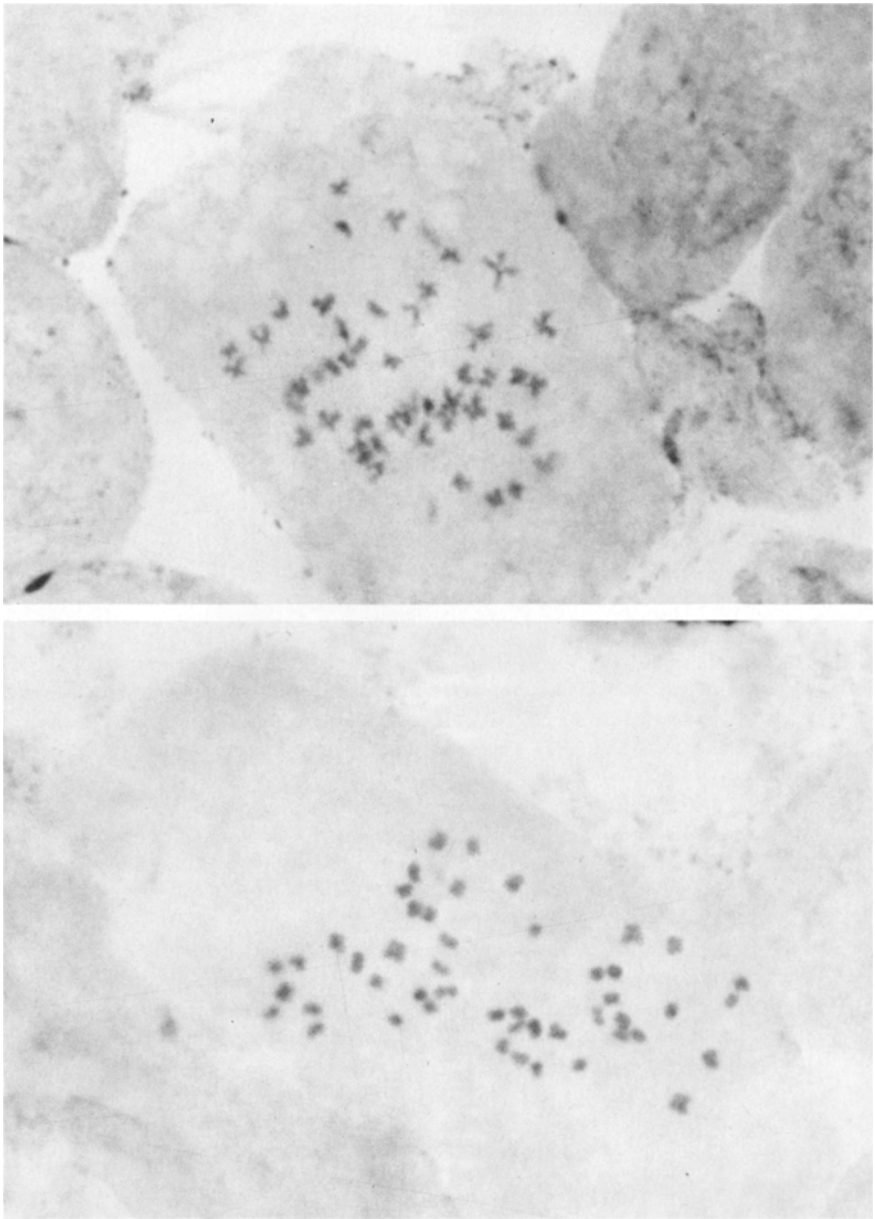

FIG. 2. A. A typical cell with spread chromosomes at metaphase by a pyrethroid treatment of 100ppm. It should be noted that the shape and size of chromosomes could be distinguished.

B. Chromosomes are more contracted like dots at metaphase by pyrethroid treatment with 200ppm but enough usable for chromosome counting.

A 4x potato clone, Serrana.INTA was used in both A and B. Photographs were taken with magnification of x1000.

a crucial point in successful observation in karyotyping and gene localization on chromosomes.

In considering cost/technology availabilities, it appears that the present finding offers an additional help to identify chromosome number and karyotyping with ease and accuracy. Better accessibility to mitotic cells and controllable chromosome sizes by this method would also help chromosome identification. Moreover, this pretreatment method is consistently applicable to other polyploid crop species in which chromosome counting is always more difficult than working on diploid genera, and pretreatment such as 8-hydroxyquinoline does not always show a desirable result. The pretreatment with pyrethroid works well on polyploid genera such as maca (*Lepidium*, 2n=?x=56), sweet-potato (*Ipomoea*, 2n=6x=90), and ulluco (*Ullucus*, 2n=3x=36) (Orrillo unpublished data).

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