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A PROCEDURE FOR OBTAINING MITOTIC CHROMOSOMES FROM MAIZE

A.L. RAYBURN, J.R. GOLD [1]

Genetics Section, Texas A & M University, College Station, Texas, 77843, U.S.A.

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ABSTRACT - A procedure is described for obtaining mitotic metaphase chromosomes from cell suspensions of maize root tips. The procedure obviates the need for squashing and coverslip removal with dry ice or liquid nitrogen, and as a consequence generally provides good harvests of well-spread, complete, cytoplasm-cleared metaphases. The manner of spreading insures cell monolayers where the metaphases are non-overlapping and easily found. A slight modification in the general procedure yields chromosomes which can be treated and/or stained to produce patterns of differential staining or banding.

KEY WORDS: Procedure, Mitotic chromosomes, Maize.

INTRODUCTION

Procedures for obtaining mitotic metaphase chromosomes from root tips of maize and other plants generally involve a squash of appropriately prepared material followed by dry ice or liquid nitrogen freezing to remove the coverslip (GILL and KIMBER, 1974; LIN, 1977; STACK and COMINGS, 1979; PRYOR *et al.*, 1980; CHOW and LARTER, 1981). While this approach is almost universal, the squash and coverslip removal steps very often result in either lost or fragmented chromosomes, or in the failure to effectively remove or clear the cytoplasm. Other difficulties include frequently overlapping metaphases due to inadequate dispersal of cells from dense meristematic tissue, long scanning times in the search for adequate metaphases, and the expense and effort involved in using dry ice or liquid nitrogen. Partially successful attempts to overcome some of these problems in other plants were made by VAN BREUGEL (1980) and HIZUME *et al.* (1980) using *Vicia faba* root tips, and by KURATA and OMURA (1978) using root tips from *Oryza sativa*. BROWN (1967) developed a smear technique for preparing maize root tip chromosomes without the squashing and coverslip removal steps. Her method, however, required that root tips be stained prior to slide making, a step which effectively

[1] To whom reprint requests should be sent.

precludes treatments to slides to produce differential staining or banding of chromosomes.

In this paper a procedure is described that bypasses the need for squashing and coverslip removal by using a root tip cell suspension from which chromosomes are then spread onto slides. The method of spreading is an amalgam of several solid tissue techniques developed for vertebrate chromosomes (FOX and ZEISS, 1961; EVANS *et al.*, 1972; STOCK *et al.*, 1972; KLIGERMAN and BLOOM, 1977), and generally produces cell monolayers where the metaphases are complete, non-overlapping, and easily found. A slight modification in the general procedure yields chromosomes which can be treated and/or stained to produce patterns of differential banding.

MATERIALS AND METHODS

Seeds are germinated in a water saturated 1:1 mixture of perlite and vermiculite and incubated in a temperature regulated growth chamber set for an 8 hour light period at 32°C and 16 hour dark period at 26°C. After 4-6 days when the secondary root tips are *ca.* 10mm long, the seeds (with root tips attached) are rinsed briefly in distilled water to remove the soil and placed in 10-15 volumes of 4×10^{-3}M (0.05%) 8-hydroxyquinoline for 2-2½ hours in the dark at 32°C. The root tips are then fixed for 24 hours at 4°C in 3:1 ethanol-acetic acid, transferred to 70% ethanol, and stored at 4°C. Chromosome preparations are generally made the following day, but storage of the fixed tissues (in 70% ethanol) for up to two months has had no apparent effects on chromosome morphology or stainability.

To prepare chromosomes, the root tips are removed from the seeds, cut in *ca.* 3mm lengths, soaked in 10-15 volumes of distilled water for 2-3 minutes, and hydrolyzed in 15-20 volumes of 5N HC1 for 45 minutes at room temperature (*ca.* 22°C). Following a 2-3 minute rinse with distilled water, each hydrolyzed root tip fragment is then placed in a depression well of an agglutination microslide (Figure 1) and treated with 4-5 drops of enzyme solution for 45 minutes at room temperature. The enzyme solution is prepared fresh and consists of 0.2g cellulysin and 0.1g macerase (both from Calbiochem-Behring Corp.) dissolved in 10ml of 10^{-3}M EDTA to a final pH of 5.1 - 5.3. By using a microslide with 12 depression concavities, several root tip fragments can be treated simultaneously under nearly identical conditions. After treatment, most of the enzyme solution is withdrawn, and the root tip fragments are gently minced in the depression concavity with a small scalpel until a visible cell suspension begins to form. Four to five drops of 60% acetic acid are added and the root tip fragments further minced for 2-3 minutes. Fifty to sixty microliters of the cell suspension is then withdrawn into a 50µl disposable accupette pipet with a rubber suction tube (Figure 1) and expelled gently onto a precleaned slide heated to *ca.* 48°C on a slide warmer. After 25-30 seconds, the suspension is withdrawn back into the accupette leaving a ring of cells *ca.* 1 - 1.5cm in diameter. The process is repeated, and generally three such rings can be made per slide. The slides are removed from the slide warmer after drying for 2-3 minutes. At this point,

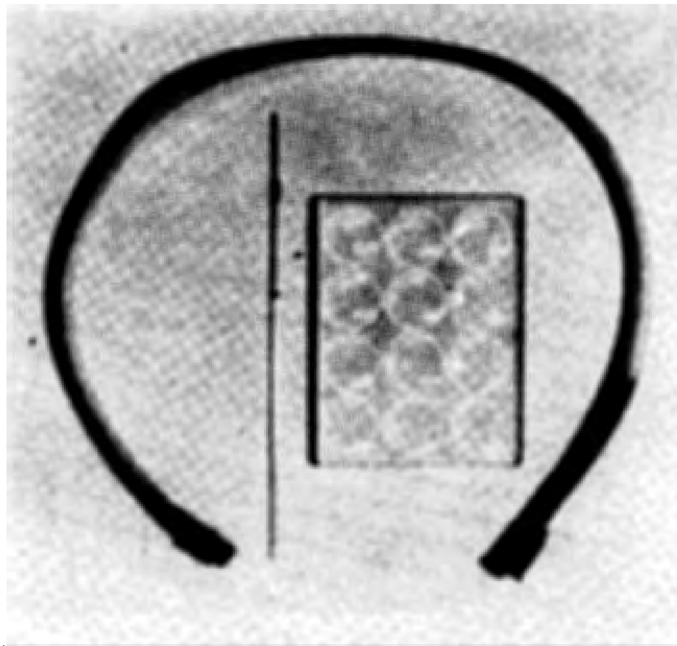

FIGURE 1. Apparatus used in enzyme treatment and slide preparation. The agglutination microslide with 12 depression concavities (ea. 16mm diameter x 1.75mm deep) is for holding root tip fragments during enzyme and 60% acetic acid treatments; the 50 µl accupette pipete and rubber suction tube are for transferring cell suspensions onto slides.

slides may be treated and/or stained, or stored dessicated at room temperature for up to 2-3 weeks with no apparent ill effects on the chromosomes. For standard karyotypes, slides are stained seven minutes with 2% Giemsa in 10^{-2}M phosphate buffer at pH 6.8, rinsed in distilled water, air dried, cleared in xylene for 5-10 minutes, and mounted in permount.

For C-banding (with Giemsa) or H-banding (with Hoechst 33258), the acid hydrolysis of root tips is carried out with 0.2N HC1 for 10 minutes at 40°C. All other root tip preparation and slide making steps are the same. Details of treatment and/or staining procedures for C- and fluorescent H-banding are to be presented fully elsewhere (RAYBURN and GOLD, 1982), but essentially follow commonly used methods in maize and other organisms (FILION and BLAKEY, 1979; STACK and COMINGS, 1979; WARD, 1980; CHOW and LARTER, 1981; HILWIG and GROPP, 1972; WHEELER and ALTENBURG, 1977).

RESULTS AND DISCUSSION

The above procedure has been used in our laboratory to preparare

FIGURE 2. Standard karyotypes of a) KYS - a standard inbred line, and b) *Zea diploperennis* - a perennial teosinte originally collected in 1979 from Manantlan, Jalisco, Mexico. Bar = 10 µm.

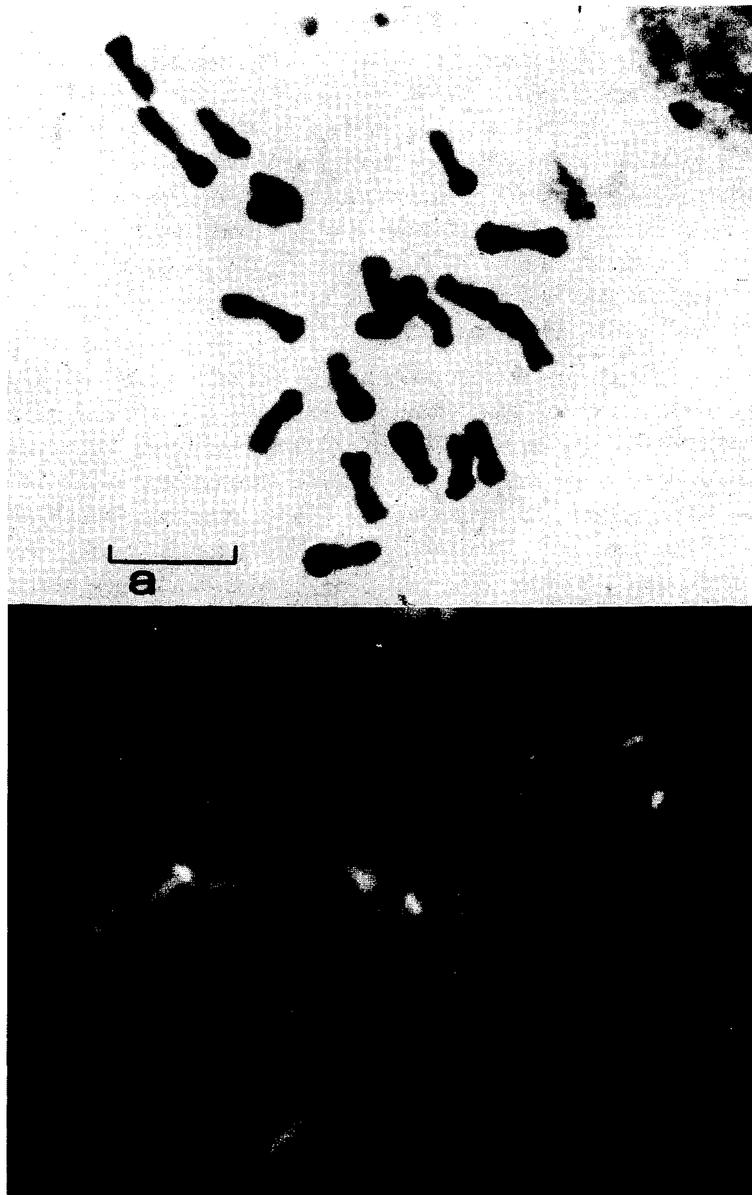

FIGURE 3. Banded karyotypes: a) C-bands of *Zea mays mexicana,* an annual teosinte from Mexico; and b) H-bands of KYS. Bar = 10 µm.

both standard and banded karyotypes of several different strains of maize (Figures 2-3). Minor adjustments in either HC1 hydrolysis or enzyme treatment times are often necessary for different strains. On the average, about 15-20 metaphases are obtained per ring (=50-60 per slide), and of these at least half are well-spread with only one or a few overlapping chromosomes. Most metaphases are complete (2n = 20). At the mitotic inhibitor level suggested (viz., 0.05% 8-hydroxyquinoline for 2-2½ hours), the majority of the chromosomes are in mid-metaphase; less or more contracted chromosomes (in prophase or late metaphase) can be harvested by appropriately altering inhibitor concentration, time of treatment, or both. Is should be noted that the procedure also has been used successfully on other plants (Figure 4). Slight modifications in the germination procedure and in the time of acid hydrolysis of root tips were all that was necessary to adapt the procedure for these species.

The central advantage of the procedure is the elimination of squashing and coverslip removal. For most plant chromosome preparations, squashing serves in part to break the pre-softened cell wall and clear the cytoplasm, and in part to detach the cells and spread the chromosomes. The problems lie in the inconsistency of achieving all these functions simultaneously, and also in the loss or fragmentation of chromosomes during coverslip removal. With the procedure described here, the chromosomes are usually free of the cell wall and separated from one another insuring both good contrast and definition of chromosomes for photography. The detachment of free, intact cells into a suspension insures both adequate dispersal of metaphases (i.e., few overlapping cells on slides) and good spreading of individual chromosomes within a cell. The manner of spreading offers the further advantage that most metaphases migrate to the periphery of the rings and can be easily and quickly located. Other benefits of the procedure are that unstained slides can be stored more or less indefinitely, different types of chromosome banding methods can be applied to slides from the same root tip, and permanent preparations are easily made.

The success of the procedure depends primarily on three factors, viz., the conditions of acid hydrolysis of root tips, the length of time the cells are in 60% acetic acid, and the temperature of the prewarmed slide. Optimal conditions for the acid hydrolysis are determined by monitoring stained slides and observing the point when maximal numbers of free, intact nuclei are produced. Details of the effects of modifying the other two variables are discussed fully in Evans *et al.* (1972), Stock *et al.* (1972), and Kligerman and Bloom (1977). Briefly, increased time in acetic acid can improve chromosome spreading, but often results in

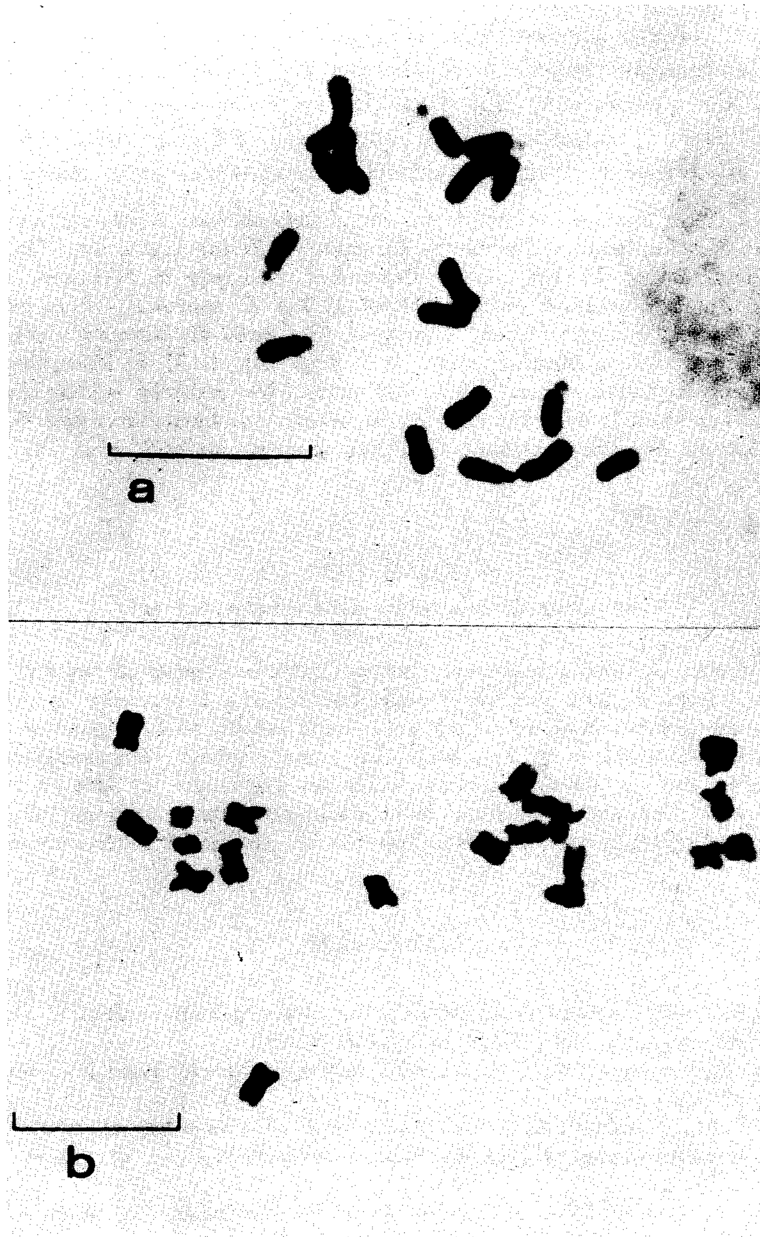

FIGURE 4. Standard karyotypes of a) *Microseris douglasii,* and b) *Arachis batizocoi.* Bar = 10 μm.

prematurely burst nuclei and scattered chromosomes; shorter times yield undetached cells and less well-spread chromosomes. Raising the temperature of the prewarmed slides can also improve chromosome spreading, but generally produces chromosomes with poor stainability and morphology. Lower temperatures impede both chromosome spreading and migration of cells to the periphery of the ring. Suffice it to say that the concentrations, times, temperatures, etc., suggested here are those empirically derived as optimal for preparing root tip chromosomes from maize.

Seeds of the maize stocks used in this study were obtained as follows: KYS - from Dr. M. P. Maguire, Zoology Department, University of Texas, Austin, Tx.; *Zea diploperennis* - from Dr. H. H. Iltis, Botany Department, University of Wisconsin, Madison, Wis.; and *Zea mays mexicana* (sent as P.I. n° 331783, *E. mexicana*) - from the USDA Regional Plant Introduction Station, Experiment, Ga. Seeds of *Microseris douglasii* and *Arachis batizocoi* were obtained from Ms. J. L. Riggs and Ms. K. S. Davis, respectively, of the Genetics Section, Texas A & M University. We gratefully acknowledge these courtesies. The research was supported by the Texas Agricultural Experiment Station at College Station, Tx.; the equipment used was provided by NSF grant DEB-8022173 to JRG.

RIASSUNTO

Una tecnica di preparazione dei cromosomi del mais

Viene descritto un procedimento per ottenere cromosomi metafisici mitotici da sospensioni cellulari di apici radicali. Il procedimento evita la necessita di operare la rimozione del vetrino con ghiaccio secco o con azoto liquido, ed è in grado di produrre ottime metafasi, complete e ben distese. La tecnica assicura l'ottenimento di strati monocellulari dove le metafasi possono individuarsi facilmente e dove esse non si sovrappongono. Una piccola modifica del procedimento generale permette di ottenere cromosomi che possono essere trattati o colorati con tecniche di colorazione particolari.

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