sister centromere separation at the first meiotic division followed by plate re-orientation of these centromeres, so that equational distribution at the first division and disjunctional distribution at the second division were possible. Results of a systematic study of defects found after application of the various irritants used show that this type abnormality seems to be associated only with treatment with ethylene glycol and related compounds. These include carbowax, a polyethylene glycol which is a common base for medicinal ointments.

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1. Differential Giemsa staining in maize.

Direct application of mammalian Giemsa banding techniques to the somatic chromosomes of maize does not result in banded chromosomes. Further, techniques employed with other plants do not yield suitable banding in maize. This report describes a series of experiments designed to obtain reproducible banding patterns concomitant with the maintenance of chromosome morphology.

Slides were prepared according to Chen[1] with the exception that a 23 hour cold treatment (4°C) was used in lieu of the 8-hydroxyquinoline treatment. Cover slips were removed by the dry ice method and the slides were air dried. The dry slides were stored in a dessicator for up to one week. Dry slides were then "pretreated" with one of various reagents or a combination thereof (Table 1), stained in Giemsa solution, air dried and made permanent.

Giemsa stain is a complex mixture of dyes and as expected, different sources, e.g., Fisher Scientific Co., Gurr R66 and Curtin Scientific Co., produced variable results. That is, different dilutions and staining times were required to yield equivalent staining; Fisher brand is used currently. Reference to Table 1 shows the range of pH, concentration and temperatures used to stain the slides. Salient points include the

following: a) the Giemsa solution should be buffered within a pH range of 6.8 - 7.0. The molarity of the buffer is important; that is, a concentration greater than 0.1M inhibits staining. b) The magenta compound[2] was present consistently if the stain was preheated to 40°C. c) We found that lower stain concentrations (1-2%) with longer times yielded more reproducible results. Staining times have not been given because these varied with the concentration of the stain and the "pretreatment" applied. In addition, fresh slides (less than one day old) required longer staining than older slides. d) The intensity of staining varied from cell to cell on any one slide. e) Only chromosomes completely outside the cell displayed clear banding, since the cell wall obscured the banding pattern. f) Banding patterns were not always present in every chromosome in any one metaphase spread.

Table 1

Band Inducing Reagents (A) and Staining Methods (B)

A. Pretreatment	Reagent	Range of pH	Range of Conc.	Range of Temp.
I Denaturant				
(i)	NaOH	12.9	0.1N	room temp.
(ii)	NaOH + NaCl	9-12	0.07N - 0.112N	room temp.
(iii)	$Ba(OH)_2$	12.9-13.5	0.1N	room temp.
Renaturant	SSC	7.0-8.2	2X	60° -65°C
II Proteolytic Enzyme	Trypsin	6.0-8.0	0.025-0.2%	4° -35°C
III Protein Denaturant	Urea	-	6M	room temp.
IV A.S.G.	SSC	7.0	2X	60° -65°C
B. Stain	Solvent			
Giemsa	Sörensen's Buffer (M/15)	6.7-7.0	1 - 20%	room temp.-50°C

C-banding

Centromeric banding was obtained by denaturing with $Ba(OH)_2$ (pH 13.5) and renaturing with 2X SSC, (pH 8.5) (Table 1) for two hours at 60°C or 65°C, and staining in 10% Giemsa (pH 6.8). These bands consistently appeared only on condensed chromosomes. Chromosome morphology was unsatisfactory due to a swollen, distorted appearance.

G-banding

No G-bands (chromosome cross banding revealed by Giemsa stain) were obtained using NaOH or NaOH-NaCl denaturants (Table 1). Depending on the length of these "pretreatments," chromosome morphology ranged from a distorted, ghost-like appearance to complete disintegration. Some large bands similar to those reported by Vosa[3] were obtained with $Ba(OH)_2$ (pH 12.9) plus 2X SSC (pH 7.0, 60°C). Again, chromosomes were distorted.

The acetic-saline-Giemsa (A.S.G.) technique produced faint G-bands after 2 hours incubation in 2X SSC at 60°C. No banding was apparent after longer incubation times (up to 24 hours).

The protein denaturant urea was used, following the technique of Döbel *et al.*[4] Moderate banding was obtained, but chromosome morphology was unsatisfactory. Further attempts with lower molarity urea solutions are in progress.

Reasonable G-banding has been obtained with trypsin (0.1%, 32°C, pH 7.5)[5], and staining with 2% Giemsa (M/15 Sörensen's buffer, pH 7.0) at 40°C. Chromosome morphology is still not totally acceptable.

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