sister centromere separation at the first meiotic division followed by
plate re-orientation of these centromeres, so that equational distribu-
tion 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 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 desiccator 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, differ-
ent sources, e.g., Fisher Scientific Co., Gurr R66 and Curtin Scientific
Co., produced variable results. That is, different dilutions and stain-
ing 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 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)

<table>
<thead>
<tr>
<th>A. Pretreatment</th>
<th>Reagent</th>
<th>Range of Conc.</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Denaturant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) NaOH</td>
<td>12.9</td>
<td>0.1N</td>
<td>room temp.</td>
</tr>
<tr>
<td>(ii) NaOH + NaCl</td>
<td>9-12</td>
<td>0.07N</td>
<td>room temp.</td>
</tr>
<tr>
<td>(iii) Ba(OH)₂</td>
<td>12.9-13.5</td>
<td>0.1N</td>
<td>room temp.</td>
</tr>
<tr>
<td>Renaturant</td>
<td>SSC</td>
<td>7.0-8.2</td>
<td>60° -65°C</td>
</tr>
<tr>
<td>II Proteolytic Enzyme</td>
<td>Trypsin</td>
<td>6.0-8.0</td>
<td>0.025-0.2%</td>
</tr>
<tr>
<td>III Protein Denaturant</td>
<td>Urea</td>
<td>-</td>
<td>6M</td>
</tr>
<tr>
<td>IV A.S.G.</td>
<td>SSC</td>
<td>7.0</td>
<td>2X</td>
</tr>
<tr>
<td>B. Stain</td>
<td>Solvent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giemsa</td>
<td>Sörensen's Buffer (H/15)</td>
<td>6.7-7.0</td>
<td>1 - 20%</td>
</tr>
</tbody>
</table>
G-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 (N/15 Sörensen's buffer, pH 7.0) at 40°C. Chromosome morphology is still not totally acceptable.

References:


W. Gary Filion