An Improved Cytogenetic Method for Maize Pachytene Chromosomes

Eveline Teixeira Caixeta and Carlos Roberto de Carvalho*

Departamento de Biologia Geral, Universidade Federal de Viçosa, 36571-000, Viçosa-MG, Brazil

Accepted March 6, 2001

Summary Chromosome analysis in pachytene has been considered a powerful tool in cytogenetic and evolutionary studies carried out in several plant species. Many morphological details of chromosomes can be observed during this stage, although overlapping of chromosomes may hinder the analysis. Considering this limitation, a new cytogenetic methodology was developed for maize meiotic chromosomes. This method consists of submitting the pollen mother cells (PMCs) to an enzymatic solution, which promotes PMC release from the anthers and subsequent digestion of the cell wall. The bivalents are mechanically released into the cytoplasm and spread out over the slide. In this slide preparation the chromosomes were isolated and spread over in the same plane of focus. The preservation of morphological traits of the bivalents, and the near complete absence of background or artifacts, resulted in high quality preparations, adequate for cytogenetic analysis. The obtainance of individual chromosomes allowed for the detailed analysis of the 10 maize bivalents. This analysis revealed additional data on the morphology and longitudinal differentiations of the chromosomes, facilitating their identification and morphological characterization.

Key words Maize chromosomes, Pachytene, Plant cytogenetics.

Cytogenetic analysis in maize has been carried out both in mitotic and meiotic chromosomes. The most useful stage for studies that require precise characterization of chromosomal morphology in terms of longitudinal differentiation has been the meiotic prophase (Rhoades and McClintock 1935, Carlson 1988). In the pachytene the chromosomes are usually slender and, therefore, exhibit many features not recognizable in condensed chromosomes of somatic metaphases (Rhoades and McClintock 1935, Swanson 1964, Dempsey 1994).

The importance of chromosome analysis during pachytene is widely recognized, however, the overlapping of bivalents may difficult the chromosome visualization (Dundas et al. 1983, Carlson 1988). Besides, in plant cells, the cell wall works as a physical barrier which blocks chromosome spreading and keeps the cytoplasmic background in conventional cytogenetic preparations. The presence of the cytoplasm obscures the structural details of the chromosomes (Shen and Wu 1989). Dempsey (1994) reports that, in some maize stocks, the pachytene chromosomes are not easily separated in squashed preparations and, when they become sufficiently compacted to allow individual pairs to be distinguished, structural details may be lost by condensation.

Considering these limitations, we developed a slide preparation technique for maize meiotic chromosomes. This new methodology aims to isolate bivalents in pachytene when they are less compact, but with enough resolution for a detailed analysis of their morphology.

Material and methods

Plant material collection and storage

Maize (Zea mays L.) seeds of the inbred line L-869 were obtained from the germplasm bank

* Corresponding author, e-mail: ccarvalh@mail.ufv.br
of Universidade Federal de Viçosa. Male inflorescences were selected in order to obtain only anthers in pachytene stage. Panicles were removed and the anthers were fixed in fresh cold methanol-acetic acid solution (3 : 1). The fixative was changed three times and stored in a freezer (−20°C) for one to several days.

**Preparation of cytological material**

Approximately 15 anthers were removed from the fixative solution, placed in a scooped plate containing distilled water and transferred to a specially adapted 0.5 ml microfuge tube (microcentrifuge filters-Sigma®), which had its original membrane filter removed and replaced with a nylon screen (100 μm pore size). The material was washed, three times, in distilled water for 15 min, in order to remove all fixation solution. After washing, the excess water was removed with filter paper. The tube containing the anthers was immersed in a not diluted enzymatic solution, enough to cover the anthers, and placed in an incubator at 35°C, for 50 min. The enzymatic solution used, Flazyme™, is a liquid product prepared by NOVO FERMENT™, which contains pectinolytic, cellulolytic and hemicellulolytic activities. After digestion, the anthers were washed again for 15 min, in 3 small Petri dishes containing distilled water. The adapted microfuge tube was then placed into a 1.5 ml microfuge tube, filled with enough distilled water to reach the nylon screen. The anthers were mechanically ground with a stylet, releasing the pollen mother cells (PMCs) into the 1.5 ml microfuge tube. The cell suspension formed in the 1.5 ml tube was centrifuged at 150 g for 5 min. The supernatant was removed, remaining 0.5 ml of the solution, and 30 μl of the previously mentioned enzymatic solution was added. The cells were carefully resuspended. After digestion (at 35°C, for 1 h), the tube was filled up with distilled water and centrifuged at 150 g for 5 min. In order to completely remove the enzymatic solution, this washing procedure was repeated four times. The supernatant was removed and, to the cellular sediment, a fresh fixative solution (methanol–acetic acid 3 : 1) was dripped, in such a way that the droplet went down the tube wall, thus avoiding chromosome disruption due to the shock between the fixation solution and the cellular suspension. After 10 min of incubation in a freezer, the fixative solution was changed twice. Slides were prepared in the following sequence: centrifugation of the fixed suspension; supernatant removal, leaving approximately 0.3 ml of material; careful resuspension; dripping in a clean, cold slide, from a height of approximately 20 cm. Slides were air-dried, placed onto a hot plate at 50°C and stained with 5% Giemsa in phosphate buffer, pH 6.8, for 7 min, then washed twice in distilled water and air dried.

**Image analysis**

Images of meiotic chromosomes were captured with an immersion objective (100×) and digitized by imaging system. Measurements of the bivalents were taken using NIH-Image SXM 1.61, a public domain software package (Rasband 1997). Absolute values of the bivalents were obtained by separately measuring the total length of the short and long arms. All values were calculated as the average of 10 measurements.

**Results and discussion**

In the standardization of the air-drying technique with enzymatic maceration, describe in this paper, an enzyme solution was used in order to release the pollen mother cells (PMCs) from the anthers and promote cell wall degradation. Both the release of the PMCs and cell wall digestion were efficient, with few damaged cells, and the morphological integrity of the chromosomes was maintained. The bivalents were mechanically released from the cytoplasm and spread out on the slide.

Preparations brought about with this technique allowed for the obtainance of pachytenees containing the 10 bivalents in the same plane of focus, with their morphology well preserved (Fig. 1a). The 10 bivalents were identified and represented as an idiogram (Fig. 1b). Chromosomes were iden-
tified based on length, on the presence of knobs and, in some cases, by the arm ratio. Based on these criteria, it was observed that, for this particular inbred line, chromosome 5 (53.26 µm) is slightly longer than 4 (50.47 µm). In the majority of the pachytenes analyzed, it was also observed that chromosome 8 is longer than 7 (Table 1). However, due to some unidentified treatment effect, the idiogram displays chromosome 7 longer than 8. Inversions in the measurements of bivalents confirm the data observed by Rhoades (1955). Bivalent 6 showed a length of only 38.36 µm, but it is conventionally identified by the nucleolus organizing region (NOR).

The values of total length and arm length of chromosomes from different pachytenic cells may display some variation, due to different degrees of compactation. Therefore, 10 measurements from each of the 10 chromosomes were taken (Table 1). By examining the data on this table, it confirms that bivalent 5, with an average length of 50.76 µm, is slightly longer than 4 (48.48 µm); 8 (45.21 µm), slightly longer than 7 (41.91 µm); and 6 (39.71 µm), shorter than 7 and 8. Table 1 also shows that the standard deviation values for the arm ratio, for all 10 bivalents, are very small, demonstrating that this feature is of great relevance for the bivalents identification. The position of the centromere, however, is not easily recognized, for many times it is confused with gaps observed along the bivalents (Carlson 1988). The importance of the centromere identifica-

![Fig. 1. a) Pachytene obtained by the air-drying technique with enzymatic maceration. Bivalents are identified with their respective numbers. b) Idiogram of the 10 bivalents containing measures and some specific features of each chromosome.](image)

Table 1. Average and standard deviation values for absolute length, long and short arm lengths, and arm ratio for the 10 maize bivalents

<table>
<thead>
<tr>
<th>Chromosome number</th>
<th>Related length</th>
<th>Absolute length (µm)</th>
<th>Long arm (l)</th>
<th>Short arm (s)</th>
<th>Arm ratio (r=l/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>216.23</td>
<td>68.61±5.83</td>
<td>35.65±4.43</td>
<td>29.72±1.76</td>
<td>1.08±0.03</td>
</tr>
<tr>
<td>2</td>
<td>177.40</td>
<td>56.29±2.83</td>
<td>32.95±4.15</td>
<td>26.57±1.37</td>
<td>1.12±0.06</td>
</tr>
<tr>
<td>3</td>
<td>175.01</td>
<td>55.53±6.85</td>
<td>38.27±4.84</td>
<td>25.02±3.14</td>
<td>1.55±0.03</td>
</tr>
<tr>
<td>4</td>
<td>152.79</td>
<td>48.48±3.35</td>
<td>29.88±2.00</td>
<td>18.60±1.83</td>
<td>1.61±0.14</td>
</tr>
<tr>
<td>5</td>
<td>159.97</td>
<td>50.76±5.76</td>
<td>25.74±2.75</td>
<td>17.26±2.05</td>
<td>2.22±0.07</td>
</tr>
<tr>
<td>6</td>
<td>125.15</td>
<td>39.71±2.54</td>
<td>27.97±1.83</td>
<td>17.74±1.00</td>
<td>1.59±0.17</td>
</tr>
<tr>
<td>7</td>
<td>132.30</td>
<td>41.98±3.70</td>
<td>30.46±2.85</td>
<td>27.97±1.83</td>
<td>2.64±0.12</td>
</tr>
<tr>
<td>8</td>
<td>142.48</td>
<td>45.21±3.11</td>
<td>34.50±2.33</td>
<td>11.52±0.93</td>
<td>3.23±0.11</td>
</tr>
<tr>
<td>9</td>
<td>116.55</td>
<td>36.98±4.40</td>
<td>24.63±3.16</td>
<td>10.70±0.84</td>
<td>3.23±0.11</td>
</tr>
<tr>
<td>10</td>
<td>100.00</td>
<td>31.73±3.28</td>
<td>22.67±2.38</td>
<td>9.06±0.96</td>
<td>2.50±0.10</td>
</tr>
</tbody>
</table>
tion highlights the requirement of a good quality cytological preparation.

This analysis revealed additional data on the morphology and longitudinal differentiations of the chromosomes, facilitating their identification and characterization, as well as allowing for a more detailed morphological analysis of the bivalents. Literature data has listed additional applications of a detailed bivalent analysis, besides chromosome identification. Cheng and Bassett (1981) mention that, in the work of Brown (1949), chromatic and achromatic segments observed in tomato chromosomes are parameters for studies the process of chromosome condensation, pairing and chiasmata frequency. These same authors used these regions in bean chromosomes for condensation studies. Blumenschein (1968) described several studies correlating knobs with morphological features of the maize plant. In this species, morphological details observed along the bivalents may help to the elucidate phenomena related with it genetic, as well as be extrapolated to other higher organisms.

Morphological analysis of bivalents has been considered to be a powerful tool in genetic and cytogenetic studies. However, the overlapping and tangling of these chromosomes may difficult the analysis. The cytogenetic technique used in this work allowed for the visualization of isolated, well defined chromosomes, evenly spread out in the same plane of focus on the slide. The morphological preservation of the bivalents and the near absence of background or artifacts resulted in high quality cytogenetic preparations.

Acknowledgements

The study was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais-FAPEMIG and CNPq, Brazil.

References


