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1. Methods for electron microscopic study of maize pachytene chromosomes.

In the course of making serial electron micrographs of maize pachytene nuclei, a number of different techniques were tried in an effort to obtain maximum contrast and ease of reconstruction of the synaptonemal complexes which hold together the pachytene bivalents. These various fixation and staining techniques may be useful to workers who contemplate ultrastructural studies of various features of the meiotic prophase nucleus.

Fixation of Anthers.

A. Aldehydes (1) 4% formaldehyde in 0.1 M cacodylate buffer + 4% sucrose; (2) 6% glutaraldehyde in 0.1 M cacodylate buffer + 4% sucrose.

Fix for ½ to 1 hour in (1), then add an equal volume of (2) to give a concentration of 2% formaldehyde + 3% glutaraldehyde. Fix a further 2 to 2½ hours. Total fixation time - 2½ to 3 hours.

0.067 M phosphate buffer has also been used with success. Sucrose may be omitted. It is important to keep the anthers beneath the surface of the fixative as they have a tendency to float, and uneven fixation results. Holding them down with a piece of tissue (Kimwipes) or fine cloth, or shaking in a rotary shaker usually improves the uniformity of fixation within and between anthers. Physical damage to the anthers should be avoided, although cutting anthers in half with a fine clean cut (e.g. a new, degreased razor blade) improves fixation with only marginal damage. Anthers are usually dissected out in buffer or the first fixative.

B. Wash 3 times ½ hour in buffer + sucrose (or buffer only).

C. OsO_4 - 2% in buffer. Fix for 1½ hours.

D. Wash 3 times ½ hour in distilled water.

E. Stain for 3 hours at 60° C in 2% aqueous uranyl acetate (Locke et al., 1971).

F. Wash thoroughly 3 times 1 hour in distilled water.

Dehydration and Embedding.

Alcoholic dehydration has been used. Infiltration and embedding with Spurr's (1969) low viscosity resin can be carried out directly from the dry absolute alcohol stage. Luft's (1961) Epon or Araldite mixtures may also be used after propylene oxide. Flat embedding of anthers allows orientation so that sectioning is carried out perpendicular to the longitudinal axis of the anther and all four locules can be examined.

Staining.

After sectioning and picking up the sections on grids, only lead staining is necessary, such as the method of Reynolds (1963).

Two techniques which give various degrees of preferential staining of the synaptonemal complex have been tried.

(1) Bernhard's (1969) EDTA Technique destains selectively the DNA but not RNA and results in the synaptonemal complex being stained but the surrounding chromatin remaining unstained. Osmium fixation is omitted; therefore omit steps B through E in the above schedule. After picking up the sections, the grids are stained in saturated uranyl acetate for 20 minutes, floated on 0.2 M EDTA for 1 to 2 hours, washed and stained with lead citrate as above. The length of time in uranyl acetate and EDTA has to be varied according to the thickness of the sections. This method gives good contrast of the lateral elements of the synaptonemal complex. Chromatin in centromeres and the nucleolus organizer also appears to stain. Care is necessary to avoid contamination of the sections by precipitation during the long EDTA step. Step E above may be included but staining with uranyl acetate after sectioning is usually still necessary.

(2) Positive Phosphotungstic Acid (PTA) - Sheridan and Barrnett (1969). This technique results in staining of basic protein residues in the absence of osmium staining. Hence steps B through E are again omitted in the fixation. After dehydration, the anthers are stained overnight in alcoholic 1% PTA at ice temperature (or in refrigerator). The time of staining appears to have some effect on the specificity as 15 hours resulted in the lateral elements of the synaptonemal complex staining much more intensely than the chromatin, whereas 19 hours found the chromatin almost as electron dense as the lateral elements. The nucleolus organizer and the centromeric chromatin are also differentially stained.

After staining, the anthers are washed several times in absolute alcohol, infiltrated, embedded and sectioned as usual. No further post-sectioning stain is required.

Both the EDTA and the PTA methods omit OsO_4 fixation and hence the preservation of membranes is not always perfect. Both are useful for allowing the tracing of synaptonemal complexes through serial sections without the hindrance of obscuring chromatin, yet they allow chromatic knobs and in particular the centromeres and nucleolus organizer to be identified. Using the PTA method, I have been able to reconstruct and identify the synaptonemal complex of entire pachytene bivalents from maize microsporocytes, including inversion heterozygote bivalents. The reconstruction technique is essentially the same as I had previously used for Neurospora crassa (Gillies, 1972).

References

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