

## A STAINING METHOD FOR LIGHT MICROSCOPIC STUDY OF NUCLEOLAR STRUCTURE IN PLANT CELLS

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Ever since the nucleolus was noted by Fontana (1781), a vast array of literature has accumulated on staining procedures for its study. However, most are for animal cells and there are few for plant cells. These techniques are reviewed by Busch and Smetana (1970). A method suggested by Das (1962) and later modified by Das and Alfert (1963) uses silver nitrate followed by photographic developers. Silver nitrate staining after hydroquinone-formalin fixation has been suggested for routine use (Gomez *et al.* 1969, Stockert *et al.* 1969). However, by these light microscopic techniques the entire nucleolus is darkly stained and intranucleolar structure is observed.

For the study of intranucleolar organization a modified silver stain is suggested here. This method was perfected using root tips of *Allium cepa*; it also has given good results with roots of *Areca catechu* L.

*Materials and Methods.* Root tips about 5 mm long from pot-grown *Allium cepa* bulbs and seedlings of *Areca catechu* L. were used for the present study. Roots pretreated with 0.01%  $\text{KMnO}_4$  were thoroughly washed in distilled water and fixed in freshly prepared and aged (2 days) 1:1 mixtures of 10% formalin and 1%, 1.5% and 2% hydroquinone separately for either 1, 2 or 6 hr. Roots thus fixed were then washed in frequent changes of distilled water for 30–45 min. Next they were stained in 2% and 3% aqueous silver nitrate solutions for 15, 24, 36 or 48 hr at 70 C in the dark. The stained roots were washed in distilled water for 30–45 min and reduced in fresh and aged 1:1 mixtures of 10% formalin with 1%, 1.5% and 2% hydroquinone. The roots were then squashed in 50% acetic acid and examined under the microscope with transmitted light.

*Results and Comments.* All the above treatments gave well stained nucleoli. When freshly prepared hydroquinone-formalin was used for fixing nucleoli stained darkly as compact or vacuolated structures, but roots fixed in the mixture aged for 2 days and stained for 36 hr showed more detail. The latter showed an outer amorphous region and a darker, inner, reticulate fibrillar region. In many cells the inner fibrillar part had two darker regions which showed continuity with the rest of the nuclear material through the amorphous region. The dark regions represent the nucleolus organizing regions from which the nucleolus is formed. Slides stained in this way are optimally observed in the first 72 hr after preparation.

Of the various combinations of fixative and staining solutions tried, the following procedure was found to yield the best intranucleolar differentiation (Fig. 1).

- 1) Shake root tips 5 mm long in 0.01% aqueous  $\text{KMnO}_4$  for 5 min.
- 2) Wash in distilled water for 10 min.
- 3) Fix in 1:1 mixture of 10% formalin and 1% hydroquinone aged for 2 days, for 6 hr at room temperature.
- 4) Wash in distilled water for 30–45 min with frequent changes.
- 5) Stain in 2%  $\text{AgNO}_3$  at 70 C for 36 hr in the dark.

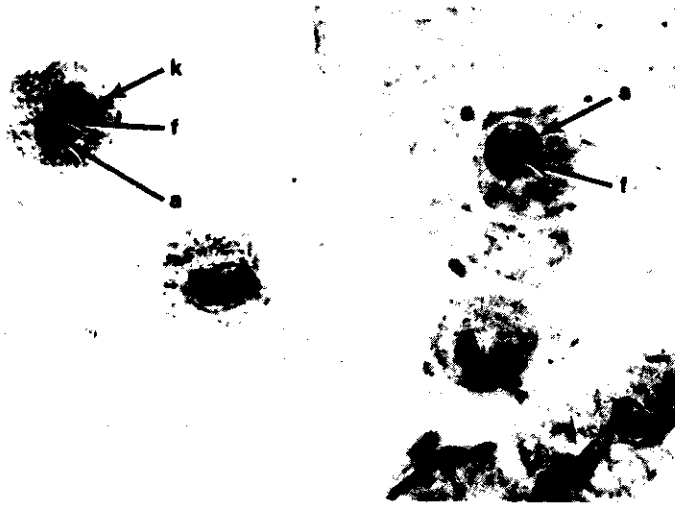


FIG. 1. Root tip nucleoli, stained as described. Left, onion; right, *Areca*: (a) outer amorphous region, (f) inner fibrillar region, (k) two knoblike dark areas (the nucleolus organizing regions) which show continuity with the nuclear material.  $\times 1500$ .

- 6) Wash in distilled water for 30–45 min with frequent changes.
- 7) Reduce in fresh 1:1 mixture of 10% formalin and 1% hydroquinone.
- 8) Squash in 50% acetic acid on a clean slide and seal the sides of the coverslip.

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