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A TECHNIQUE FOR PERMANENT CHLOROPLAST PREPARATIONS

THE chloroplast count technique is a rapid and reliable method of determining ploidy levels in a plant species¹. It is easier to study chloroplasts in the guard cells, following reduction of silver nitrate. The staining principle is based on M. Lisch reaction^{2,3}. This method requires an immediate count as the stained chloroplasts lose their colour when exposed to light. On keeping the preparation for longer time, the entire epidermal layer becomes reddish-brown. A method to prepare permanent mount is not available. In plants like okra, the mucilage interferes in staining. A method was, therefore, developed for the preparations of permanent chloroplasts of *Abelmoschus* sp.

Procedure

Adaxial epidermis of leaves of a plant exposed to sunlight for four hours was stripped off and placed on a glass slide. A drop of 2% silver nitrate solution was added on the strip for a minute. The strip was then washed thoroughly with distilled water and fixed for 5 minutes in a few drops of hypo solution (25 g sodium thiosulphate and 0.1 g potassium metabisulphate dissolved in 200 ml of distilled water). After washing in distilled water, it was mounted with canada

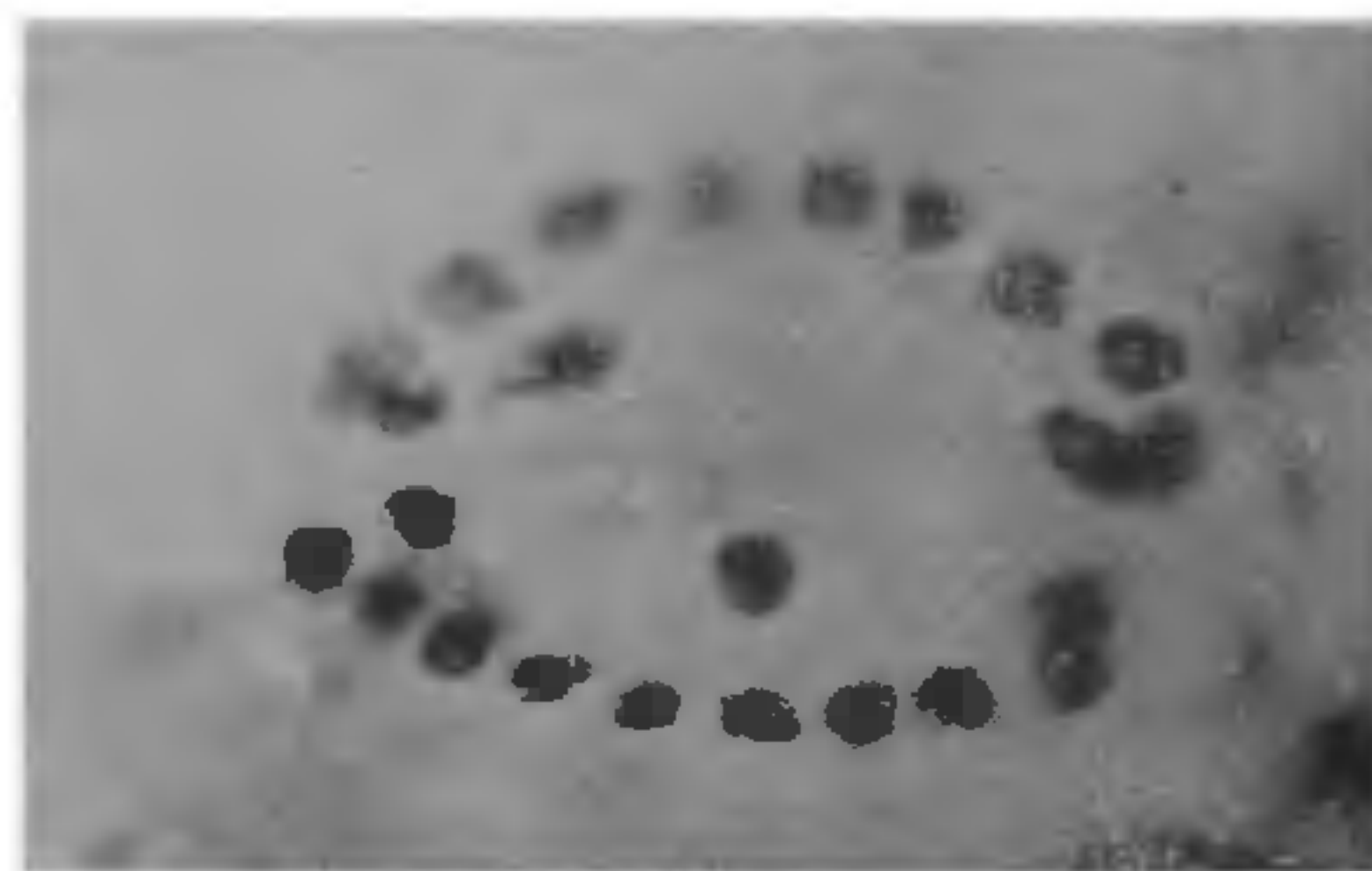
balsam. The slide was then dried and observed under a microscope.

The preparation retained stain for a few days after which there was cloudy appearance due to the release of the mucilage. To overcome this, the slides were made permanent by adopting the following additional step:

After washing the strip, free of hypo, it was dehydrated by passing through butanol-acetic acid series (1:1, 3:1) and finally through *n*-butanol, keeping for 2 minutes in each. Then it was mounted in canada balsam. A preparation of guard cell chloroplasts of *Abelmoschus* sp. stained by this method is illustrated in Fig. 1.



(a)



(b)

FIG. 1. Chloroplasts in stomatal guard cells of *Abelmoschus* sp. a, diploid; b, tetraploid ($\times 3,850$).

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