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Short Technical Note

A simple and rapid permanent squash technique for bulk-stained plant material

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There are a variety of methods for making permanent squash preparations, each of which has certain advantages as well as disadvantages. Many cells may be lost when the coverslip is detached from the slide by the acetic acid method (Haskell & Wills, 1968). The quick-freezing method of Conger & Fairchild (1953) requires a readily-available supply of dry ice (solid CO_2). The cellophane method of Murin (1960) requires the exposure of the squashed material to formaldehyde vapours for 45 min in order to make the tissue adhere to the slides. During the preparation of *Lens* root tips for chromosome counts, a simple and rapid method was developed for making permanent squash preparations of bulk-stained material.

MATERIALS AND METHODS

Several primitive cultivars of *Lens culinaris* Medik. were germinated in 'Pyrex' Petri dishes on moist filter paper under natural illumination. The radicles were removed after 3–4 days of growth and immediately placed in a saturated solution of paradichlorobenzine (PDB) for *ca* 3 h in order to contract the chromosomes for better visualization. This pre-fixation treatment in PDB was immediately followed by a 3-h fixation period in acetic-alcohol (1 : 3). The fixed root tips were hydrolyzed in 1 N HCl for 10 min at 60°C, quickly rinsed in distilled water, and placed in Feulgen stain for 3–24 h. At the end of the staining period, roots which had an intense purple stain at the tip were placed in three changes of tap water instead of sulfurous acid as a rinsing agent (Demalsy & Callebaut, 1967) for $\frac{1}{2}$ h/change. Rectangular pieces of moistureproof polypropylene film (22 × 30 mm) (Winfield transparent film wrap, obtainable from F. W. Woolworth & Co. Ltd) or thin gauge polythene film from polythene bags were placed in distilled water at the same time that the stained root tips were placed in the tap water rinses.

The terminal 1–2 mm of the Feulgen-stained root tip was placed on a microscope slide which had been subbed with Mayer's albumen and then covered with a piece of polypropylene or polythene film which had been presoaked in distilled water for $1\frac{1}{2}$ h or longer. The root tip was squashed into an oval shape under the film by rolling a test tube forward and backward over it several times. The slide was then immersed flat in absolute ethanol with the film-side up for a period of 2 min. The test tube was again rolled forward and backward over the tissue, the slide was placed flat in absolute ethanol for a second time and the edge of the film was lifted to allow the alcohol to come in contact with the tissue for *ca* one minute. The film was then gently and slowly peeled off of the slide while under the

alcohol thus leaving the tissue adhering to the slide. Occasionally the tissue would adhere to the film in which case the tissue was again pressed against the slide with the test tube. The tissue normally stuck to the slide after this repeated pressing treatment. The excess ethanol was drained from the slide and the tissue was permanently mounted with Euparal or cleared in xylene and mounted in a resinous medium.

OBSERVATIONS AND DISCUSSION

Figures 1 and 2 show the clarity of the chromosomes ($2n = 14$, see Darlington & Wylie, 1955) prepared by the method described here. The simplicity and the rapidity of the method (*ca* 5 min per slide) make the technique useful for research as well as for teaching purposes. This technique is not, however, intended to replace the dry-ice method of Conger & Fairchild (1953) or the cellophane

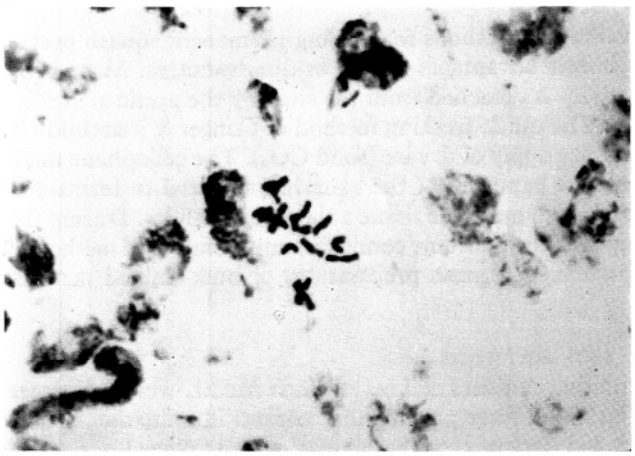

Fig. 1. Permanent squash preparation showing the clarity of the chromosome preparation of one cell. Bright-field microscopy. × 1000.

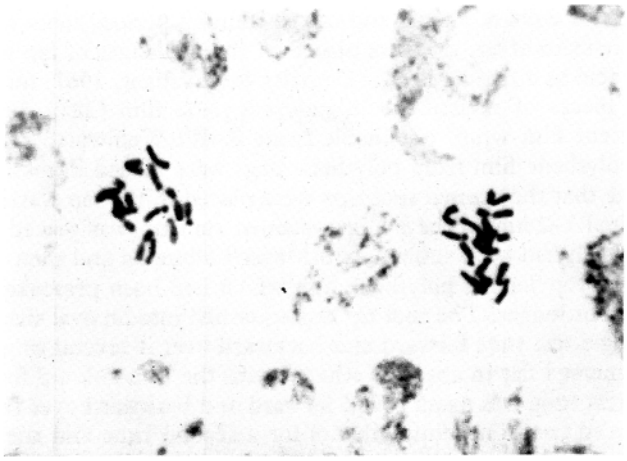

Fig. 2. Permanent squash preparation showing the clarity of the chromosome preparations of two cells. Bright-field microscopy. × 1000.

technique of Murin (1960) since it was developed primarily for use with material that has been stained in bulk prior to squashing. Tests on root tips of other plant species gave the same consistent and reproducible results as did the *Lens* root tips with respect to the clarity and permanency of the preparations. However, the prefixing treatment with PDB used for contracting the chromosomes was found to vary with the different species of plants.

The technique for making permanent squash preparations of bulk-stained plant material described here was also found to be successful with root tips that were bulk-stained according to the aceto-orcein-HCl method of Tjio & Levan (1950). However, absolute ethanol should not be used on the orcein-stained material as it has a solvent action on orcein. Consequently, a dehydrating agent which does not have a solvent action on orcein and which can be cleared in xylene for mounting the tissue in a resinous medium should be used instead of absolute ethanol. Cellosolve was found to be ideal for making permanent squash preparations of the aceto-orcein-stained material.

ACKNOWLEDGMENT

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