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A NOTE ON THE STAINING OF SOMATIC CHROMOSOMES OF THE COCONUT PALM

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SUMMARY

Pre-treatment of actively-growing coconut root-tips with 0·1 per cent aqueous colchicine, fixation in 1:3 acetic/alcohol, and staining with Feulgen preceeded by hydrolysis in 1 N.Hcl gives a satisfactory stain of somatic chromosomes. It is possible to keep the stained material without deterioration for about 14 days in 45 per cent acetic acid at deep-freeze temperatures. This is advantageous as the preparations can then be made at leisure, besides maintaining a reserve of stained material for further preparations if required.

INTRODUCTION

Chromosome study in palms is considered to be difficult and some of the common techniques often fail to give the desired results with this material. The varieties and forms of the coconut palm (*Cocos nucifera* L.) grown in Ceylon were screened for their somatic chromosome complement and this paper describes the techniques that were employed. Successful karyotypic study requires pre-treatment, fixation, suitable stains and maceration techniques.

MATERIAL AND METHODS

Actively-growing root tips from seedlings (or from adult palms provided with a satisfactory rooting medium) are incised about a centimetre from the apex and split lengthwise. The root cap is carefully removed as its presence greatly reduces the intensity of the stain of the meristematic tissues.

(*a*) *Pre-treatment:* Prefixatives like α-bromo-naphthalene, aesculin and colchicine were tried, and the last named when used at a concentration of 0.1 per cent for 2 hours provided a satisfactory spread for somatic chromosomes of the coconut. According to Sharma (1956), "One of the limitations of the colchicine technique is that tissues require thorough washing before subsequent fixation for observation of chromosome structure as otherwise some of the alkaloid remains on the tissue surface obscuring the cell constituents". A further snag is that "most of the dividing cells enter into the interphase during the time required for washing which is from at least 30 minutes to one hour". With coconut root-tips pre-treated with 0.1 per cent colchicine, three 5 minute changes of distilled water were sufficient to wash off surplus colchicine.

(*b*) *Fixation:* Abraham and Mathew (1963), report the successful use of Carnoy's fixative in cytological studies of coconut endosperm. Fixation for 12 minutes in 1:3 glacial acetic acid/absolute alcohol, a rapidly penetrating fixative, gave good results with coconut root tips.

(*c*) *Staining Techniques:* A mixture of 2 per cent acetic orcein/N.Hcl (9:1) has been successfully used to stain somatic chromosomes of palms, (Sharma and Sarkar, 1955; Nambiar and Upadhya, 1961). Abraham and Mathew (1963) used aceto-carmine for chromosome study in

coconut endosperm. The successful use of Feulgen stain for palm chromosomes has not been reported.

Freshly pre-treated and fixed coconut root tips are hydrolysed in 1 N.Hcl at 60°C for 9 minutes and left for 5 hours in Feulgen at 4° to 5°C. Highly satisfactory preparations can be obtained employing familiar squash techniques (Fig. 1). The above staining technique works only with freshly fixed material and fails to answer if the material has been left in alcohol even for a few hours. This has its drawbacks as the entire schedule has to be completed in about 7 hours, and it is not always possible to prepare a large number of slides from several samples.

(*d*) *Storage:* The above drawback may be successfully overcome by cold storage. It is reported that Feulgen stained material can be stored in distilled water in tightly stoppered vials at 4° to 10° C for long intervals (Flagg, 1961). With coconut root tips the intensity of stain is lost in about 7 days. Alternatively the material could be stored at deep-freeze temperatures in 45 per cent acetic acid (Ford and Hamerton, 1956), where with coconut root-tips the intensity of stain remains for about 14 days. After 21 days the staining is weak and does not have sufficient contrast for any critical work.

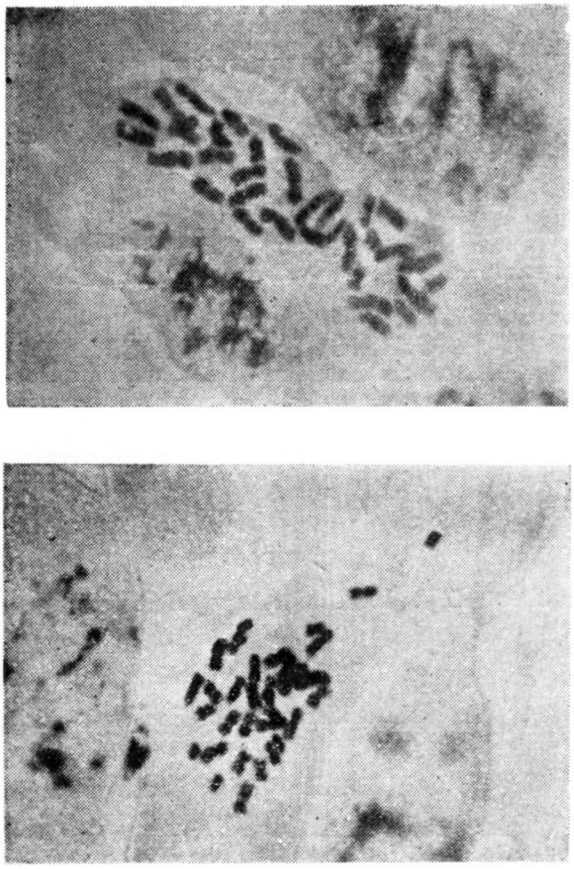

Fig. 1. Micro-photographs of metaphase plates of coconut root-tips.

Summarised Procedure

1. Cut root-tips from actively growing roots about a centimetre from the apex and split lengthwise. Remove the root-caps.
2. Pre-treat in 0.1 percent aqueous colchicine for 2 hours. Change of solution after 15 minutes would remove much of the tannin.
3. Wash in three 5 minute changes of distilled water.
4. Fix in glacial acetic acid/absolute alcohol (1:3) for 12 minutes.
5. Hydrolyse in 1 N.Hcl for 9 minutes at 60°C. Stain in Feulgen for 5 hours, preferably at low temperatures (4° to 5°C) and away from light.
6. Transfer the material to distilled water and remove the deeply stained tissue on to a slide.
7. Squash the material in a drop of 45 per cent acetic acid, and film cover glass into position, tap with a needle and warm judiciously.
8. Apply finger pressure evenly to the cover glass over several thicknesses of blotting paper, preventing any lateral movement of cover glass or slide.
9. Seal the edges of the cover glass with rubber solution and store the preparations overnight.
10. Remove rubber solution, and leave preparation "face down" in 40 per cent alcohol in a ridged smearing dish.
11. When slide and cover glass separate (usually 3-6 minutes), take each through 80 per cent alcohol and two 3 minute changes of absolute alcohol.
12. Recombine slide and cover glass with "Euparal".
    If it is desired to store the stained root-tips, transfer to 45 per cent acetic acid after (5) store at 0°C, and complete the rest of the schedule before stain deteriorates.

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