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LACTOPROPIONIC ORCEIN AS A SUITABLE STAIN FOR MITOTIC CHROMOSOMES OF ORCHIDACEAE

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Orchidaceae represents one of the most successful families of flowering plants. However, our knowledge of its cytological aspects is indeed very poor, especially with respect to the tropical species. The staining techniques used in orchid cytological studies are laborious and time consuming. In this study, an attempt has been made to develop a rapid and more convenient staining technique for orchid mitotic chromosomes, using lactopropionic orcein stain.

Keywords : Lactopropionic orcein; Mitotic chromosomes; Orchidaceae.

Introduction

Orchidaceae represents one of the most successful families of flowering plants, as is clear from the wide distribution it enjoys and the innumerable number of species, spread all over the world[1]. Considering this fact, it is to be admitted that our knowledge of its cytological aspects is indeed very poor, especially with repect to the tropical species[2]. The cytology of Indian orchids, especially the north-eastern species has received considerable attention[3-6]. The chromosome numbers of 87 species of south Indian orchids have also been studied[7].

Abraham and Vatsala[2] and Tanaka and Kamemoto[8] recommended acetocarmine and acetoorcein for obtaining good preparations of mitotic chromosomes of orchids. However, these staining techniques were laborious, involving several steps of pretreatment and fixation. An easy and rapid staining technique for mitotic chromosome study of orchids is yet to be described. In view of this, an attempt was made to develop a staining technique, for orchid mitotic chromosomes, which was simpler, more rapid and more convenient, compared to other existing techniques.

Materials and Methods

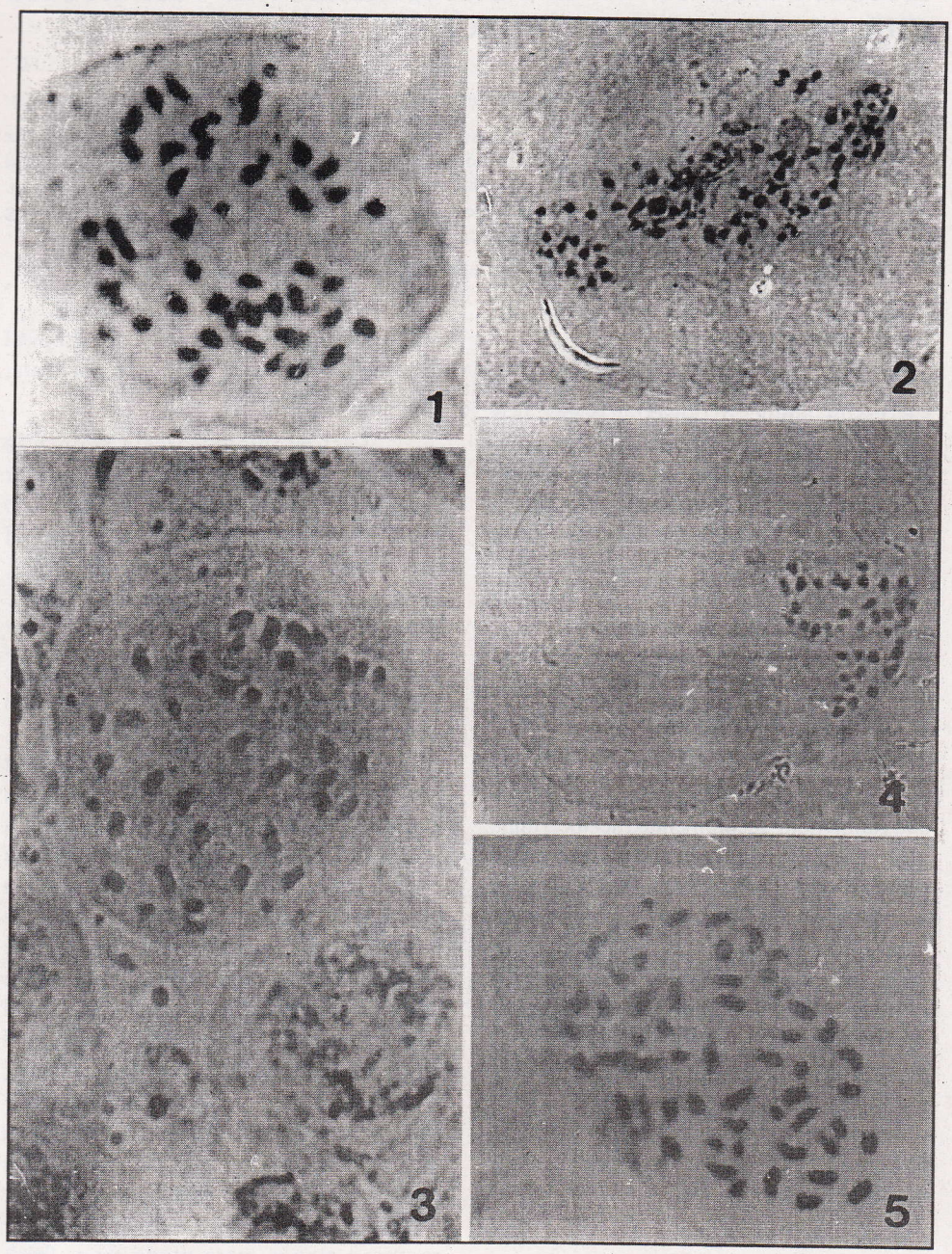
Four orchid species, viz. *Habenaria crinifera* L., *Nervilia aragoana* Gaud., *Vanda coerulea* Griff. ex. Lindl., *Renanthera imschootiana* Rolfe, and one elite orchid hybrid, *Phalaenopsis* 'Chuck Hagan' were used for this study. All these orchid plants were grown in the orchidarium of the Tropical Botanic Garden and Research Institute, Trivandrum (India).

Lactopropionic orcein stain was prepared by dissolving 2 gm orcein powder (SD Fine Chemicals, Mumbai, India) in 100 ml of 1:1 lactic acid and propionic acid and later diluting it to 45% with distilled water, as reported elsewhere[10]. It was stored at 4°C until use.

The root segments, containing the root tip, measuring about 1 cm were collected form the orchid plants, at around 10 a. m. and brought to the laboratory. The root segment was kept on a clean microslide, in 2 drops of the lactopropionic orcein stain. It was softened by boiling over the flame of a spirit lamp (1 min). The stained root tip (2 mm long) was then carefully cut off from the rest of the root segment and quickly placed on another clean microslide, in a fresh drop of lactopropionic orcein. It was covered with a coverslip. The coverslip was pressed down between the folds of a blotting paper, with the thumb, after gently warming the slide, so as to get a thin spread of cells, form the softened root tip, with the excess stain removed. The slide was then ready for observation. Photomicrographs of the required stages were made from fresh preparations.

Results and Discussion

Well spread out and intensely stained chromosome preparations were obtained by this technique. The background cytoplasm



Figs. 1-5 : Somatic chromosomes of Orchidaceae (x 1500) 1. *Habenaria crinifera*, 2n = 42; 2. *Nervilia aragoana*, 2n = 68; 3. *Renanthera imshootiana*, 2n = 38; 4. *Vanda coerulea*, 2n = 38; 5. *Phalaenopsis* 'Chuck Hagan', 2n = 57.

was not stained. The Chromosomes were intact and there were no artefacts.

The root tip squashes revealed a mitotic chromosome complement of 2n = 42 for *H. crinifera*, 2n = 68 for *N. aragoana*, 2n = 38 for both *V. coerulea* and R. *imschootiana*, and 2n = 57 for *Phalaenopsis* hybrid 'Chuck Hagan' (Figs. 1-5).

Orchids are generally recognised as cytologically difficult material[2]. The main drawbacks in studying their somatic chromosomes are the very slow rate of growth and consequent reduction in the number of dividing cells in their roots. Previously employed cytological techniques for orchids[2-8], involved several laborious and time consuming steps[9] of pretreatment and fixation of the orchid root tips. The present method, differs from them. in that there are no pretreatments with 8 - hydroxyquinoline, to condense and spread out the chromosomes, and no fixation procedures with Carnoy's fluid involved[2,7]. The root tip is directly boiled and squashed with the lactopropionic orcein stain. The use of ferric acetate, for the chromosomes to take additional stain is also not necessary, in this technique. The whole process of staining required not more than 10 min, with present technique.

The staining of chromosomes was intense, thier structure was well preserved. There was no cytoplasmic staining, thus rendering a clear background for viewing the chromosomes with increased contrast. Avoiding the use of fixative helped to keep the tissue soft enough for squashing and further softening by maceration was not necessary. Elimination of cytoplasmic staining was also achieved by warming the material in lactopropionic orcein during squashing.

The orchid root tip is a very delicate tissue. Lactopropionic orcein is recognized as a more useful alternative to acetic orcein for delicate materials[10]. Perhaps, that is the reason for its suitability to stain orchid chromosomes. George and Geethamma[10] used this technique to stain chromosomes of Oleaceae, but they had employed pretreatments with 8 - hydroxyquinoline and fixation methods with Carnoy's fluid.

Propionic acid readily dissolves orcein and the stain penetrates the cytoplasm without staining it[10]. At the same time, it stains the chromosomes, more effectively and uniformly. Rupturing of cell membranes, scattering of chromosomes and deterioration of stain does not take place, because of the presence of lactic acid.

It may be concluded that the lactopropionic orcein method of chromosome staining, described herein, is rapid, convenient and particularly useful for chromosome study in root tips of the Orchidaceae.

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