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PROPIONO-CARMINE SQUASH TECHNIQUE FOR MEIOTIC STUDIES IN ROSE

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Rosa is a large genus among the ornamental plants comprising more than 120 species ranging from diploid (2X) to octaploid (8X), with the basic chromosome number X=7. Cytological investigations in this genus were initiated in 1920 [1]. Since then several methods have been tried to analyse meiosis in roses but the cytological preparations have not been satisfactory. Swaminathan et al. [2] developed a simple propiono-carmine pollen mother cell (PMC) smear method for the plants with small chromosomes which was utilized by Shahare and Shastry [3] to study meiosis in garden roses with small chromosomes. The present study deals with an improved technique for preparation of PMC smears in roses. The experimental material consisted of *Rosa hybrida* cv. Folklore and its twelve induced mutants.

For preparing PMC smears the following method gave good results:

FIXATION

1) Uniform flower buds, about 8–12 days old, were selected for fixation.

2) Flower buds of right stage were stripped free of perianth at 8–9 A.M. during December–March.

3) The material was then kept in Carnoy's fluid for 1 h as pretreatment, after which it was put in fixative.

4) The fixative consisted of 3 parts of ethyl alcohol and 1 part of glacial acetic acid (saturated with 2% ferric acetate solution). The buds were kept in this solution for 2 h.

5) The flower buds were then stored in acetic acid: alcohol (1:3) without ferric acetate at 14°C for 24 h.

6) Finally, the fixed flower buds were stored in 70% ethyl alcohol in a refrigerator.

STAINING

Propiono-carmine (1% and 2%) was used for staining the chromosomes. The schedule for staining and squashing was as under:

1) **Hydrolysis**. The fixed anthers were immersed in a solution made from 12 drops of 2% propiono-carmine and 3 drops of 1 N HCl and kept at 60°C in an oven for 20 min. The anthers were washed 3–4 times with 1% propiono-carmine to remove the HCl residues.

2) **Squashing**. Smears were made by using 1% propiono-carmine solution. The hydrolized anthers were squashed in 1–2 drops of propiono-carmine on the microslides. A good spreading was obtained by applying gentle pressure on the cover slip and slide.

SEALING

DPX mounting solution was used to seal the slides temporarily.

PHOTOMICROGRAPHS

Photomicrographs were taken by using an Olympus BH-2 research microscope with uniform magnification of 1000 x on Orwo M-8 ortho-chromatic black-and-white 35 mm film. Agfa (hard and normal) paper was used for printing.

PERMANENT SLIDES

The following steps were taken for making the slides permanent:

1) DPX mountant strip was removed from the slide and coverslip.

2) Three marks were made by ink pen on the slide and coverslip around the tissue.

3) About 30 ml solution of aceto-butanol (1:1) was taken in a Petri dish and the slide was kept inverted in the solution with the help of a glass rod, till the coverslip separated from the slide.

4) The separated slide and coverslip were transferred individually into pure butanol for 1–2 min.

5) 1–2 drops of eupral were placed on the slide and the coverslip was replaced carefully on the previous marks, to avoid overlapping of the tissues.

6) Slides were kept at 30°C in oven for 24 h and then stored at room temperature.

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