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STUDY OF POLLEN MITOSIS AND DETERMINATION OF GAMETIC CHROMOSOME NUMBER IN OLEACEAE

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STUDY of nuclear division in pollen is interesting and important for the correct determination of the gametic number of plant chromosomes. A convenient and effective technique of staining chromosomes in pollen for observing their division, form and behaviour has not so far been standardised. Light miscroscopic examination of pollen mitosis is usually hindered by the presence of the exine enveloping the inner protoplast. There are several plants like the many members of Oleaceae whose pollen although stainable by dyes do not ordinarily germinate under *in vivo* or *in vitro* conditions[1]. As such, their nuclei or chromosomes in division are unavailable for study at the time of pollen tube formation. This necessitates separation of the inner protoplast from the exine. It is extremely inconvenient and difficult to achieve their separation by mechanical means like pressing or tapping with needles. Further, when the protoplast is taken out by such methods the intine and the inner cell contents are usually broken asunder resulting in the scattering of chromosomes. A more convenient method of separating the exine from its inner protoplast with intact intine at the same time staining the nuclei has been developed[2]. This method has been taken advantage of presently for staining and observing the chromosomes in pollen mitosis and determining the gametic chromosome number of several species of Oleaceae.

The materials for the present investigation were the pollen from several members of Oleaceae collected from different places in South India. Pollen grains from either freshly collected flowers or from those fixed in 1 : 3 acetic ethanol were placed in a drop of LPO (lactopropionic orcein previously prepared by dissolving 2 g Gurr's natural orcein in 100 ml 1:1 lactic acid and propionic acid and diluting it to 45% with distilled water[3]) on a slide, covered with a cover glass, sealed and kept for 2 hr before observation under the light microscope.

It was observed that the protoplast of the pollen bulged out through the pore and got ejected completely out of the exine without rupture of the membranous intine or any apparent injury or alteration of the inner cell contents. At the same time, the chromosomes at different stages of division such as metaphase (figure 1) or anaphase were intensely stained and distinctly discernible. By this method the gametic chromosome numbers of several members of Oleaceae have been determined. *Jasminum calophyllum* Wall., *J. chinensis* L., *J. pubescens* Willd., *J. primulinum* Hensl., *J. grandiflorum* L., *J. sambac* Wight. (Var. 'gundumalli' double whorled and single whorled) had 13 each, *Ligustrum ovalifolium* L., *L. lucidum* Ait., *L. robustum* Blume., *Linociera courtallensis* Bedd., and *Olea dioica* Roxb. had 23 each and *Nyctanthes arbor-tristis* L. had 22 gametic chromosomes. By comparison with the gametic numbers both somatic and meiotic numbers of different species of Oleaceae could further be confirmed.

The above method of staining chromosomes in pollen is simple, rapid and extremely convenient; at the same time it preserves the structure of the cell without producing artefacts. It enables microscopic observation of gametic chromosomes on a clear cytoplasmic background with sufficient contrast. In addition to ejection of protoplast as observed presently it is further noted that rupturing of cell mem-

Figure 1. Pollen protoplast of *Jasminum pubescens* treated with LPO showing 13 gametic metaphase chromosomes × 3000.

branes, scattering of chromosomes and deterioration of stain are avoided by the presence of lactic acid in the LPO staining fluid as observed by Dyer[4]. This method is particularly suitable for a correct determination of the haploid number of chromosomes in cases of plants where the meiotic number is variable through meiotic abnormalities[1]. It is by far the most convenient method of studying pollen mitosis and determining the gametic number of chromosomes inside the pollen which fail to produce pollen tube.

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IMPROVED PRODUCTION OF CALCIUM GLUCONATE BY MUTANTS OF *PENICILLIUM FUNICULOSUM*

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A PROJECT was undertaken to obtain the mutant strains of *Penicillium funiculosum* with a better yield of calcium gluconate than the parent strain isolated[1]. Solution of $NaNO_2$ (2 M) in acetate buffer (pH 4.5) was used as a mutagen.

The parent strain of *P. funiculosum* used in the investigation was selected as the best out of 15 isolates of *Penicillium*. It produced 26.2 g of calcium gluconate from 35 g of glucose per 100 ml of medium. The fermentation medium used was the modified medium[2] and had (g/l); glucose 35, glutamic acid 0.50, KH_2PO_4 0.19, $MgSO_4 \cdot 7H_2O$ 0.05 (pH 6.5). Calcium carbonate (1 g for every 4 g of glucose) sterilized separately, was added to the sterilized medium. To avoid precipitation of calcium gluconate, due to higher concentration of glucose, boric acid at 25 mM level was added to the medium[3–5]. Fifty ml of the medium were taken in 500 ml Erlenmeyer flasks, inoculated with 1 ml of aqueous suspension of spores (10^7/ml) and incubated on a rotary shaker. Calcium gluconate was measured by estimating calcium in the broth by the EDTA titration method[6]. The identity of gluconic acid in the culture filtrate (after removing calcium by ion exchange resin treatment) was confirmed by paper chromatography as well as by co-chromatography using an authentic sample of gluconic acid as standard. Ethanolic solution (0.04%) of bromophenol blue was used as a spraying reagent for the development of spot on the chromatogram.

For the development of high yielding mutants the parent strain of *P. funiculosum* (93 g) was treated with 2 M solution of $NaNO_2$ for 0, 5, 10, 15 and 20 min at 37°C. The method for mutagenesis was essentially that of Calvori and Morpurgo[7] and of Das[8]. Treated spores were washed thoroughly with 0.1 M phosphate buffer (pH 7.2) and finally plated on optimized mineral salt agar medium containing 0.04% alcoholic solution of bromocresol green as an indicator and were incubated for 3–4 days at 30°C. The number of colonies was counted and the acid unitage of the surviving strains was determined to select the high yielding mutants. Acid unitage was calculated by following the formula of Roy and Das[9]

$$\text{acid unitage} = \frac{\text{mycelial zone} + \text{acid zone}}{\text{mycelial zone}}$$

Of the surviving strains, 300 clones having acid unitage greater than the wild type were selected and tested for their ability for calcium gluconate production by growing them in 50 ml liquid medium in 500 ml flasks for 11 days when growth and calcium gluconate were estimated. Among the clones tested the mutant strain N10-287 was found to be the best and produced 29.2 g of calcium gluconate per 100 ml of fermentation broth and this yield amounted to an increase of 11.2% over the parent isolate. From the experimental results it is evident that $NaNO_2$ is an effective mutagen for this fungus and is able to produce mutants with improved production of calcium gluconate.

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