

Feulgen staining of somatic nuclei of *N. crassa*

J. Weijer

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Abstract

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karyokinetic cycles was achieved as follows.

Wild type strain 74A and the aconidial strain 46a of N. crassa are commonly used. Separation of the three independent somatic

1. Juvenile cycle. The fungus was grown on agar slants containing minimal medium for 3 days at 30°C. The slants were then stored for 5 days at 4°C. After storage, a 250 ml. conidial suspension in minimal medium with a density of approximately 10^6 cells/ml. was prepared. The Erlenmeyer flask containing the conidial suspension was incubated at 30°C on a shaker, and small samples were taken out at 30 minute intervals. These samples were transferred onto slides and dried on a slide warmer set at approximately 60°C.

2. Maturation cycle I. Several solidified agar plates containing minimal medium were inoculated in the center with a minute fragment of hyphal tissues taken from a stock culture. Four square (22 mm.) cover slips were placed edge-to-edge in such a manner that the inoculum on each plate was located at the common point of the four coverslips. The plates were incubated for at least 6 hours at 30°C after which a sample plate was chosen. The four coverslips were carefully removed in order not to disturb the adherent fungal growth, and dried on a slide warmer set at approximately 60°C. Subsequent samples were taken at 30 minute intervals.

3. Maturation cycle II. A similar procedure as that described for Maturation cycle I was followed. By extension of the incubation time to approximately 12 hours or more (depending on the strain employed and culturing methods) the culture will initiate microconidia formation. Since Maturation cycle II provides the microconidium with its nuclear content, sampling has to be started several hours in advance of the actual release of these conidia. Samples were taken at hourly intervals and fixed by the drying method described above.

The preparations were Feulgen stained according to the following schedule, after drying on the slide warmer at approximately 60°C, thereby omitting an additional fixation stage in acetic alcohol. It was found that the above schedule greatly enhanced the staining density of the karyokinetic configurations without altering their morphological appearance. After heat fixation, the slides were immediately hydrolyzed in 1 N HCl at 60°C for 8 minutes and stained for 3 hours in Feulgen. After staining the slides were rinsed for 10 minutes in tap water after which they were transferred to absolute alcohol for a few seconds. They were then mounted using a mixture of 1 part Euparal and 1 part Euparal essence. The Feulgen reagent was prepared by dissolving 1 g. basic fuchsin and 1.9 g. $\text{Na}_2\text{S}_2\text{O}_5$ in 100 ml. of 0.15 N HCl. Shake on a mechanical shaker for 2 hours. Add 0.5 g. fresh activated charcoal, shake for two minutes, and filter. If the reagent becomes pink, add charcoal, shake, and filter. Store the pale yellow reagent in a refrigerator. - - - Department of Genetics, University of Alberta, Edmonton, Alberta, Canada.