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BRIEF COMMUNICATION

A Macerating Ammonium Oxalate Fixative and Schedule for Some Root Meristems

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Abstract. 0.25 % ammonium oxalate in a mixture of ethyl alcohol, chloroform, formaldehyde, acetic acid and hydrochloric acid works as a very fast and efficient macerating fixative for the root meristems of *Allium cepa*, *Allium sativum*, *Ornithogalum thyrsoides*, *Pisum sativum* and *Vicia faba*. Slicing the tip region of excised meristems hastens spindle poison pretreatment, fixation and staining. Owing to the dissolution of the middle lamella and eliminating a physical barrier to permeability an extremely rapid fixation and staining schedule involving several stains has thus been developed.

It has been earlier recommended by Ford that application of ammonium oxalate in combination with hydrogen peroxide following osmic acid fixation softens the root meristematic tissues (WOLFF 1964, DARLINGTON and LA COUR 1969). Yet the meristems required the conventional acid hydrolysis at 60 °C for Feulgen staining while the use of other stains was out of the question. During our studies with different plant materials the desirability of dispensing with hydrolysis and also Feulgen staining has been felt in certain contexts and circumstances. This resulted in the improvement of an earlier fixative (SHARMA *et al.* 1976) by incorporating ammonium oxalate, and in development of a schedule involving several stains. The schedule incidentally happens to be the fastest known so far (SHARMA and SHARMA 1972).

Materials chosen for the study are the root meristems of *Allium cepa*, *Allium sativum*, *Ornithogalum thyrsoides*, *Pisum sativum* and *Vicia faba*. Ammonium oxalate is weakly soluble in water (LANGE 1956). After conducting trials with different combinations of (a) effective concentration of ammonium oxalate treatment prior to fixation, (b) concentration of the salt as an ingredient of several fixatives like acetic alcohol. Carnoy's fluid, Battaglia's fluid and Sharma's fluid (SHARMA *et al.* 1976, SHARMA and SHARMA 1972), (c) duration of fixation and (d) staining and squashing media, on the cap-incised root meristems of the above materials, a fixing fluid and a staining schedule have been developed.

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Fixing Fluid

Add 0.25 g of ammonium oxalate to 100 ml of the following fixative (SHARMA *et al.* 1976):

Absolute ethylalcohol	5 parts
2 N hydrochloric acid	2 parts
Acetic acid	1 part
Formaldehyde	1 part
Chloroform	1 part

Schedule

1. Slice off the extreme cap region of the excised root meristems.
2. Treat in any pretreating agent for chromosome spreads but preferably in 0.05 % colchicine for about 45 min.
3. Wash and fix in the above fixative for 10—20 min (*Allium cepa*, *A. sativum* and *Ornithogalum thyrsoides*) or for 20—40 min (*Pisum sativum* and *Vicia faba*).
4. Store doubly excised tips in 70 % alcohol or proceed directly.
5. Wash and stain in acetocarmine, acetoorcein, or aqueous haematoxylin and squash in the same medium, or
6. stain in crystal violet or toluidine blue, rinse and squash in 10 % acetic acid.
7. The wax ringed slides are stored for a day and made permanent by the conventional acetic acid — butanol series. It is desirable not to store the temporary slides beyond 4—5 days.

Ammonium oxalate macerates the tissue by dissolving calcium pectate of the middle lamella. The saturated solution *i.e.* 2.5 % recommended earlier (WOLFF 1964) is presently found to be cytotoxic while 0.5 % and 0.25 % employed for 15 and 45 min respectively gave satisfactory results. But 0.25 % salt solution in Sharma's fixative effectively macerated the tissue with much less treatment sometimes within 10 min in some materials while simultaneously fixing them. This may be due to the cumulative effect of the ammonium salt and the hydrochloric acid component of the fixative.

Excision of the extreme cap region resulted in a great reduction in the duration of the macerating fixation in the new formulation as well as pretreatment in colchicine. The apices are stained well in all the stains within 2—3 min. Thus well spread C-metaphase configurations have been obtained within 40—90 min in different materials due to rapid penetration caused by the new schedule. The fixed materials can be stored in 70 % and temporary slides remained intact up to 4—5 days. In the case of toluidine blue and crystal violet stained roots it is necessary to wash off excess stain in 10 % acetic acid before squashing in the same medium.

The extreme rapidity of the whole process is due to the compound effect of (a) incorporation of ammonium oxalate in the fixative so as to dissolve middle lamella and (b) excision of the root cap so as to eliminate a barrier of entry, both facilitating rapid penetration of all the agents of maceration, fixation, chromosome spreading and staining.

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BOOK REVIEW

NIGON, V., LUEKEN, W.: **Vererbung. Allgemeine Biologie Band 4.** — Gustav Fischer Verlag, Stuttgart 1976. 213 pp., 32,— DM.

This book represents the 4th volume of "Biologie Générale" edited by Pierre-Paul Grassé, Paris. The originally French edition includes further: Structure and Function of Cells (A. Hollande), Reproduction and Sexuality (P. Laviolette and P. P. Grassé), Experimental Embryology (E. Wolff) and Evolution (P. P. Grassé). The French version of this book, written by professor V. Nigon (University of Lyon) and published in 1966 by the publishing House Masson et Cie, Paris, was destinated to be translated by professor D. W. Lueken, university of Giessen. The delay in the translation of about 9 years ("from various reasons") led the translator to revise both the concept and the content of most chapters. This resulted in a "new" book with two authors.

About 200 pages are divided into 15 chapters, 3 appendixes, recommended literature and subject + author index. The explanation of genetics has the following schedule: Chemistry of nucleic acids and their components, biochemistry of DNA replication, repair, recombination and mutations, synthesis and structures of RNA species, mechanism, phases and regulation of transcription, proteosynthesis, genetic code (up to this point 45 pages), gene action, its regulation, structure of genophores in procaryotes, recombination in procaryotes (which includes host: parasites pecifity, role of plasmidesm, bacteriophages, transformation *etc.*), structure of genophores in eucaryotes (chemistry and morphology of chromosomes, cell cycle, mitochondrial and plastid genophores), chromosomal recombination in eucaryotes (diploidy, meiosis, alleles, autogamy, crosses, autosomal and gonosomal heredity in diploids, special cases of heredity like trisomics, predetermination, gene mapping *etc.*), cytoplasmic heredity (mitochondrial, plastid, plasmal, infection), mutations (up to this point 154 pages), genetics of populations and human genetics. One appendix includes the description of the most frequently used models in pro- and eucaryotes, the second one the statistical and biochemical methods like ultra centrifugation, de- and renaturation and hybridisation of nucleic acids, autoradiography, sequence analyses and gene synthesis. At the end of each chapter the important literature is quoted — mostly reviews and books.

The described schedule, although not usual in the majority of genetical textbooks, was extremely well exploited by both authors for logical explanation of general genetics. Two further advantages make the book valuable: the high scientific level and concise style.

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