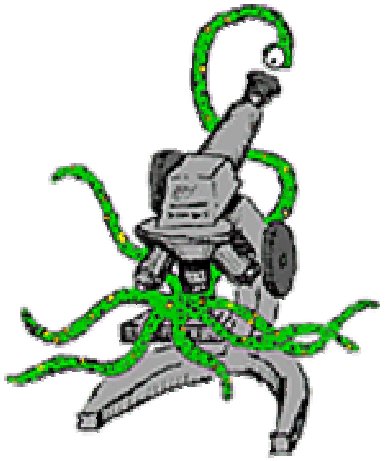
Aim of the plan

Few books exist about the technique of preparation of plant tissues for the observations under the microscope, and it's difficult to find information on Internet, too. This information isn't often complete, and the procedural choices aren't explained. I'm interested in microscopy, I want to start studying plant anatomy, so I began a personal experimental research to develop the research and the bibliography in this field. I want replace toxic reagents with other less dangerous ones for the study of plant histology, too.



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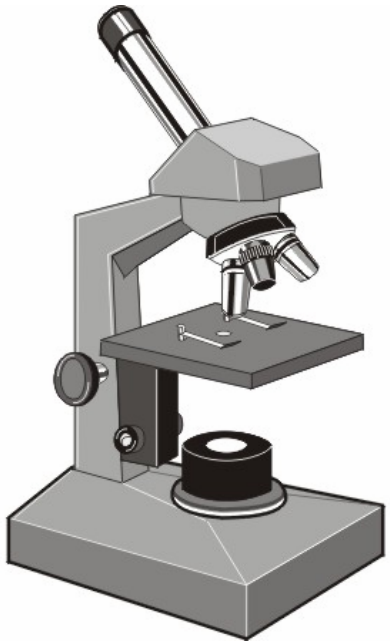
2004

RESEARCH FOR EFFECTIVE METHODS ABOUT THE STAINING OF PLANT TISSUES



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RESEARCH FOR EFFECTIVE METHODS ABOUT THE STAINING OF PLANT TISSUES

My work is divided into six phases:

PHASE 1: to identify a method of double polychrome stain for cross-sections of petioles and leaves: by leaving from a method of staining found in a microscopy book, I have carried out some tests in order to determine the concentration of stains to obtain a procedure of effective staining. At the end of this phase the tests have allowed me to emphasize a procedure of staining for sections of plant tissues that differentiated the types of the present tissues clearly.

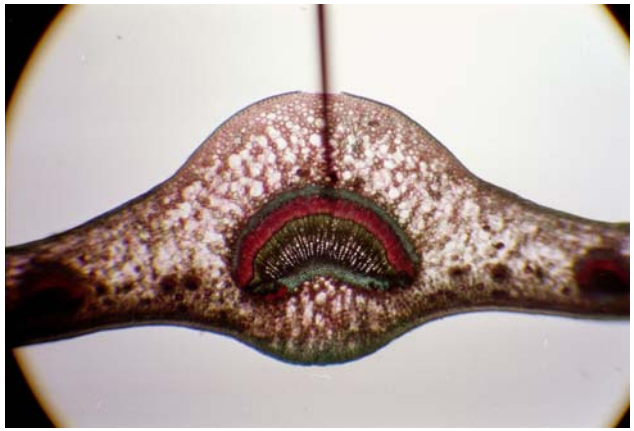
Cross-section of Camellia japonica's petiole (unstained)

PHASE 2: to test a procedure for the permanent mounting of the stained sections: I have tested some mounting mediums for the permanent mounting of the stained sections.

PHASE 3: to confront various procedures in order to characterize general rules for double polychrome stains of plant tissues sectioned: by comparing the obtained procedure in phase 1 and information found through Internet, I have formulated four hypotheses. Their experienced verification has allowed me to find some general rules in the use of stains that allow the operator to change their types in the method without altering the efficacy of the staining.

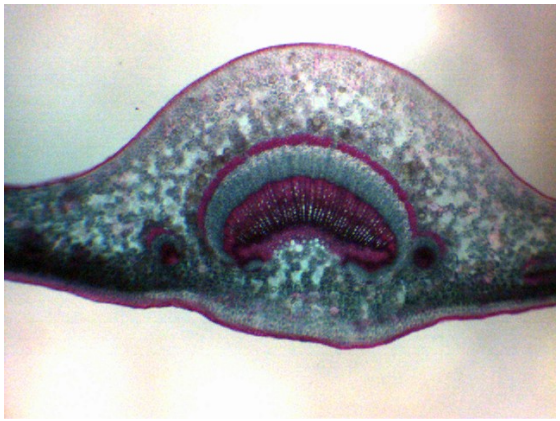
PHASE 4: to apply methyl green – Congo red method on different plant materials: I verify with some experiments that the same tissues are emphasized in the same way in different specimens stained with methyl green – Congo red method.

PHASE 5: method of staining with different dyes: I made new procedures that use different dyes to apply the general rules find in phase 3. I've tried eight methods: malachite green – Congo red, gentian violet – Congo red, methylene blue – Congo red, safranin – light green, basic fuchsin – light green, neutral red – light green, safranin – aniline blue, basic fuchsin – aniline blue. The different tissues present in cross-section have a different affinity as to acid dyes and basic dyes. In each procedure it's possible to change a dye with another one, but you must find the best time of exposure and concentration for each dye.



Cross-section of Camellia japonica's fillode (methyl green-Congo red method)

PHASE 6: research of a law that put in relation the times of exposure, the concentration of dye solutions and their intensity: I've already qualitatively observed that "Stronger stains must be used in a smaller concentration and the time of exposure must be shorter". I want verify if a mathematical tie exist between the times of exposure, the concentration of dye solutions used and their intensity. Dyes' intensity it's measurable with a spectrophotometer (absorbance).



Cross-section of Camellia japonica's fillode (basic fuchsin - light green method)