

SECTION A, BIOLOGICAL SCIENCES

Subsection Botany

Clearing and Staining Plant Materials with Lactic Acid and Pararosaniline Hydrochloride

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Over the years plant anatomists have found it advantageous to study vascular structure in thick, cleared sections or whole plant organs rather than use the tedious method of reconstruction from serial sections. For the many technics that have been invented to achieve this end, the reader is referred to Lersten's bibliography (1967). Apparently most investigators develop their own favorite routines. For the past 25 years, my students and I have been experimenting with the lactic acid-basic fuchsin method originally reported by Debenham (1939). This paper reports some refinements on the older method.

There is no question that 75% lactic acid (3 parts Lactic Acid U.S.P. to 1 part water) will clear many plant materials. There may remain, however, a problem with various residual pigments. We have found that commercial bleaches such as "Chlorox" and "Purex" may be used, but that they must be very carefully employed because they tend to macerate many materials. The bleaches should be tried before the specimens are placed in lactic acid. A good general rule is that the smallest concentration of bleach that will do the job is best. Even so, some specimens may disintegrate in the bleaching solutions.

Lactic acid itself softens many delicate plant organs and such organs as petals and sepals must often be treated with great care to bring them through the clearing process. The task with such delicate organs is to decant the various liquids from them as carefully as possible. For this we use an infants' rectal syringe.

Cleared specimens may be stored almost indefinitely in lactic acid, since this compound is not volatile. For many studies, this is enough. However, if photomicrography is contemplated, staining becomes a useful adjunct.

Debenham (1939) used basic fuchsin; we have found that pararosaniline hydrochloride is more precise and manageable. Also, we have been able to reduce the concentration of NH_4OH and thus also to alleviate further softening of the specimens.

We use a stock solution of 1% pararosaniline hydrochloride (National Aniline and Chemical Co., Lot No. 5049) in 95% alcohol. This keeps fairly well, but it may deteriorate within a year or so, for which reason it is not wise to make up large quantities.

The stain is prepared as follows:

1. 20 cc concentrated NH_4OH
2. 80 cc HOH
3. 2 cc of a 1% solution of pararosaniline hydrochloride in 95% alcohol

4. Filter before use in order to remove undesirable precipitates. The solution should be a light, dull pink. If it has a deeper color it may overstain.

Pour this solution over the cleared materials and leave it for at least 24 hr, longer with thick specimens.

At the end of the staining time, it is very likely that there will be very little evidence of success. However, when the staining solution is replaced with 95% alcohol the color will begin to appear. We prefer 2 changes in 95% alcohol, followed with 2 changes in 100% alcohol. Each change should be allowed 12 hr or more. After this, we decant the alcohol and pour methyl salicylate over the specimens for clearing and storage. Almost any other clearing agent will work, including xylene and toluene, but the low volatility of methyl salicylate is a definite advantage. Furthermore, most stained materials retain their bright colors longer in methyl salicylate than in other clearing reagents we have tried. Even so, there is much difference in the way specimens from different species react to this clearing and staining method. Some retain their colors for years while others fade disconcertingly soon.

It is also possible to go from most of these clearing reagents to the various brands of bioplastics now on the market. So far we have experimented very little with bioplastics, but we have done enough to know that these compounds have much promise for the preparation of permanent cleared sections.

Illustrations of materials prepared by the above clearing and staining technic may be found in Boke (1963, 1966).

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