STAINING PLANT SECTIONS

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Here's the classical procedure I use which so far gives acceptable results, even though I'm still tweaking it. It's one of those methods that requires an alert practitioner and can't be left alone in critical moments.

Ingredients you need are safranin O, fast green FCF, saturated aqueous picric acid, 70% ethanol with some concentrated ammonia, 70%, 95% and 100% ethanol, xylene and Canada balsam.

Recipes are available in Files section of this group.

Picric acid is vastly superior but can be substituted with 10% acetic acid or water acidulated with hydrochloric acid, although results won't be as good and destaining can be too hard.

Ethanol can be substituted with isopropanol with the downside of longer waiting as ethanol is less fatty so it penetrates into tissues faster.

Using watch glass and pipettes minimizes reagent use.

1. Cut very thin sections (50 µm is possible on a DIY handheld microtome) and drop them immediately in safranin solution in a watch glass. I rely on larger numbers so that after the procedure is done, I can pick the best and discard the rest. Cover the watch glass and leave it like that for some six hours or more, for the dye to profoundly overstain the sections.
2. Do few brief washings with 70% ethanol to get rid of excess dye pooled in the tissue.
3. Partially destain by adding one drop of concentrated picric acid in a small pool of alcohol your sections are swimming in. Start swirling and carefully observe how the acid attacks and removes safranin from tissues that don't have affinity for it.
4. When you feel it's enough, better sooner than later(!), stop the reaction by adding few millilitres of ammonified ethanol and swirl. It usually takes half minute so be careful.
5. Quickly decant and rinse with 70% ethanol three times.
6. Bring the sections to 95% ethanol.
7. Decant, then flood with fast green. Exact time depends on your preferences but 1 min is usually well over enough.
8. Decant, rinse with 95% ethanol three times, fast.
9. Decant and rinse with xylene three times, waiting several minutes before each washing. No need to be fast. Xylene won't remove any stains.
10. Inspect the sections carefully. Discard faulty ones: badly cut, thick, understained, etc.
11. Mount sections in a drop of warm Canada balsam with a tiny drop of xylene. Carefully cover it with a coverslip and put a small weight on it; AA battery will do. Plant sections can curl in the mountant, dislodging the coverslip so force is necessary.
12. After an hour, mountant edges are sufficiently dry not to move on their own. Inspect under microscope, label and and store flat for at least several months.

There's no need for embedding plant tissue blocks into wax like animal tissue is. When I collect a plant, I cut the stem into pieces and store it in alcohol. After few days they're permeated and fixed.

I assemble the microtome, pour wax on the bottom of the cavity and affixed stem into it. Then more wax is dripped around it, forming structural support. Top is sealed with wax to prevent alcohol evaporating away.

After everything solidifies, it's ready for pushing out and cutting. Works like a charm.

Plant tissues are already very sturdy and don't require wax permeation like animal tissue does.