Selected staining methods for plant tissue

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Figure 7-2 Microscope slide staining dishes. A: Staining dish and slide rack; B: metal slide

Staining staining and in situ hybridization; C: Coplin jar; D: staining dish with slots. Adapted from Preece (1972).

SELECTED STAINING METHODS FOR PLANT MICROTECHNIQUE

The following are examples of staining schedules for plant microtechnique organized into functional groups (histology, cytology, etc.) As there are thousands of published staining procedures this list is obviously not exhaustive. It is presented here only as a starting point for your investigations. See Johansen (1940), Sass (1958), Emmel and Cowdry (1964), Gurr (1965), Gray (1973), Pearse (1980), Clark (1981), and Pearse (1985) for details of these and other staining schedules.

JOHANSEN’S SAFRANIN AND FAST GREEN

Johansen’s method (Johansen, 1940) differs from most other Safranin/Fast Green protocols in the use of additions to the stain and clearing solutions to enhance and differentiate tissue structure. (See the staining schedule for notes on the purpose of the additions.) It is widely agreed that this method yields a more brilliant staining of plant tissues than almost any other schedule. Safranin O is a regressive dye and requires destaining and differentiation with picric acid or HCl. The Safranin-stained tissues are counterstained with
Fast Green — a progressive dye. At the concentration used, Fast Green stains the tissues within 10–15 s, so it is best to test the procedure on one or two slides before staining your entire set. In plant tissues stained with this method, Safranin O appears brilliant red in chromosomes, nuclei, lignified, suberized, or cutinized cell walls. Fast Green appears a brilliant green in cytoplasm and cellulosic cell walls. Fast Green turns blue in basic solutions. It appears blue to bluish-green in the stems and leaves of aquatic plants and most gymnosperms (Johansen, 1940). An aqueous alternative to Fast Green FCF is Alcian Blue.

**Staining Procedure**

1. Deparaffinize in Histo-Clear or xylene and bring slides to 70% using a graded EtOH series. Coating is optional and is used only if test sections fall off the slides.

1. Stain 2–24 h in Safranin O staining solution.

1. Wash out excess stain for a few moments with DI water. You may use running water but take care not to dislodge sections. A good technique is to run water through a flexible tube into the bottom of the staining dish.

1. Dehydrate 10 s in 95% EtOH plus 0.5% picric acid. Picric acid will cause Safranin O differentiation.

1. Wash 10 s to 1 min (no longer) in 95% EtOH + 4 drops ammonium hydroxide per 100 ml to stop picric acid action. Excessive EtOH washing will completely remove Safranin O staining.

1. Dip briefly (10 s) in 100% EtOH to finish dehydration.

1. Counterstain 10–15 s in Fast Green FCF staining solution. Test staining on a single slide and dilute the Fast Green solution if it is too concentrated.

1. As the Fast Green staining solution evaporates with use, add additional solvent, not dye solution to maintain the correct dye concentration.

1. Rinse excess Fast Green with “used clearing solution.” You can use either a Coplin jar for a few slides or a staining dish for many slides.

1. Wash slides in Clearing solution by dipping the sections for 5–10 s.

1. Remove clearing solution by dipping for a few moments into xylene (not Histo-Clear) plus 2–3 drops 100% EtOH\(^4\) (to remove residual water).

1. Clear in xylene for two changes.

1. Keep the slides in the final xylene solution while you mount the coverslip one slide at a time. Do not allow the slides to dry before mounting coverslip, because tissue damage may occur.

**ALTERNATE MICROWAVE PROCEDURE**

1. Remove paraffin from sections using RT xylene and hydrate sections to 70% EtOH.
1. Stain the slides in preheated Johansen’s Safranin solution by microwaving 15 min at 60°C. Place the slides in an uncovered Coplin jar which itself is placed in 300 ml water in a 600-ml beaker. Slides should be totally immersed in the stain.

1. Dehydrate 5–10 s in 95% EtOH plus 0.5% picric acid.

1. Wash 2 s in 95% EtOH + 4 drops ammonium hydroxide per 100 ml to stop picric acid action. Excessive EtOH washing will completely remove Safranin staining. Slides may be agitated.

1. Dip briefly (2 s) in 100% EtOH to finish dehydration.


1. Wash slides in Clearing solution by dipping the sections for 5–10 s. Slides may be agitated.


1. Mount coverslip with Permount or some other toluene-soluble mountant. Mount coverslip one slide at a time.

*Carbol-xylene will also work. However, be certain that the EtOH contains no residual water, as water will cloud xylene or carbol-xylene.

**Staining**

<table>
<thead>
<tr>
<th>Safranin O solvent</th>
<th>Fast Green solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl cellosolve</td>
<td>Methyl cellosolve 1 vol</td>
</tr>
<tr>
<td>EtOH 100%</td>
<td>Abs EtOH 1 vol</td>
</tr>
<tr>
<td>DI</td>
<td>Methyl salicylate 55 1 vol</td>
</tr>
<tr>
<td>Sodium acetate (intensifies stain)</td>
<td>1% (w/v)</td>
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<tr>
<td>Formalin (mordant)</td>
<td>2% (v/v)</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Safranin O staining solution</th>
<th>Fast Green FCF staining solution</th>
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</thead>
<tbody>
<tr>
<td>Safranin O</td>
<td>Fast Green FCF 0.05% w/v</td>
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</table>

**Clearing solution**

<table>
<thead>
<tr>
<th>Clearing solution</th>
<th>Used clearing solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl salicylate</td>
<td>“Used” clearing solution 2 vol</td>
</tr>
<tr>
<td>EtOH (100%)</td>
<td>EtOH 1 vol</td>
</tr>
<tr>
<td>Xylene (not Histo-Clear)</td>
<td>Xylene 1 vol</td>
</tr>
</tbody>
</table>

**SASS’S SAFRANIN AND FAST GREEN**

This method of Sass (1958) uses fewer additions than Johansen’s method (above) but works adequately. Some workers (Popham, et al., 1948; Schmid, 1978) note, however, that aqueous or weak alcoholic solutions of Safranin O yield markedly inferior results compared to Johansen’s method.
1. Deparaffinize in Histo-Clear and bring slides to water as per the usual method. (Celloidin treatment is optional.)

1. Stain 1–12 h in aqueous Safranin O (1% w/v).

1. Wash in DI until no more dye is removed from the sections.

1. Dehydrate in 30, 50, 70, and 95% EtOH, 2–5 min/step.

1. Dip 5–30 s in 95% EtOH plus Fast Green FCF (0.1% w/v).

1. Wash 2Z 2 min each step, in 100% EtOH.

1. Dip 5 seconds in carbol-xylene or a mixture of methyl salicylate and xylene (1:1) to remove the last traces of water.

1. Clear in 100% xylene for two changes.

1. Mount coverslip.

SAFRANIN O AND ORANGE G

The Sharman staining series is a good general purpose stain for meristematic tissues. In plant tissues stained with this method cell walls stain blue-black, nuclei stain yellow to orange, starch grains appear black, and lignified cell walls stain brilliant red (Foster, 1934; Sharman, 1943).

Procedure

Tissues may be preserved with any fixative.

1. Deparaffinize in Histo-Clear or xylene followed by hydration in a graded EtOH series.

1. Transfer for 1 min to filtered 2% ZnCl2 (aq).

1. Wash in DI for 5 s.

1. Stain for 5 min in Safranin O staining solution.

55 Johansen (1940) used clove oil instead of methyl salicylate.

Staining

1. Wash in DI for 5 s.

1. Transfer to Orange G staining solution and stain for 1 minute.

1. Wash in DI for 5 s.

1. Transfer to tannic acid solution for 5 min.

1. Wash in DI for 1–3 s.
1. Transfer to 1% aq iron alum [(NH₄)₂Fe(SO₄)₂, filtered] for 2 min.

1. Wash in DI for 5–15 s.

1. Dehydrate through 45, 90, 100% EtOH, about 10 s each step.

1. Transfer to EtOH/xylene or 1:3 methyl salicylate:xylene for >1 min.

1. Transfer to xylene (not Histo-Clear) and mount coverslip as usual.

<table>
<thead>
<tr>
<th><strong>Safranin O stock solution</strong></th>
<th><strong>Orange G staining solution</strong></th>
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</thead>
<tbody>
<tr>
<td>Safranin O 1 g DI 50 ml</td>
<td>Orange G 2 g Tannic acid 5 g HCl (conc) 4 drops DI to 100 ml</td>
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</table>

Add thymol, phenol, or azide (0.03% w/v) to inhibit microorganism contamination. Azide can be used at 0.03–0.1% w/v.

Filter before use

<table>
<thead>
<tr>
<th><strong>Safranin O staining solution</strong></th>
<th><strong>Tannic acid solution</strong></th>
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</thead>
<tbody>
<tr>
<td>Safranin stock (2%) 1–1.5 ml DI 500 ml</td>
<td>Tannic acid 5 g DI to 100 ml</td>
</tr>
</tbody>
</table>

Add thymol, phenol, or azide (0.03% w/v) to inhibit microorganism contamination.

Filter before use