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Histochemical Staining of Plant Tissues

Sameer S. Athreya

Student of St. Joseph's College, Bangalore

Abstract

Histochemistry is a subfield of histology that focuses on identifying the chemical components of cells and tissues. Starch deposition occurs throughout the plant body, but seeds, the parenchyma of secondary vascular tissues in the stem and root, tubers, rhizomes, and maize are notably affected. The protoplast's main ergastic components are starch and proteins. Tannin, a diverse collection of phenol derivatives that are usually related to glucosides, is prevalent in the leaves (xylem) of many plants. Saponins are a type of saponin that is extremely rare. Fats are broadly dispersed throughout the plant body, and they are likely present in minute quantities in each plant cell. Fats are a common reserve resource in meristematic cells such as seeds, spores, and embryos. The breakdown product of carbohydrates is glucosides. Alkaloids are the byproducts of protein breakdown. Many plants have secondary products that are medicinally valuable.

Using stains, markers, and light and electron microscopy, histochemistry studies the identification and distribution of chemical substances within and between biological cells.

Histochemistry is a methodological approach that allows the chemical analysis of cells and tissues in relation to their structural organisation , but for plant secretory structures, a broad histochemical analysis is required because the same gland and even the same glandular cell can produce multiple metabolites at the same time.

Staining methods

The plant organs are stored in FAA solution for fixation. They are then embedded in paraffin wax and sectioning is done using microtome.

The use of the homemade plastic mold to embed the stems in 7% agarose proved to be fast and easy .The two parts of the embedded vial system make it simple to easily release the stem embedded in agarose as the agarose does not stick to the vial parts that are inert, keeping the system clean. The vials can be reused multiple times for years. The convenience of storing the embedded stem also makes the next steps easier. In order to save sometime, similar embedded stems can be grouped (up to 3) on a single sectioning plate for sectioning.

Staining with Toluidine Blue O

1. To get a 0.02 percent solution, dissolve 0.02 g toluidine blue O in 100 mL distilled water. NOTE: The toluidine blue O solution can be kept at room temperature for two weeks in a dark bottle.

2. Place stem portions in a microcentrifuge tube with a 2.0 mL capacity. Fill the tube with 1 mL of the 0.02 percent toluidine blue O solution. Pipette up and down the 0.02 percent toluidine blue O solution gently. Then incubate for 5 minutes at ambient temperature before repeating the pipetting. NOTE: Do not pipette the sections into the pipette tip, as this will cause damage to the sections. Allow the microcentrifuge tube to remain stationary until the sections have settled. Because the solution is black, it may be difficult

to see, so place the tube containing the sections on a test tube rack and put aside for 2 minutes at room temperature.Use a 1 ml pipette to draw out 700 µl of 0.02% toluidine blue O solution. Add 700 µl of distilled water to rinse out the toluidine blue O solution. Repeat 3-4x or until the wash solution is clear. Use a 1 ml pipette with the pipette 1. Draw out 700 l of 0.02 percent toluidine blue O solution with a 1 ml pipette. Rinse away the toluidine blue O solution with 700 l of distilled water. Rep 3–4 times more, or until the wash solution is clear. Use a 1 ml pipette with a pipette tip cut so that the sections may be pipetted out easily without being damaged.

2. Pipette portions onto the tip of the pipette and onto a microscope slide, then cover with a coverslip. Under bright-field lighting, examine the portions.

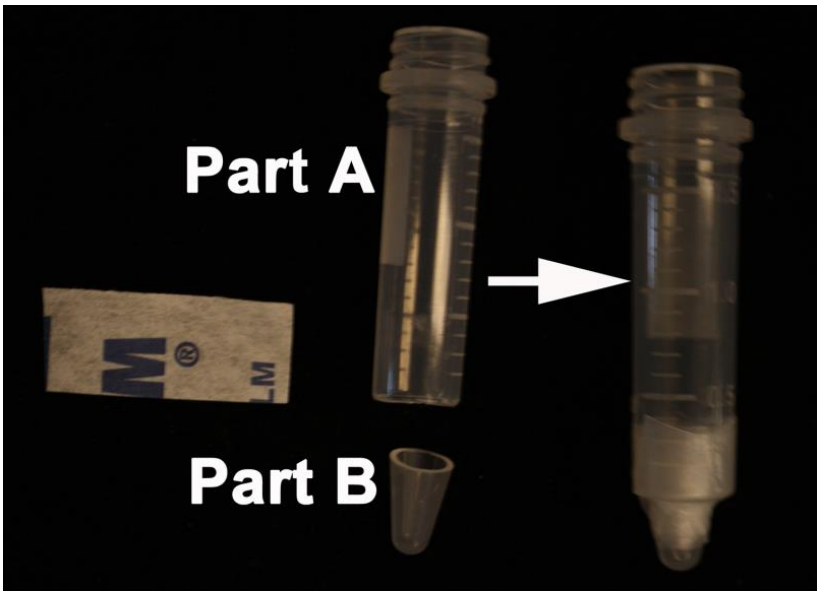


Figure 1. Homemade mold for embedding the stems. On the left side; top of the 2 ml screw-cap microcentrifuge tube (Part A) and the bottom from the 0.6 ml microcentrifuge tube (Part B). On the right side; the Parafilm holding the two parts; A and B to form the final mold.

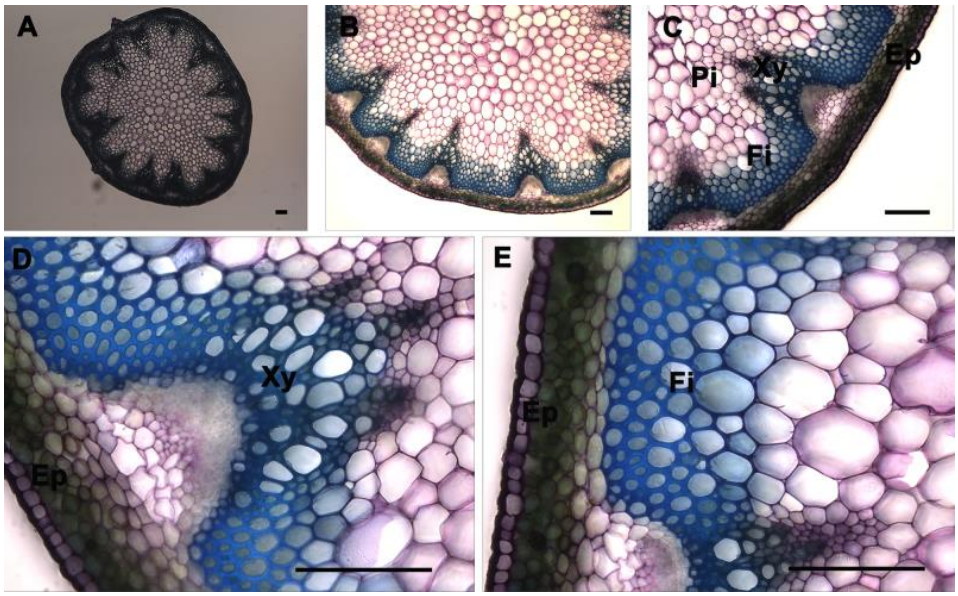


Figure 7. Toluidine blue O staining of *A. thaliana* stem cross-sections. Toluidine blue O staining of a wild type *A. thaliana* stem section showing the normal lignin deposition in the walls of interfascicular

fibers and xylem cells (**A**-**E**). The positions of the epidermis and cortex (Ep), interfascicular fibers (Fi), pith (Pi), and xylem (Xy) are indicated. Magnifications: **Panel A**: 5X; **Panel B**: 10X; **Panel C**: 20X; **Panels D and E**: 40X. Bar = 100 µm.

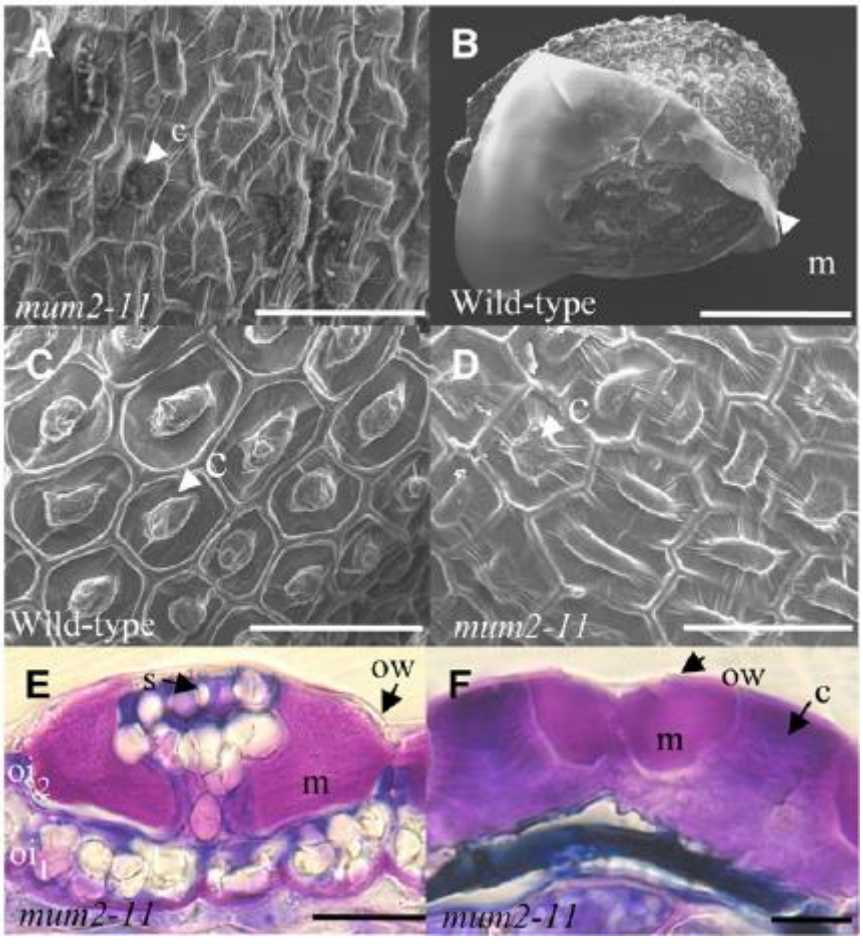
Detection of Hydrophilic substances :

Mucilage:

Ruthenium red staining:

Acidic mucilages, pectins [12, 13], and nucleic acids are stained magenta or crimson using this approach (Figure 1a).

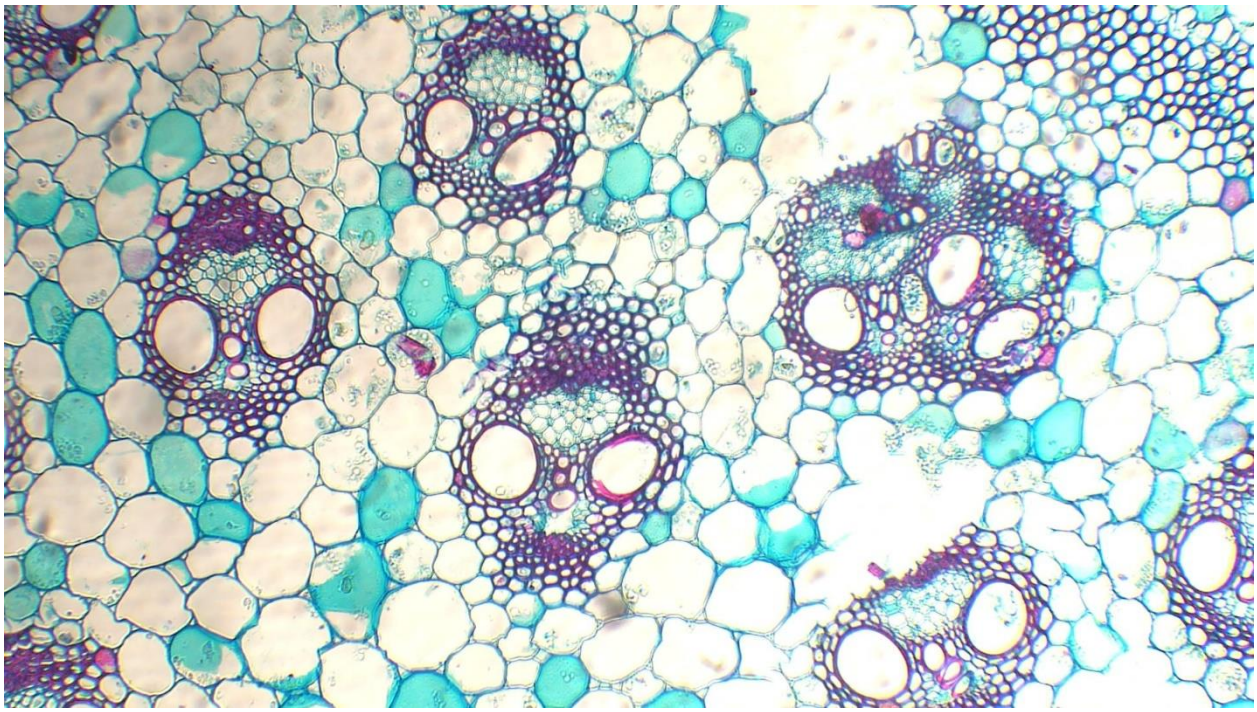
1. For 5 minutes, apply 0.1 percent ruthenium red to portions.
2. Remove excess stain by washing parts twice in distilled water.
3. Use glyceringelatin to adhere the parts between the slide and the coverslip.



Alcian Blue Staining:

This test produces results similar to ruthenium red, staining acidic mucilages, pectins ,and nucleic acids light blue

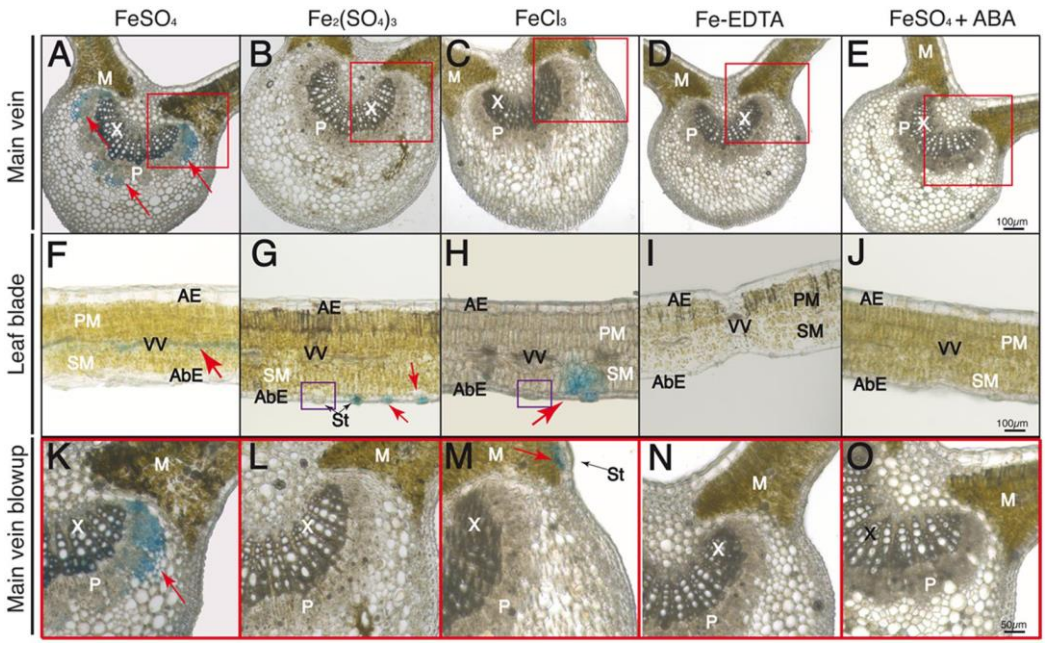
1. For 30 minutes, stain portions with 1% Alcian Blue.
2. Remove excess stain by rinsing areas twice with distilled water.
3. Use glyceringelatin to mount the slide.



Tannic Acid and Ferric Chloride:

This approach is based on the reaction of tannic acid with mucilages and pectins, which are then revealed by ferric chloride, resulting in a grey to black hue (Figure 1c).

1. Apply 5% tannic acid for 20 minutes.
2. Rinse with distilled water for a few seconds.
3. Soak parts in ferric chloride (3%) for 5 minutes.
4. Remove excess ferric chloride by washing twice in distilled water.
5. Use glyceringelatin to adhere the portions together.
6. Comparison: Compare the results of the test to those of sections treated just with tannic acid or ferric chloride.

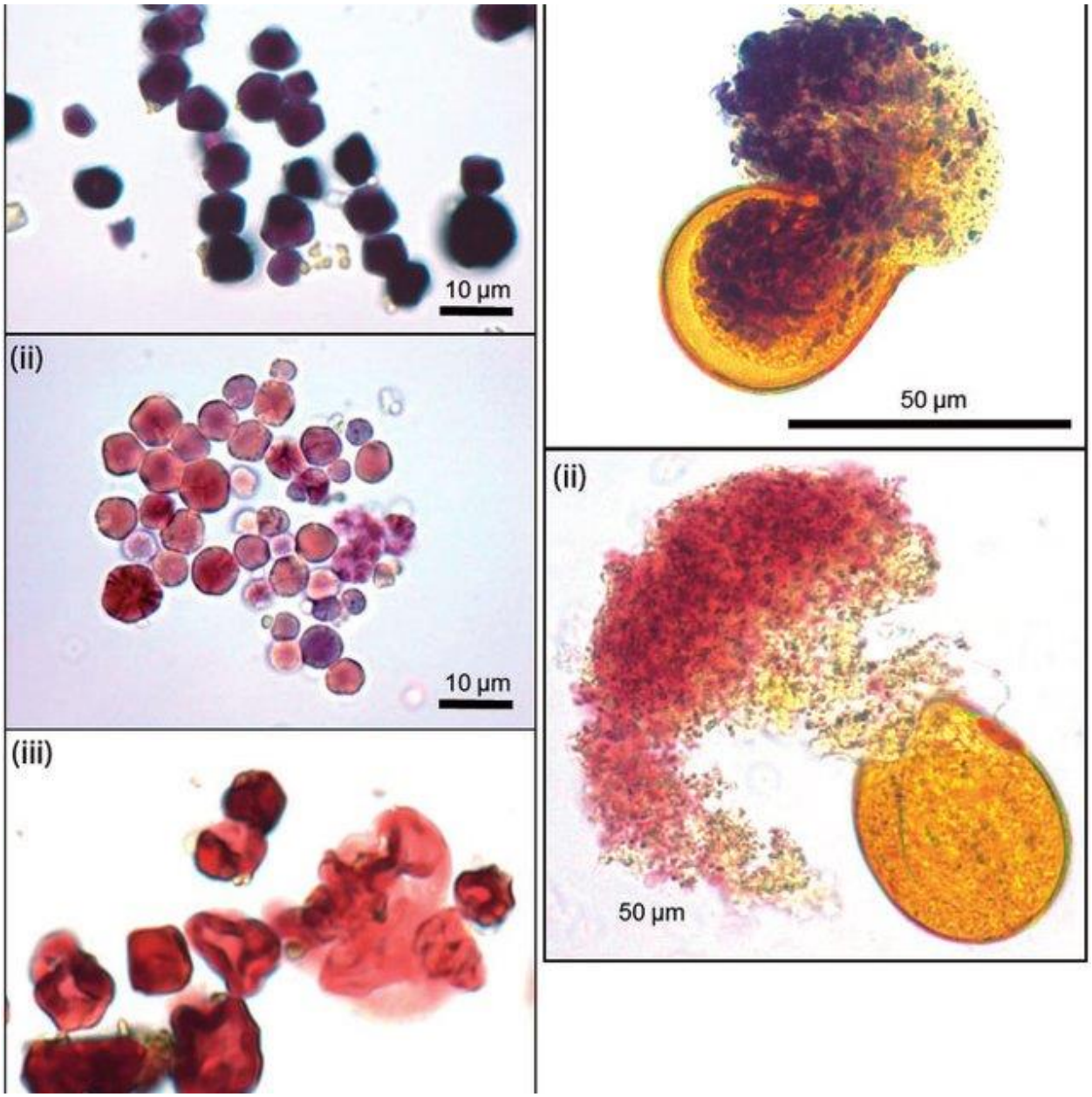


Starch

Lugol's Reagent:

The starch grains are highlighted in dark blue to black .Almost all other constructions have a yellow stain, but this hue has no special significance.

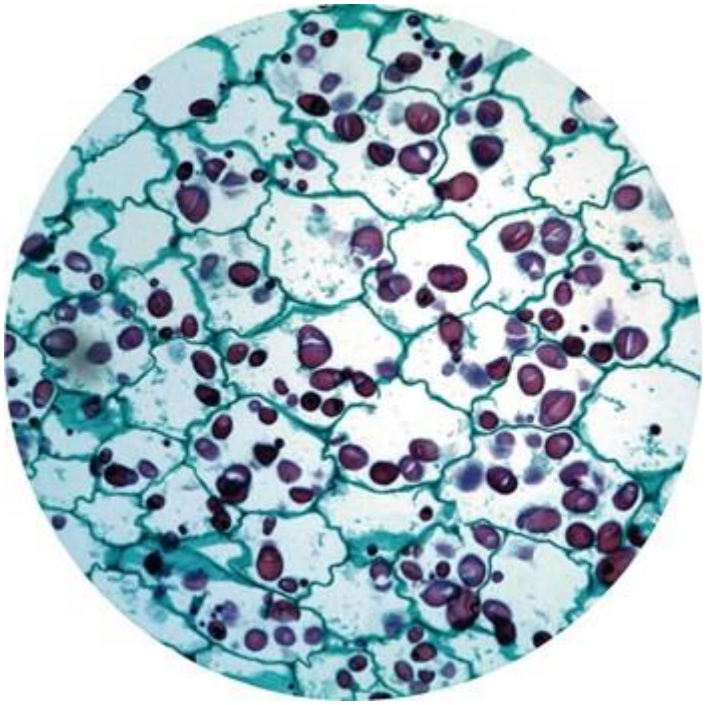
1. Soak the parts for 10 minutes in Lugol's reagent.
2. Rinse with distilled water for a few seconds.
3. Use distilled water or Lugol's reagent to mount the slides.



Triple Staining for Starch Detection:

This triple staining was created to simultaneously evaluate structural tissue components and starch grains .Starch grains turn black, acidic compounds (such as nucleic acids and lignin) turn brown, and non-lignified cell walls turn green when safranin, astra blue, and iodine–potassium iodide solution are applied

1. For 1 minute, stain the pieces with 1 percent safranin.
2. Remove excess discoloration by rinsing three times in 50 percent ethanol for a few seconds each time.
3. For 1 minute, stain with 1% astra blue.
4. Remove excess stain by washing three times in distilled water for a few seconds each time.
5. Wait 10 minutes before applying the iodine–potassium iodide solution.
6. Quickly dip pieces in distilled water. 7. Fill the slide with the least amount of water possible.

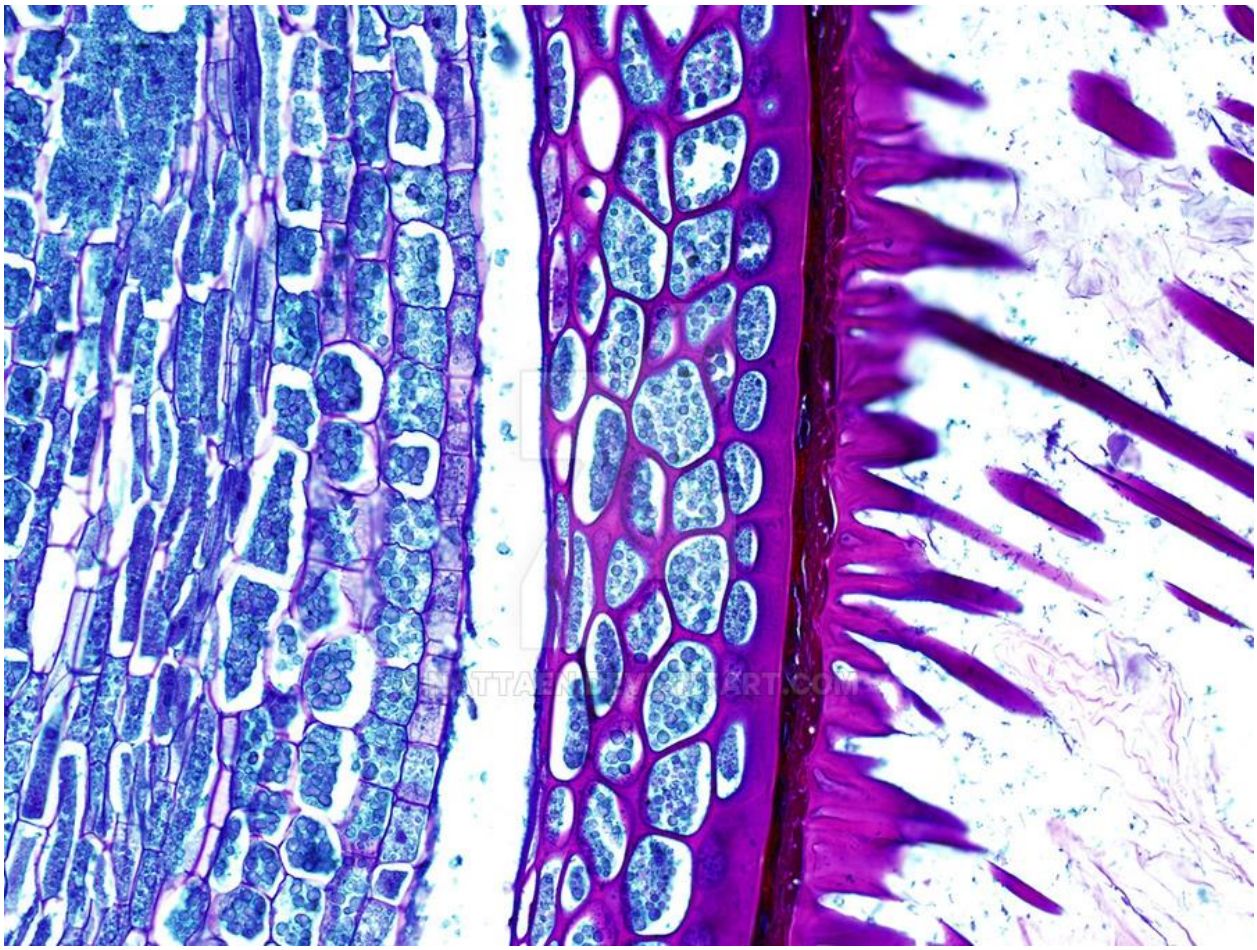


Carbohydrates

PAS Reaction (Periodic Acid: Schiff's reagent):

The Schiff's reagent reveals carbonyl groups formed when periodic acid reacts with carbohydrates, generating carbonyl groups. Carbohydrates have a magenta colour

1. For 30 minutes, apply 1 percent sodium tetraborate (freshly produced).
2. Submerge parts in 1% periodic acid for 10 minutes.
3. Rinse with distilled water for a few seconds.
4. In the dark, apply Schiff's reagent for 15 minutes.
5. Soak the parts for 10 minutes in sodium metabisulfite.
6. Rinse for 10 minutes with tap water.
7. Use glyceringelatin to mount the slides.
8. Repeat the test with the exception of step 2 as a control (periodic acid).



Aniline Blue Staining:

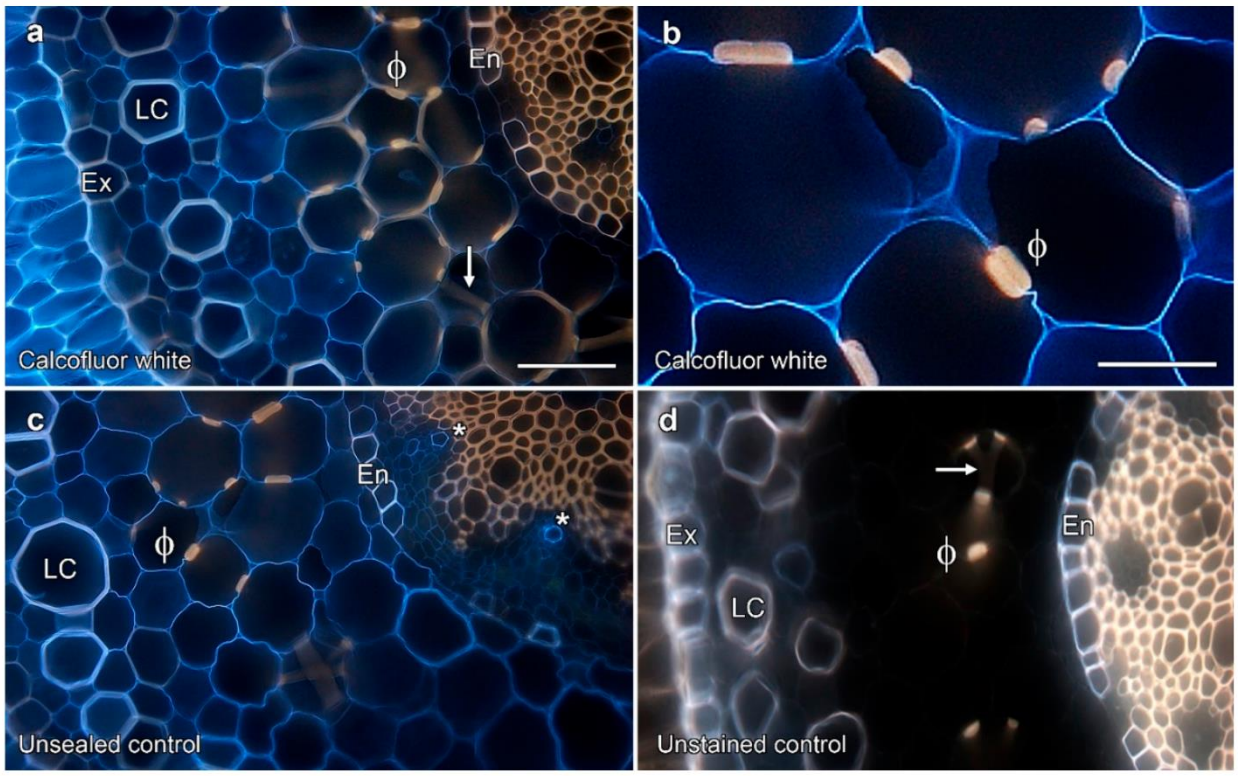
This staining identifies callose, which emits a green fluorescence when exposed to UV light

1. For 10 minutes, apply 0.05 percent aniline blue.
2. Rinse with distilled water for a few seconds.
3. Place the slide in the same staining buffer as before.

Calcofluor White Staining:

This assay is used to detect cellulose in cell walls, which emits a light blue fluorescence when exposed to UV light

1. Dip pieces for 10 minutes in 0.01 percent calcofluor white.
2. Rinse with distilled water for a few seconds.
3. Submerge the mount in distilled water.



Proteins

Aniline Blue Black Staining:

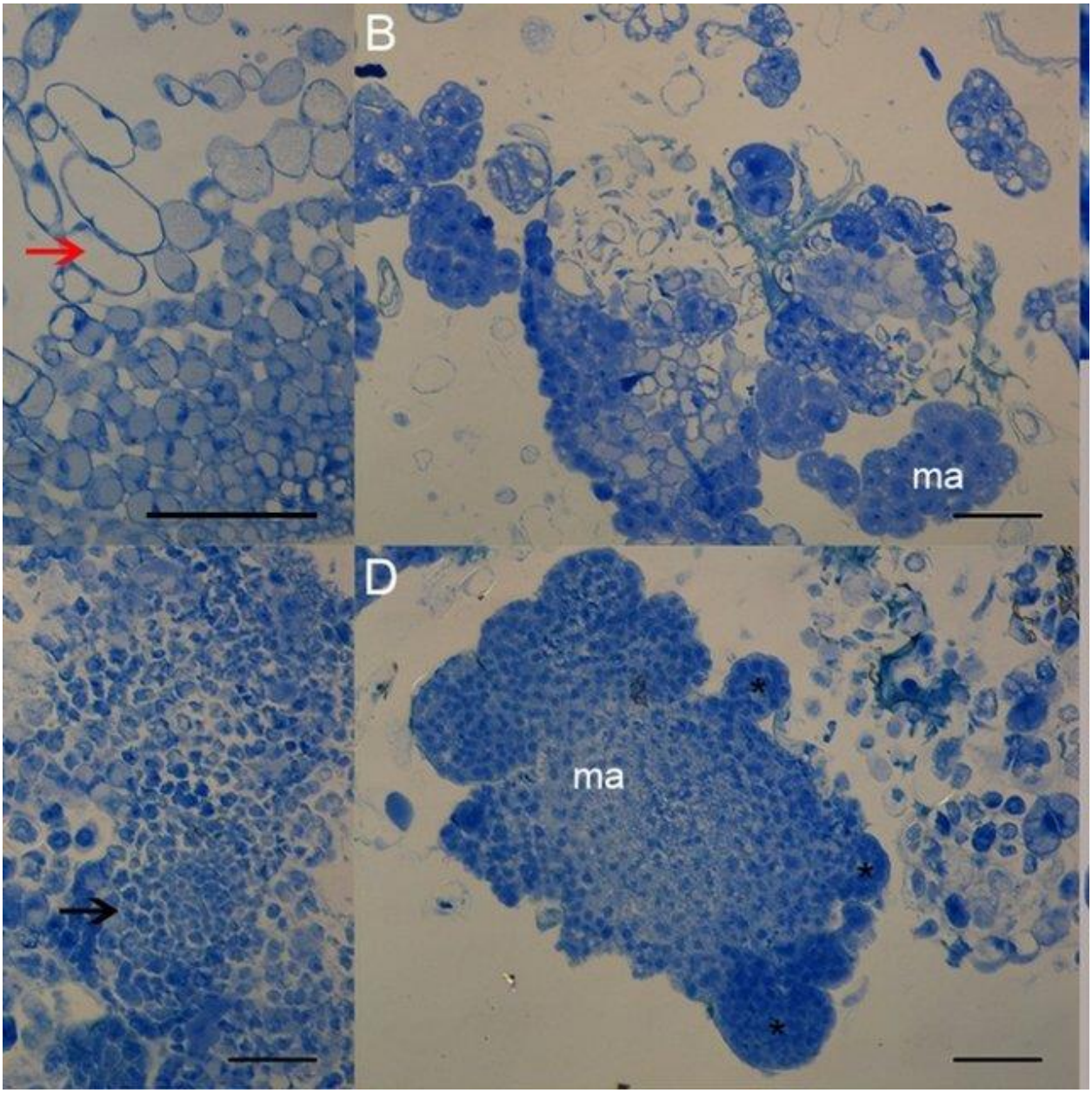
This stain identifies blue proteins, whether structural or active in primary or secondary metabolism

1. For 1 minute, dip sections in 1 percent aniline blue black.
2. Remove excess discoloration by washing twice in 0.5 percent acetic acid.
3. Rinse with distilled water for a few seconds.
4. Dehydrate portions by swiftly running them through 90 percent ethanol, 100 percent ethanol, a 1:1 mixture of 100 percent ethanol and xylene, and ultimately pure xylene.
5. Use synthetic resin to mount slides.
6. Control: Before staining, soak sections in a solution of acetic anhydride and pyridine (4:6, v/v) for 6 hours.

Coomassie Blue Staining:

This approach dyes proteins blue and yields a result similar to aniline blue black.

1. Stain for 15 minutes with 0.25 percent Coomassie blue.
2. Separate in a 7 percent acetic acid solution.
3. Rinse with distilled water for a few seconds.
4. Apply glyceringelatin on the surface.
5. Control: Before staining, soak sections in a solution of acetic anhydride and pyridine (4:6, v/v) for 6 hours.



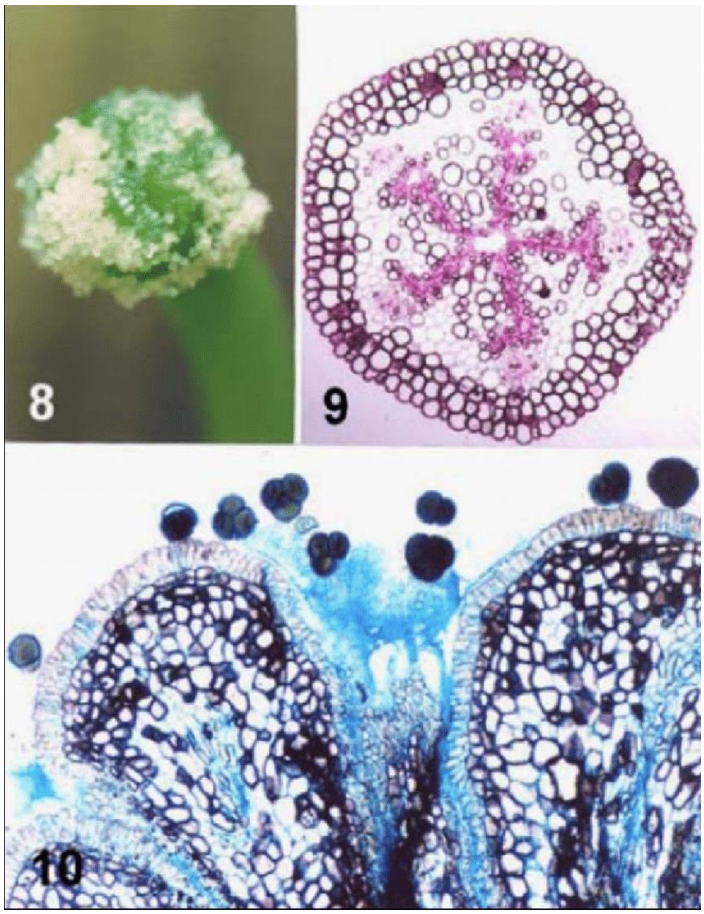
Lipids

Sudan Black Staining :

This is a typical approach for staining lipids, which results in a dark blue to black coloration

1. Stain for 20 minutes with Sudan black B.
2. . Rinse with 70% ethanol for a few seconds. 3. Use distilled water to clean. 4. Apply glyceringelatin on the surface.

3 Control: Depending on the content of the secretion, sections should be maintained in the extraction solution for 6 hours or more (determined empirically). Following this time, the portions should be transferred to distilled water and cleaned for 4 hours (4 1 hour). The staining will next proceed as specified.



Sudan IV Staining:

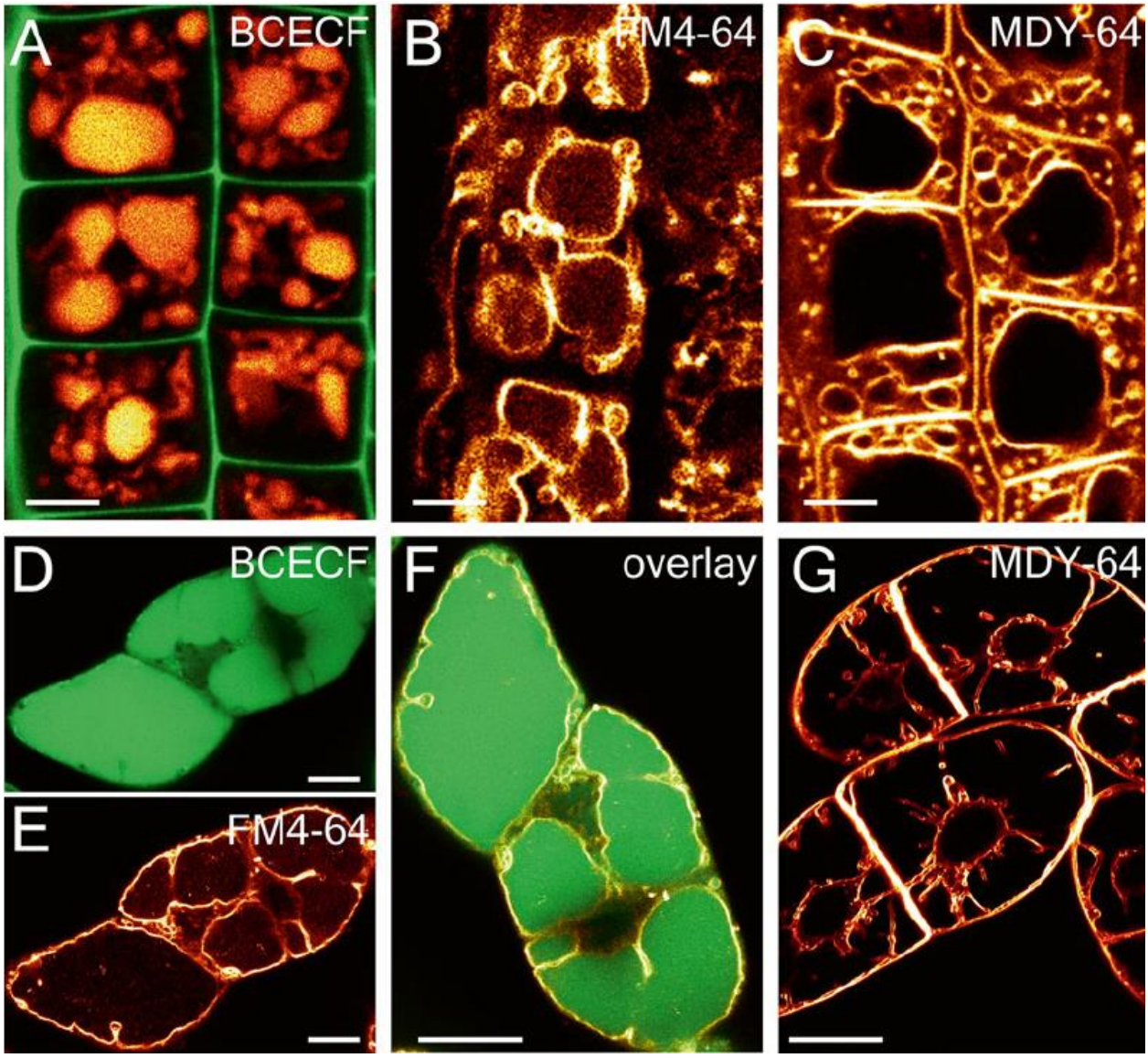
Sudan IV also stains lipids, which turn crimson or red-orange in general [14].

1. For 30 minutes, apply Sudan IV.
2. Rinse with 80 percent ethanol for a few seconds.
3. Use distilled water to clean.
4. Apply glyceringelatin on the surface.
5. Control: The sections should be kept in the extraction solution for at least 6 hours, just like Sudan black B.

Neutral Red Staining:

Depending on the lipid makeup, this fluorochrome produces distinct colours Secretion lipids glow yellow or green under blue light ,cuticle fluoresces yellow, and lignified cell walls fluoresce red.

1. Stain for 20 minutes with 0.1 percent neutral red.
2. Rinse with distilled water for a few seconds.
3. Submerge the mount in distilled water.
4. Control: The sections should be kept in the extraction solution for at least 6 hours, just like Sudan black B.

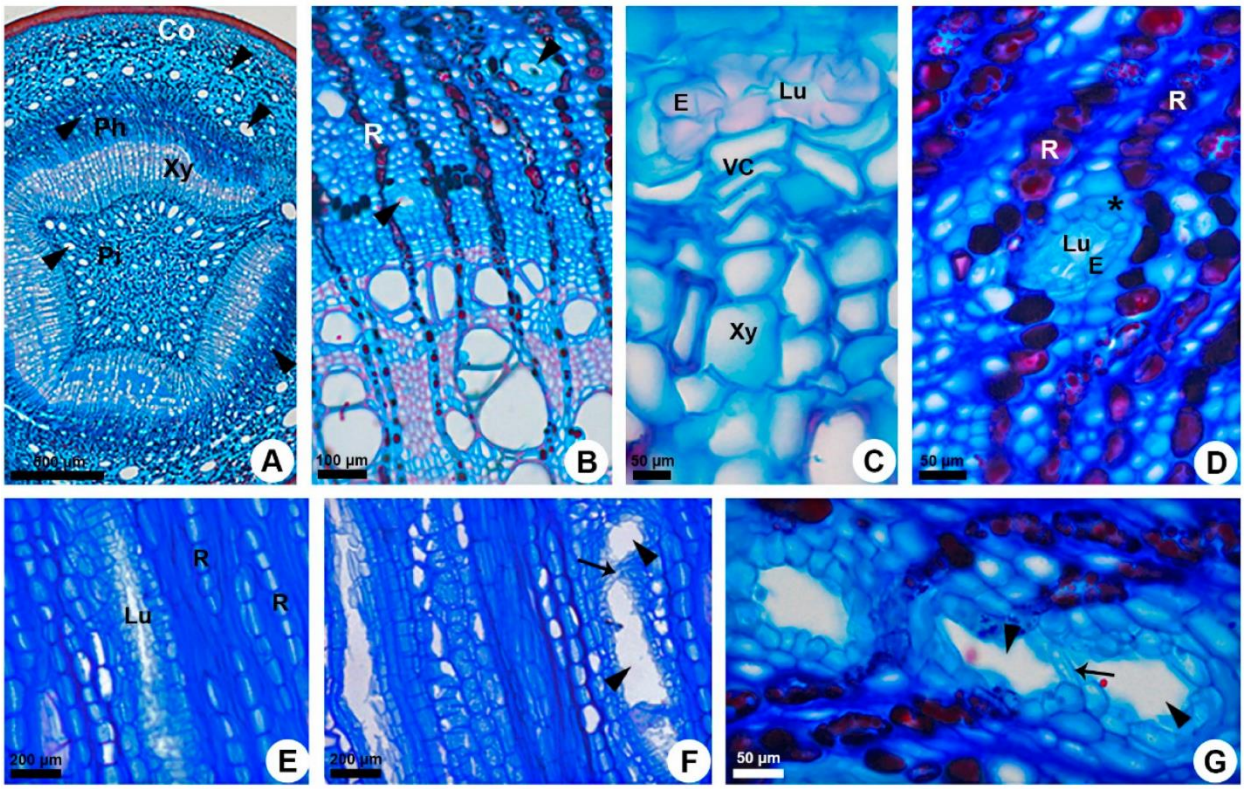


Acidic and Neutral Lipids:

Nile Blue Staining:

Nile blue separates acidic lipids, which stain blue, from neutral lipids, which stain pink because lipids were discovered in the sample.

1. Stain for 5 minutes at 60°C with Nile blue solution.
2. At 60°C, wash twice with 1 percent acetic acid.
3. Rinse well with distilled water.
4. Apply glyceringelatin on the surface.
5. Control: The sections should be kept in the extraction solution for at least 6 hours, just like Sudan black B.

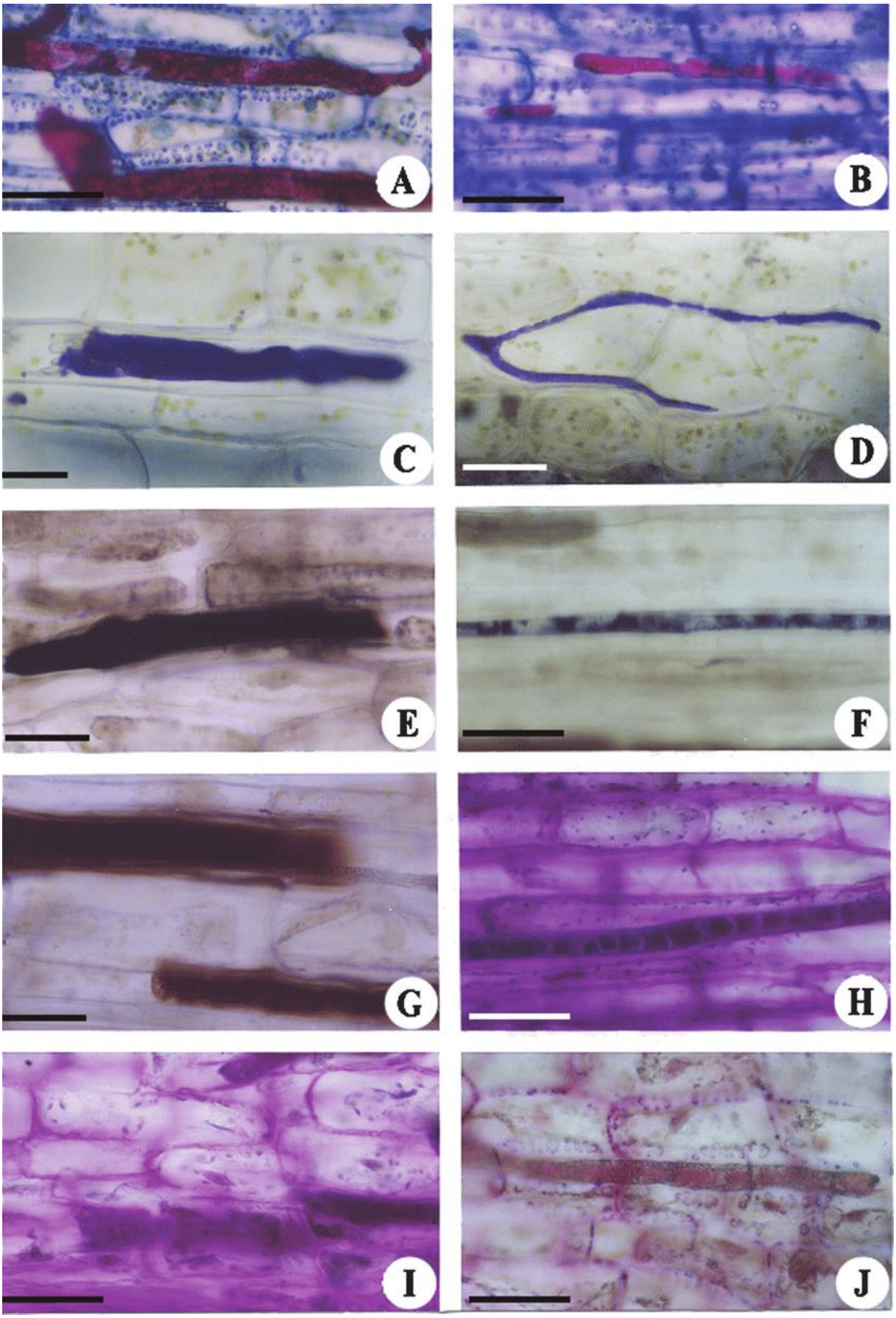


Fatty Acids:

Copper Acetate and Rubeanic Acid Staining:

This lipid identification method is slightly more specific than the Sudan tests, and it identifies fatty acids by reacting copper acetate with acidic lipids, which then turn dark green when exposed to rubeanic acid

1. For 3 hours, soak parts in 0.05 percent copper acetate.
2. For 5 minutes, apply 0.1 M Na2 EDTA (EDTA acid disodium salt solution).
3. Soak for 5 minutes in distilled water.
4. Soak parts for 20 minutes in 0.1 percent rubeanic acid (freshly produced).
5. Soak for 5 minutes in 70% ethanol.
6. Rinse well with distilled water.
7. Apply glyceringelatin on the surface.
8. Control: The sections should be maintained in the extraction solution for at least 6 hours, just like Sudan black B.

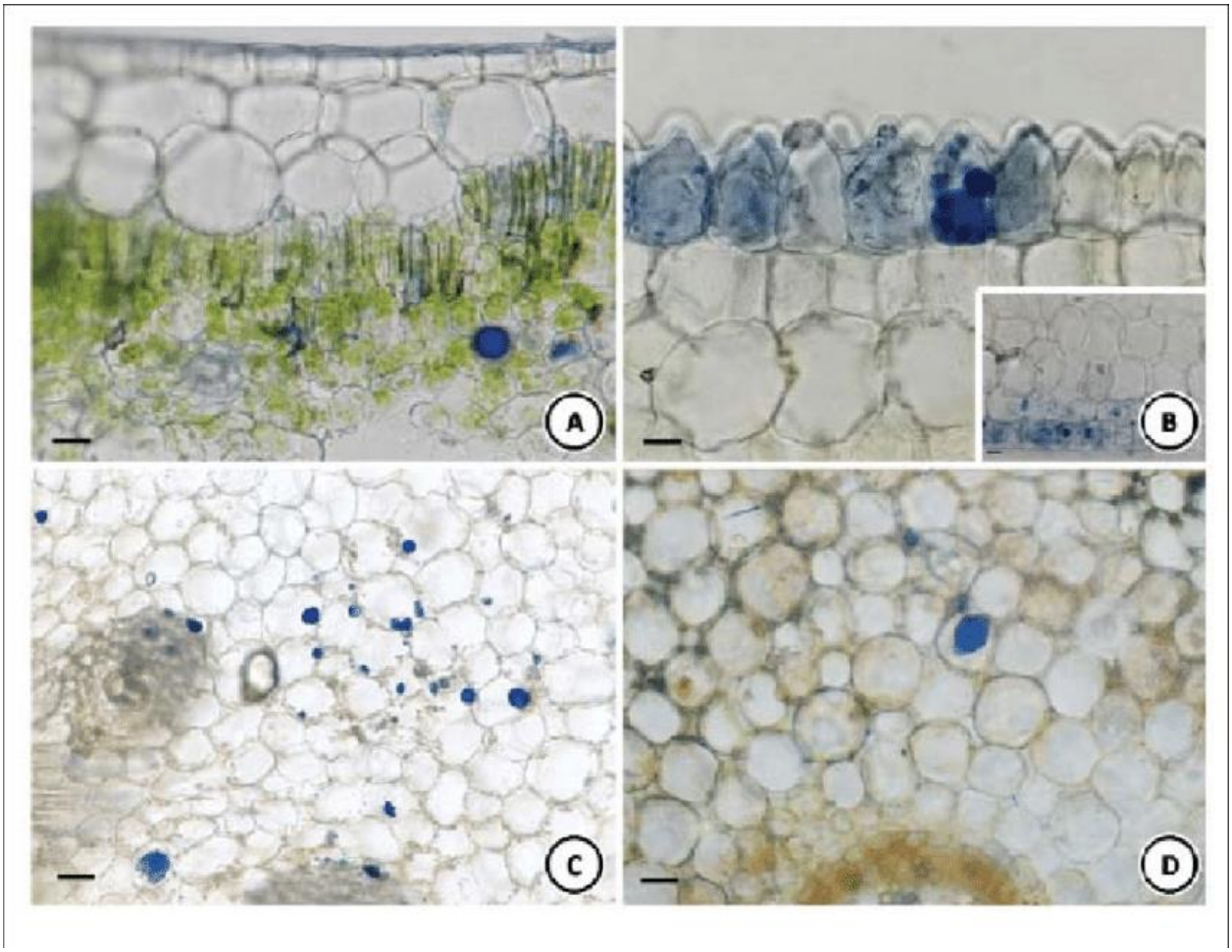

A
B
C
D
E
F
G
H
I
J

Terpenes

NADI Reaction:

Essential oils (; monoterpenes and sesquiterpenes) and resins (diterpenes, triterpenes, tetraterpenes, and derivatives) stain blue, whereas resins (diterpenes, triterpenes, tetraterpenes, and derivatives) stain red. Various hues of violet to purple are produced by combining essential oils and resins, depending on the amount of each compound present.

1. In the dark, apply NADI reagent for 1 hour.
2. Soak for 2 minutes in sodium phosphate buffer (0.1 M, pH 7.2).
3. Use the same buffer for mounting.
4. Control: The sections should be kept in the extraction solution for at least 6 hours, just like Sudan black B



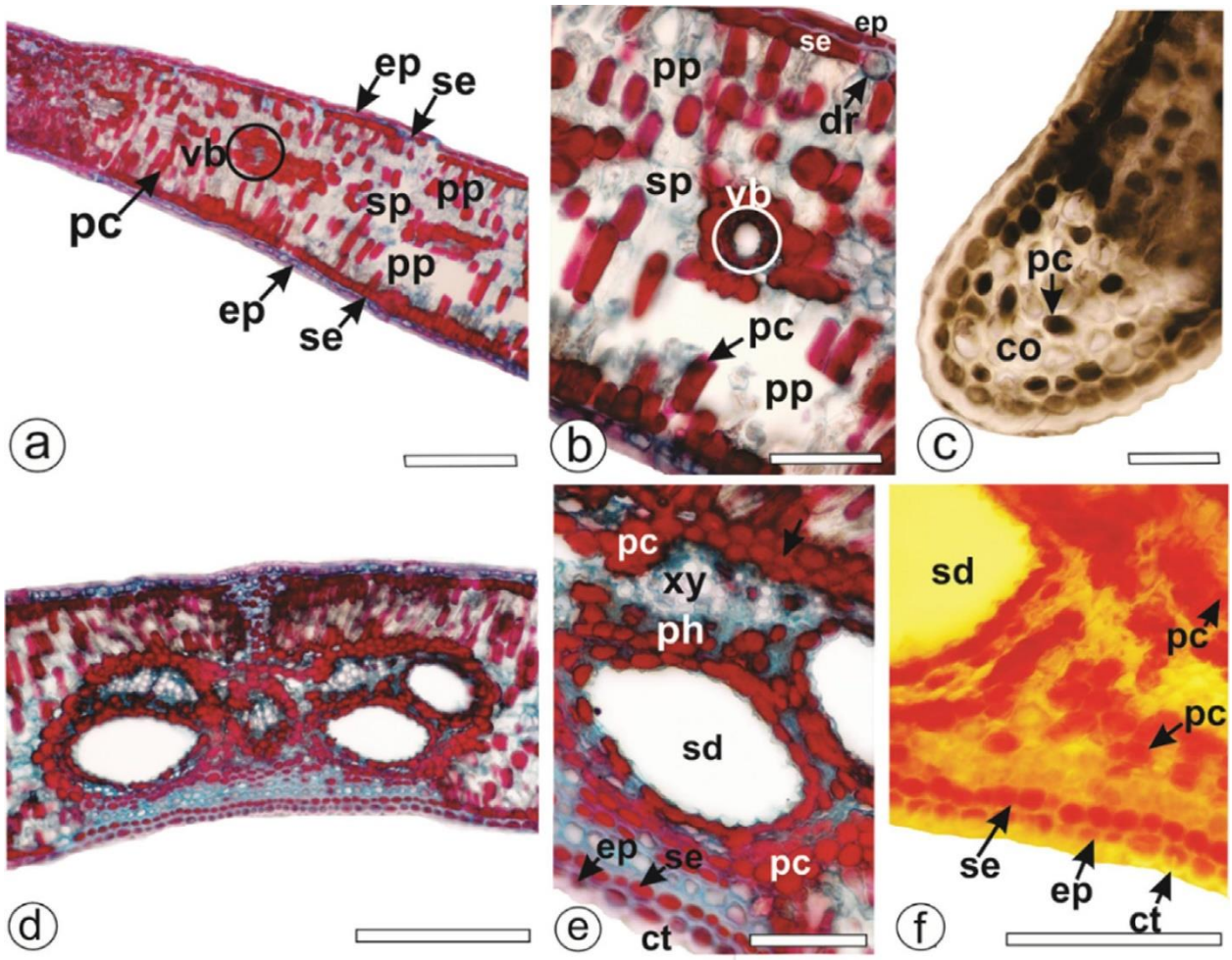
Detection of Phenolic Compounds and Alkaloids:

Phenolic compounds:

Ferric Chloride Staining

This method highlights phenolic compounds through iron precipitation, producing a dark color usually black sometimes brown.

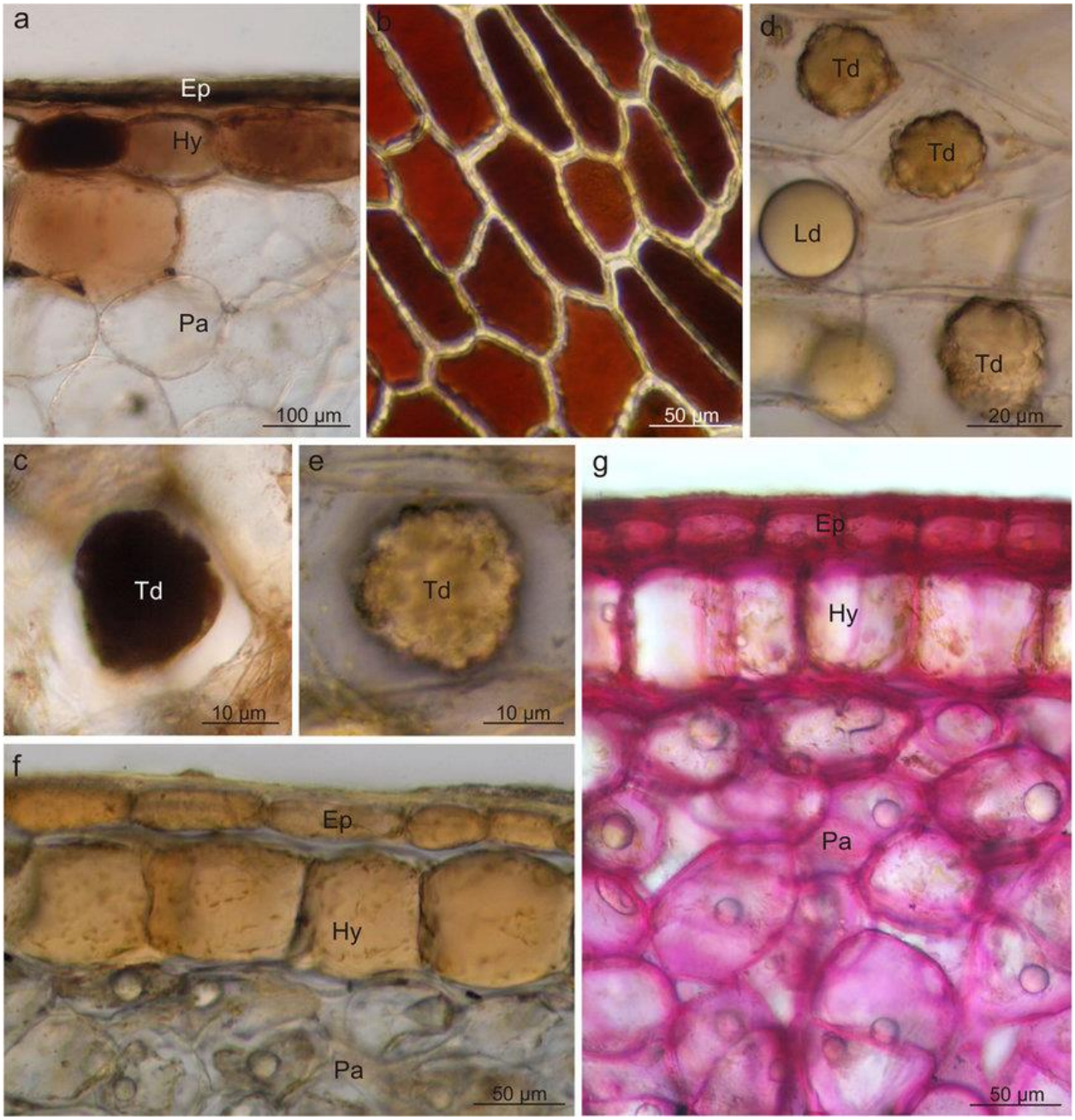
1. Apply 10% ferric chloride for 30 min.
2. Wash twice in distilled water to remove surplus ferric chloride.
3. Mount in glyceringelatin.



Potassiu m Dichromate Staining:

This method also highlights phenolic compounds; in general producing a brown or red-brown color

1. Apply 10% potassium dichromate for 30 min.
2. Wash twice in distilled water to remove surplus reagent.
3. Mount in glyceringelatin.



Ferrous Sulfate–Formalin Fixation:

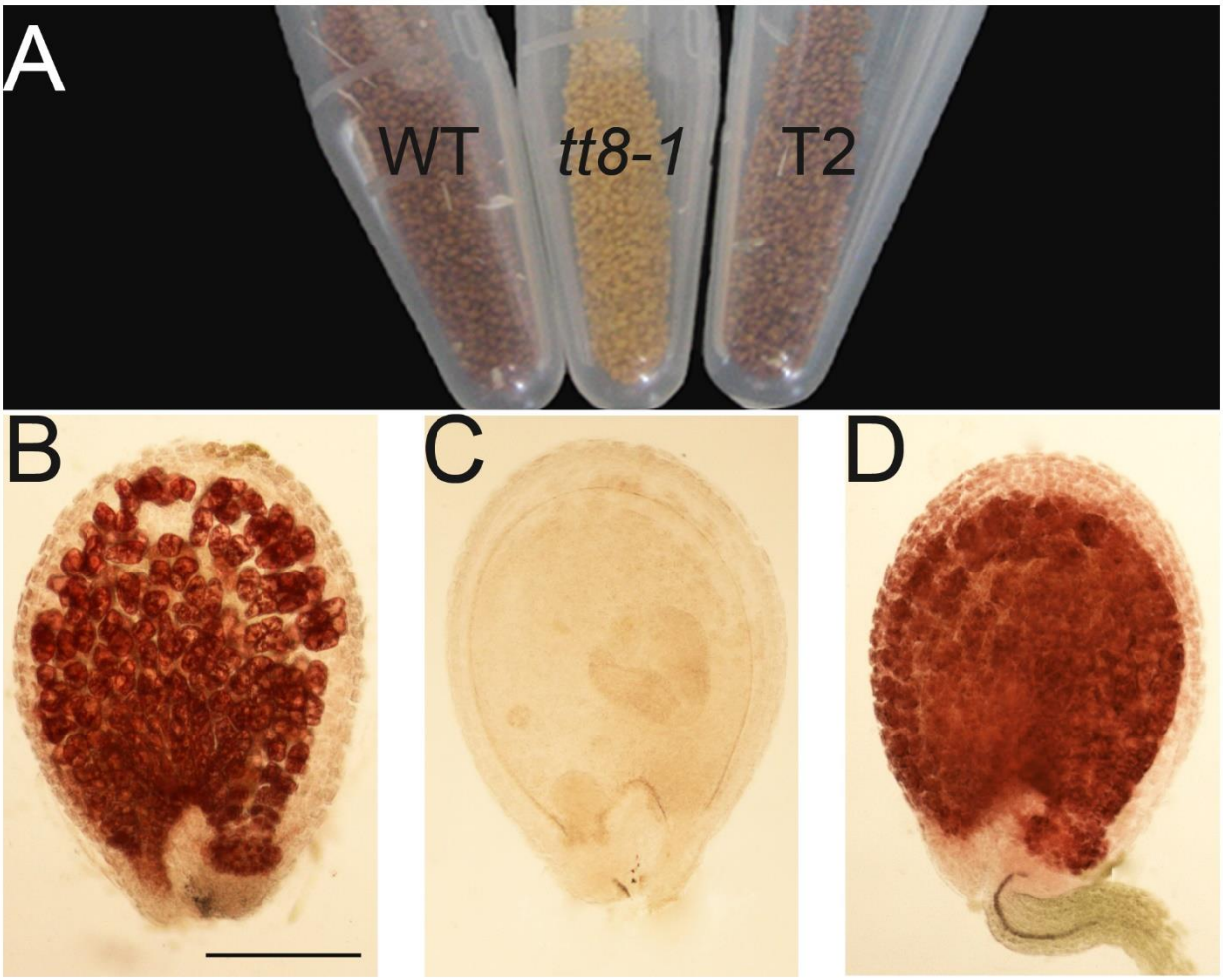
Iron salts in the fixative are the best way to identify phenolic compounds because the iron component fixes and stains the phenolic compounds

1. Fix the samples in a ferrous sulfate–formalin solution for 48 hours under vacuum.
2. Wash in distilled water for 4 2 hours (a total of 8 hours).
3. Dehydrate the material for 12 hours in 30 percent, 50 percent, and 70 percent ethanol, respectively.
4. Embed the material using the procedure of your choice (Paraplast, Historesin, or PEG), and then section it with a microtome.

Vanillin– Hydrochloric Acid Staining for Tannins:

This test is more specific for some phenolic compounds, staining tannins red Use only sections of fresh material.

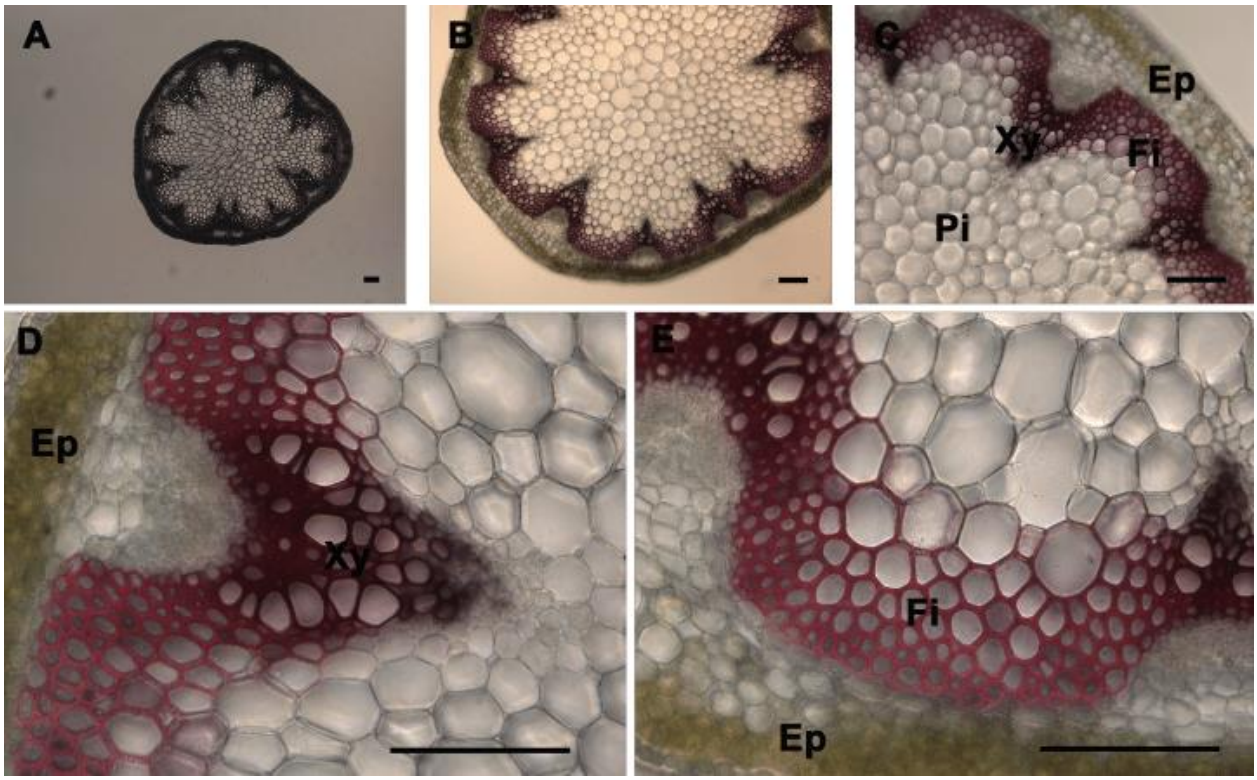
1. Treat with 0.5% vanillin for 20 min.
2. Mount the slide using 9% hydrochloric acid.



Phloroglucinol–Hydrochloric Acid Staining for Lignin

Phloroglucinol in an acidic medium stains lignin in cell walls pink to red It is possible to use either fresh or embedded material.

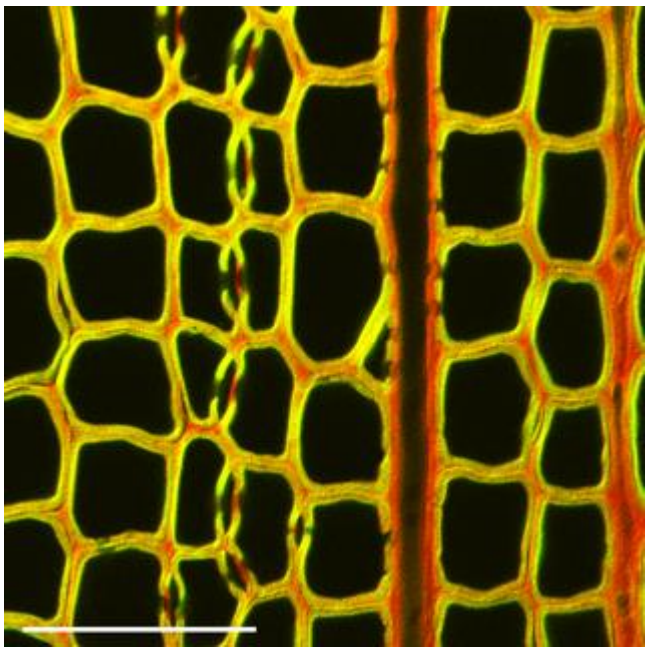
1. Apply 10% phloroglucinol for 15 min.
2. Mount the slides carefully with 25% hydrochloric acid.



Acridine Orange

In an acidic solution, phloroglucinol colours lignin in cell walls pink to crimson (Figure 3e) [12]. Fresh or implanted materials are also acceptable options.

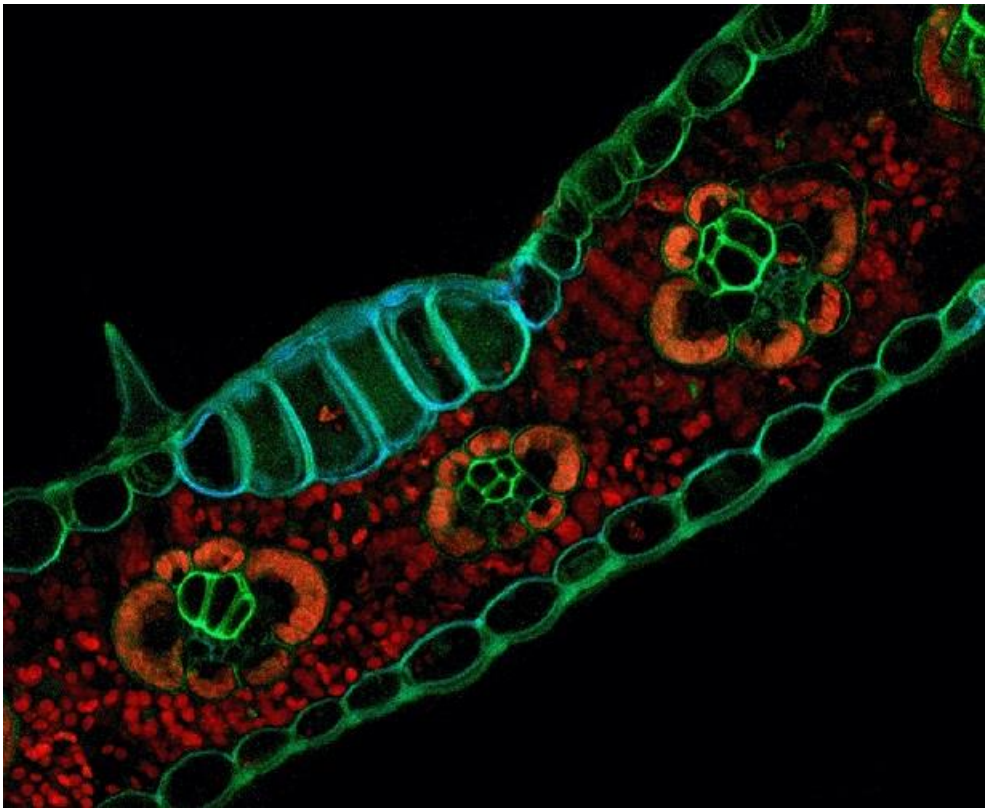
1. Use a 10% phloroglucinol solution for 15 minutes.
2. Mount the slides



Autofluorescence

Plant tissues have various autofluorescent components that allow them to be studied under UV light [30]. Many phenolic compounds (including lignin) emit a blue or blue-green fluorescence when compared to secondary metabolites (Figure 1g, 3f). However, caution should be exercised when using autofluorescence

to identify substances because several alkaloids and terpenoids may also generate fluorescence in the blue band [32].



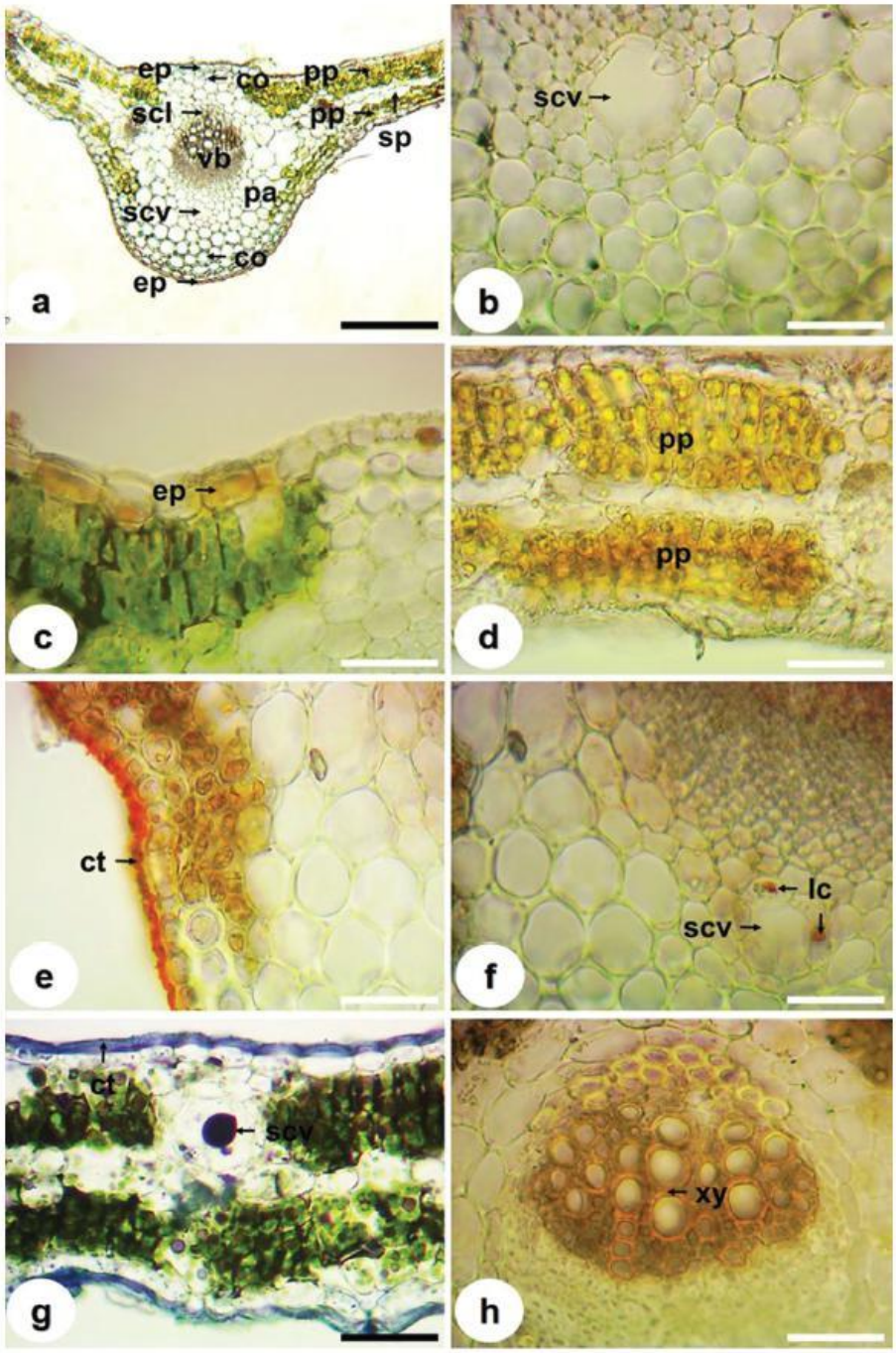
Alkaloids

Reagent:

Dragendorff:

This reagent leaves a red-brown stain on alkaloids (Figure 3h) [31]. This process can be utilised with both fresh and fixed material, however fixed material loses a lot of alkaloids and staining colour when compared to fresh material.

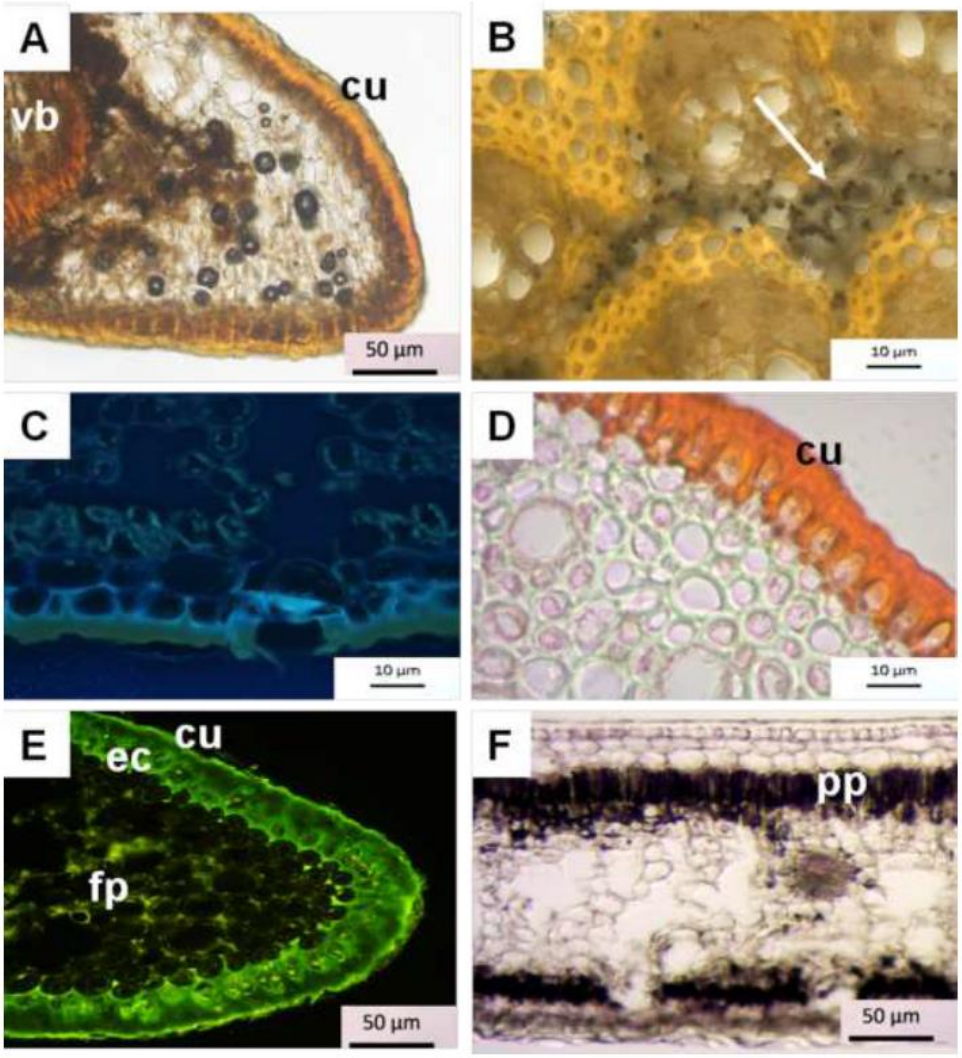
1. For a 20-minute treatment, use Dragendorff's reagent.
2. Rinse in a 5 percent sodium nitrite solution for a few seconds.
3. Submerge the mount in distilled water.
4. Repeat the staining technique after treating sections with 5% tartaric acid in 95% ethanol for 72 hours.



Wagner

Alkaloids are also stained red or redbrown using this approach. For this test, it is advised that fresh material be used.

1. Wait 20 minutes after applying Wagner's reagent.
2. Rinse with distilled water for a few seconds.
3. Submerge the mount in distilled water.
4. Repeat the staining technique after treating sections with 5% tartaric acid in 95% ethanol for 72 hours.



Acknowledgments

My thanks to guides and professors of Botany department worldwide to have given me references encouraged to write this paper and see photos for the same.

Citations

1. Histochemical Staining of *Arabidopsis thaliana* Secondary Cell Wall Elements By Prajakta Pradhan Mitra [1,2] and Dominique Loqué [1,2]
2. Plant Histochemistry: A Versatile and Indispensible Tool in Localization of Gene Expression, Enzymes, Cytokines, Secondary Metabolites and Detection of Plants Infection and Pollution Farid A Badria* and Walaa S Aboelmaaty
3. Google images for Photos
4. Photo of Alcian Blue was captured in Botany Research Laboratory (PG Block) of St. Joseph's College Bangalore.