HISTOCHEMISTRY

- Histochemistry is the branch of histology dealing with the **identification of chemical components** of cells and tissues
- Histochemistry is devoted to study the **identification and distribution** of chemical compounds within and between biological cells, using stains, indicators and light and electron microscopy
- Histochemical analysis is essential for the study of plant secretory structures whose classification is based, atleast partially, on the composition of their secretion

MINERALS

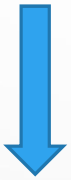
- Minerals occur in plant cells in diverse forms, soluble, insoluble or forming part of complex **organic substances**
- Minerals may be localized within the cell by several techniques of which the most important are micro-incineration, crystal formation and chemical coloration.
- More than **14 minerals** are present in plants
- Deficiency of minerals leads to chlorosis or necrosis
- **Calcium** is one of the most commonly encountered mineral substances of plants
- Calcium may be present as protein- **calcium complexes, calcium pectates, calcium carbonates, calcium salts of phosphates, carbonates, oxalates etc.**

- Calcium carbonate may either occur as free deposits or in specialized structures known as **cystoliths**
- Silicon is another material which is very common in plants. It occurs either as an incrustation or impregnation of the cellwall or silica bodies. They are also called as silica grains
- **Aluminium, magnesium, boron, copper and phosphorus** are also present in some plants
- The exact role of these minerals is not yet very clear, but the deficiency leads to some disorders in plants

Test for Aluminium- Aluminon reagent method

- Fresh or formalin fixed tissue is used

Fresh or formalin fixed material is used for free hand sectioning



Sections were boiled in the **aluminon reagent** (aurin tricarboxylic acid)

Result: development of red color indicates the presence of aluminium

Test for Boron- Crystal formation method

Fresh or formalin fixed tissue is used

10µm thick section is mount on clean glass slide without adhesive

↓

Microincinerate at 350°C, for 3 hours in muffle furnace and cool it to room temperature

↓

Add a small drop dil. HCl followed by a drop of Concentrated Potassium iodide

↓

After several hours add Concentrated Potassium chloride

↓

Finally stain the resulting crystals with 50% ethanol alkaline turmeric acid.

Result: Boron crystals are colored to an **yellowish tinge**

Test for Magnesium- Quinalizarin-Titian Yellow method

Section the material, deparaffinise and bring down to water

↓

Add 1 or 2 drops of **quinalizarin reagent**

↓

Add 1 or 2 drops of 10% **Sodium hydroxide**

Result: Magnesium develop **blue color** after several hours (24 hours)

Test for Calcium- Alkaline- Pyrogallol method

– Fresh or chemically fixed and paraffin embedded tissue

Section the material, deparaffinise and bring down to water

↓

Treat it with alkaline Pyrogallol reagent for 5 minutes

↓

Wash in distilled water to remove the reagent

↓

Leave section in water for several hours, color develops slowly

Result: Calcium stains to a **yellowish brown color**

Test for Calcium oxalate- Silver- Hydrogen peroxide method

- Fresh or formalin fixed and paraffin embedded tissue is used

Section the material, deparaffinise and bring down to water

Treat with 2N acetic acid for 15 minutes to remove phosphate and carbonate

Treat with 1% silver nitrate in 15% hydrogen peroxide for 15 minutes at 22°C.

Wash with distilled water

Counterstain with 2% safranin for 1 to 3 minutes

Dehydrate, clear and mount

Result: Calcium oxalate deposits and **crystal stain black** while **background is red**

Test for Silica- Methyl Red method

– Fresh tissue preferable, sections or peels can be used

Immerse tissue in 50% sulphuric acid for 2 to 5 minutes

Rinse in tap water and dehydrate in benzene

Treat the tissue with the dye solution and observe

Preparation can be counter stained with fast green

Result: The background appears **green** and silica appears **bright red**

CARBOHYDRATES

- Carbohydrates are **polyhydroxyketones** or **polyhydroxyaldehyde** or compounds which can be hydrolysed to these substances using dilute mineral acids
- They all share in common some chemical properties and some biological functions
- Carbohydrates are classified into **Monosaccharaides, Disaccharides and Polysaccharides**
- They also include substances such as **lipids and proteins that form complexes** with the carbohydrate proper
- Monosaccharaides and Disaccharides are **difficult to get localized** when histochemical tests are used.

- The polysaccharides are high-molecular weight molecules and may be linear or branched
  - **Homopolysaccharides-** on hydrolysis yield only one type of monomer

    Ex: Cellulose, Starch, Callose, Chitin
  - **Heteropolysaccharides-** on hydrolysis yield not only monomers containing **C, H** and **O** but also monomers which additionally have **N** and **S**

    Ex: Pectins, Hemicelluose, Gums etc.

Test for Insoluble polysaccharides- Periodic acid-schiff's reaction or **PAS reaction**

- Fresh, Frozen or chemical fixed and paraffin embedded tissue is used

Section the material, deparaffinise and bring down to water

↓

Block the tissue aldehydes in a saturated solution of **DNPH (2, 4 Dinitrophenylhydrazine)** for 30 minutes

↓

Oxidize the sections in **0.5% to 1% periodic acid** for 5-30 minutes

↓

Place the sections in **Schiff's reagent** for 10-30 minutes

↓

Transfer the sections quickly and directly to 3 successive baths of 0**.5% sodium bisulphate**, 2 minutes each

↓

Rinse in running water for 5-10 minutes, dehydrate and mount

Result: Polysaccharides stain a **purplish red to magenta** color. Starch grains react very strongly

Test for Callose- Soda Method

- **Fresh tissue preferable**

Section the material

↓

Place the section in a 4% aqueous solution of **Soda or Sodium bicarbonate** (Na_2CO_3) for 10 minutes

↓

Transfer the sections to **glycerin and mount**

Result: Callose changes to a **bright red**. If the color is very deep, transfer to 4% soda again. This will remove the stain bound to cellulose but not from callose

Test for Callose- Rosalic acid method

- **Fresh tissue preferable**

Section the material

↓

Stain in **1% rosalic acid** solution, in **4% aqueous sodium carbonate**

↓

Mount and observe

Result: Callose stains a **red color**

Test for Cellulose- Potassium iodide- iodine sulphuric acid method

- **Fresh tissue alone should be used**

Section the material

↓

Stain the sections in **potassium iodide-iodine** solution for 15-60 minutes

↓

Mount in the solution itself

↓

Result: Cellulose stains **yellow**

↓

Slowly add **60 to 75% sulphuric acid** through the sides of the coverslip until it diffuse into the section. Then observe

Result: Cellulose walls swell and take a **bright blue color**

Test for Pectin- Ruthenium Red method

- Fresh, Frozen or FAA fixed and paraffin embedded tissue is used

  Section the material, deparaffinise and bring down to water

  ⬇

  Place the sections in **aqueous ruthenium red** solution and observe

  Result: Pectin stains **red** or **pink**

Lignins

- Lignins are **phenolic polymers** present in the cell walls of the land plants
- Especially in **woody** dead cells such as the **tracheary elements, xylem and phloem fibres, and sclerenchyma**
- They fill up the space between the **micellae** of the cellulose framework of the cell wall
- Approximately **20%** of the cell wall materials of the **secondary xylem** of dicotyledons **and 35% of that of gymnosperms** constitute the lignins

Potassium iodide- iodine- sulphuric acid method

– Fresh tissue is used

Section the material

↓

Stain the sections in **potassium iodide-iodine solution** (Lugol's solution)

↓

Transfer the sections to **60- 70% sulphuric acid** solution

Result: Lignin becomes **yellow, yellowish-orange, or brown**

Schiff's reagent method

- Fresh or formalin fixed tissue is used. Fixative containing heavy metals are not suitable

Section the material, deparaffinise and bring down to water

↓

Stain the sections directly in **Schiff's reagent** for 15 minutes to 4 hours

↓

Wash, dehydrate, clear and mount

Result: Lignin stains **pink or majenta** (purplish red)

Toluidine blue O method

- Fresh, Frozen or chemically fixed and paraffin embedded tissue is used

Section the material, deparaffinise and bring down to water

Stain the sections in aqueous **toluidine blue O solution**

Wash in running water, dehydrate, clear and mount

Result: Lignin stains a **greenish blue color**