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Histological Specimen of Plant Parts by Microtomy- An Advance Technique

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ABSTRACT

Term microtomy is derived from two words- Micro and Tomy. Micro means very small (in microns) and Tomy means to cut. Microtomy is a procedure by which we can cut the section of giving sample in microns and by staining and microscope we can identify the given part. Plant anatomy plays an important role in identification. Knowledge of plant structure help to solve unknown contamination also. This points are most important because in Crude drug market a good number of drugs are either substituted or adulterated. For all such specimen microscopic structure helps in identification.

Microtomy is a method for the preparation of thin sections for materials such as bones, minerals and teeth, and an alternative to electropolishing and ion milling. Preparing leaf anatomy slide is a routine procedure in physiology and anatomy plant research. A standard microtechnique method frequently adopted for doing such procedure is Johansens'Method.

KEYWORDS – Microtome, Microtomy, Johansen's method, Plants

I. INTRODUCTION

Studying botany involves the needs for investigating the anatomy and physiology of plant.[1] Microtomy, which is defined as "sectioning" or "slicing," has been an essential component of microscopy for over a century. Microtome sections can be made thin enough to section a human hair across its breadth, with section thickness between 50 nm and 100 µm.[2] Ever since human curiosity peered through the microscope, methods and tools have been developed to slice anything of interest (animals, insects, plants, etc.) and place it on the stage for viewing. Cutting sections thin enough to view with a microscope was found nearly impossible to do by hand; therefore, machines that offered precision cuts were invented and have been fine-tuned over the decades. The almost universally adopted method of producing thin sections is by microtomy. This is a well-established procedure in the biological and medical fields and the basic techniques are extensively documented.[3] A standard microtechnique procedure frequently adopted for doing sectioning or paraffin protocol in plants is the Johansen method.[4]

II. MATERIAL AND METHOD

AIMS & OBJECTIVES

- By this method we can see the section of plant parts in microns.
- We can identify the given part.

We can identify weather the given part is real or a substitute by studying the microscopic structure.

PROCEDURE

Following procedure is adopted for making a Histological plate of specimen:

1.FIXATION
2.DEHYDRATION
3.CLEARING
4.EMBEDING AND BLOCK FORMATION
5.TRIMMING
6.SECTION CUTTING
7.SLIDE FORMATION
8.STAINING PROCEDURE

1.TISSUE FIXATION

Fixation is a complex series of chemical events that differ for the different groups of substance found in tissues

The aim of fixation:

a- To prevent autolysis and bacterial attack.
b- To fix the tissues so they will not change their volume and shape during processing.
c- To prepare tissue and leave it in a condition which allow clear staining of sections.

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d- To leave tissue as close as their living state as possible, and no small molecules should be lost.
Following chemicals are used as tissue fixative:
Formaline, Acetic acid, Glutaraldehyde, Alcohal etc. The Ideal fixative is formaline because of the following reasons:

- ❑ Rapid penetration in tissue.
- ❑ Tissue is fixed by forming cross linkage in the protein between lysine residue, without destroying protein structure.
- ❑ Tissue can remain in formalin for prolonged period without compromising histology.

2.DEHYDRATION

The aim of this is to remove water from tissue and replace with a medium that solidify it and to allow for thin section cutting.

Under this process tissue pases through different percentage of Alcohal solution.

70 % Alcohol , 90% Alcohol. Rectified spirit, Absolute Alcohol.

Duration

1 mm. thick - up to 30 minute in each alcohol.

5 mm. thick - up to 90 minute or longer in each change.

Normally time used :

- 70 % Alcohol - 3-4 hour
- 90% Alcohol - One night
- 95% Alcohol - 1 hour
- Absolute Alcohol I- 1 hour
- Absolute Alcohol II - 1 hour

3.CLEARING

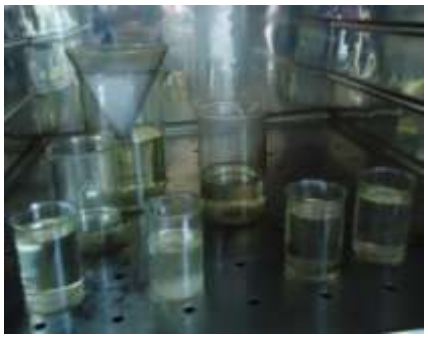
Replacing the dehydrating fluid with a fluid that is totally miscible with both the dehydrating fluid and embeding medium.

Some clearing agents:

Xylene, Toluene , Chloroform, Benzene, Petrol
Normally xylene is taken

4.EMBEDDING IN WAX AND BLOCK FORMATION

Process by which tissue are surrounded by a medium such as agar, gelatin or wax which when solidified will provide sufficient external support during sectioning.




5.TRIMMING OF PARAFFIN BLOCK

Trimming is making the pyramid like structure of paraffin wax block.


- That pyramid is mounted on a wooden block.
- Wooden block is kept for cooling.

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6.SECTION CUTTING

- Section Cutting is done by microtome.
- How much micro section you need can be adjusted in microtome.

The blade of microtome should be very sharp.


7.SLIDE FORMATION

- A well rubbed albumin slide is taken and cutting section is placed on it.
- Now a drop of dist. water from an edge is spreaded in length .

Finally slide is put on a hot plate for over night.

8.STAINING PROCEDURE

- The most accepted method for plant tissue staining is JOHANSEN'S method.[5] This protocol is adopted from the routine procedure of plant sectioning methods as described in Prawasti et al.[6]
- It is multistep procedure
- Take the slide in a coupling jar and keep this jar in xylene till whole wax becomes clear.
- Now put the block in absolute Alcohol, Rectified spirit Alcohol 90 % Alcohol 70% for in each.
- Wash the slide with water

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2-24 hour. Stain with **Safranin-0**

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Wash with dist.water

↓

10sec Dehy.witH 95%eth.alc.+.5% **picric acid**

↓

10sec wash with 95%eth.alc.+4drops**NH4OH**

↓

10sec wash with 100%alc.

↓

15sec counterstain with **Light green** solution

↓

10-15 sec Wash slides with clearing solution

↓

Few sec. dipping slides in xylene+100%alc.

III. RESULTS & DISCUSSION

Safranin-o - Gives brilliant red colour in lignified or cutinized cell wall, nuclei

Picric acid -Give Light green - Gives brillient green colour in cytoplasm and cellulosic cellwall

Now dry the slides with filter papers and put a drop of DPX Over it and cover with cover slip.

Now again put the slide's over hot plate for over night.

Next day the slide is ready for microscopic study.

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