Botany

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Plant Microtechnique

Introduction

Microtechnique is an important experimental science that has led and continues to lead a great service for each branch of the life sciences: microbiology, genetics, embryology, morphology and science, also plays an important role in the development of medical studies of human anatomy. This includes knowledge of the preparations microscopic sample, whether plant or animal. Sass (1958) was defined it as the science consists of three overlapping activities with each other: (1) Sample Preparation for microscopic study. (2) the proper use of the microscope and related devices help to explain the study samples. (3) codification of results and drawing which was replaced in modern imaging cameras , because of its major role in the transfer of the real image of the sample, so no interference from the researcher or the examiner in few modifications. And for the development and diversity of this science, it was divided into three sections: in which plant microtechnique is one of them.

Plant Microtechnique is not limited to the preparation of the samples, but includes the selection of necessary equipment, reagents and materials, selection of appropriate dyes, and knowledge in ways that prepared and get good samples. It depends on innovation and experimentation.

Preparation of large plant Material

The Large plant Materials: either the Plant or part of it retains its dry or wet for reference when needed, either in Herbariumor museums and classrooms. The preparation divides into two types:

1- Dry preparation

2 - Wet preparation

Dry preparation: The Plant, or a branch represents a complete plant, can be used for preparation, these samples or plant material must include stem and leaves, flowers or fruits, to identify the sample. In addition, the plant samples characterized by Morphological characters which must be concerned to remain for study. Since these samples will become dry and fragile, it must take care when used for the purposes of research and study.

To prepare the samples in dry preparation the following method can use

Pressing Method

Preparation of most plant material for display in a dry state, either as a whole plant or parts of it: 1 -by placing a plant or a part of it on a sheet of paper or newspapers leaves . 2- The plants are placed in a manner showing the external Morphology leaves ,flowers or fruits If they were overlapped to be spread.

Each plant material must be separated from other by few paper to prevent the plants from sticking to each others when removed. 4-Strand press used to process which may locally manufactured or bought from abroad which consists of two frames of metal or wood, either as a net or plate shapes. 5-then tie the two frames together including plant materials. 6-keep the plant materials to be dried in room temperature.

Wet preparation :

Plant samples need initial treatment with a fixative solution before it ready for display. When the plant removed or part of it, the tissues begin to decay or dry up, and then deform the morphology of the plant, and there are several reasons, including: 1. Loss of moisture from the samples that are exposed to the air. 2. Dead tissue exposure to a large number of micro-organisms, particularly bacteria and fungi. 3. The cells digest itself after the sample is separated from the plant, followed by the death of plant organ.

Sample preparation for display This is done by fixing plant sample to a plates of glass fits museum Jar or placed plant sample directly into the preservative Solution in museum Jar. 1 - Use a thin thread of nylon through certain holes in the board, and it must in this case be hidden behind the board and is not visible from any side in front of the display. 2. Use adhesive or adhesive tape do not dissolve in the solution

Retain the natural colour of the sample

To retain the natural colour of the Plant sample follow the steps: 1 -One liter of 20% glacial acetic acid solution 2 –Saturated the solution with Copper acetate in a large beaker and placed in a water bath at boiling point. 3 - then immersed fresh plant sample directly in the hot acid. 4 - notes that the sample take brown colour, but as soon as the green color back to its natural state. 5 – removed plant sample from the solution and washed in 50% Isopropyl Alcohol. 6 - then placed the sample in a preservative solution.

Collection of microscopic plant samples

Microscopic samples of plant material are small samples of the plant, which examined under a light microscope to illustrate the details of their structures which can not be seen and examined with the naked eye or manual lenses.

A- Flowering Plants Plant body is different in this group (trees, shrubs, herbs), it may be a large body or small, so collected depends on the size of its body, if the plant is large in size sufficient part of it should be collected so that represents a complete plant, as the branch has a stem , leaves and flowers, but small plants can be fully collected:

B- Liverworts & Mosses : Remove groups of Plant material with abundant amount of soil and placed in a bowl and then wetted with water from time to time. In the laboratory remove soil, taking care not to damage Plant material. Choose the appropriate sample and place it in a fixing solution.

C-Algae: Often algae live in the water or the waterlogged ground, so it collected with the amount of water that grow in it, and keep in a cool place. Some filamentous algae decompose and rot if they remained in the lab for along, so holding adequate control of lighting and proper temperature in the lab for the species under study, If is best to fix algae directly into the killer solution and keep for certain types of study.

D- Pathological materials Particular care should be exercised to ensure that the condition of host tissue is not altered by handling the samples. When collecting these samples placed in a wet containers be careful not contaminated with bacteria or fungi or mixed with any other secondary organelles. Collect disease- free samples from the same plant to compare the infected samples, and must choose the best technology to preserving the the normal condition of the host before starting to interpret the results of Pathological materials.

Types of sample preparation:

Sample preparation depends on the need of examination required , were divided into three types:

1- Temporary preparation
   Sample usually mounted with water: 1-Place a drop of water on a clean glass slide. 2- then place the sample and covered by the cover slide. 3 – Immediately, examined under a light microscope. Because of the water fast evaporation. 4- To prevent the evaporation of water place such as Petrolium jelly on the edges of the cover slide . 5- To keep a sample of a temporary preparation for longer replaces water with Glycerin which is less evaporation .

2 -Semitemporary preparation

Glycerin may be used as it is in temporary preparation , but also used Lactic acid and Phenol, particularly when preparing algae, fungi and ferns and thin sections and minute plant specimens, where the use of certain concentrations of fluids, including: A- Lactophenol: B - Phenol-glycerin C - Glycerin – Jelly (glycerin gel)

3-Permanent preparation

It means the preparation of plant sample, whether whole small plant or parts of large ones such as sections of individual organ . Large plant sample can not be mounted on a slide for examination under a microscope. Permanent preparation of samples retain their shape and structures for several years and can be consulted when need arises, e.g. samples are used for practical lessons or research or samples kept for reference. Note: Permanent preparation of plant samples and permanently dyed be better than samples prepared by temporary and semi-temporary preparation.

Methods of microscopic samples and their applications

Preparation of microscopic samples of different plantsdepend on plant sizesand complexity of their structures. There are several methods, including:

1 -Squash Method

2 -Smear Method

3 -Whole Mount

4 - Maceration Method

5 -Sectioning Method

Preparation of Pollen Grains : There are three methods followed for the preparation of pollen grains depending on the quality of microscopic examination: 1 –Preparation of pollen grains, examined under a Light Microscope. 2 -Preparation of pollen grains, examined under a scanning Electron Microscope. 3 -Preparation of pollen grains, examined under the Transmission Electron Microscope.

Whole mount: This method is used for minute plant specimens (small size) that do not need cutting or sectioning, e.g., filamentous algae, algal thallus , small leaves , parts of the Large leaf, stripped leaf and stem epidermis , hairs or pollen and parts of Flower.

Maceration Method:

When studying individual or isolated cells, the transverse or longitudinal sections of the samples are not enough to clarify those cells, so Maceration Method used. Various solutions are used to separate a tissue into its individual cells. These solutions dissolve or weaken the middle lamella so that the cells are easily shaken or teased apart.

1-Boiling water:

This method is used to macerate samples of a few soft woody tissues and cells and its cell walls consist of Pictec and cellulosic substances or separating limited layers of tissue, for example, Parenchyma tissues

2 - 5% sodium or potassium hydroxide solution: This method is used to separate individual cells, Parenchyma tissues or lignified elements within Parenchyma tissues or a group of a few lignified cells. 3 - 10% chromic acid solution and 10% nitric acid This method is used to macerate some of the tissue that consists of a single layer of cells as Almgannh in his palace some seeds or group of cells Almgannh submerged in the textile Albornchimi.

4 -Schultze's Maceration Method. Schultze used strong nitric acid and potassium chlorate:

1 - Put the material, which should be in very small pieces, into a test-tube.

2 - pour on just enough nitric acid to cover it, and then add a few crystals of potassium chlorate.

3 - Heat gently until bubbles are evolved, and let the reagent act until the material becomes white. Four or five minutes should be sufficient. The fumes are disagreeable and are very injurious to microscopes.

4 - Pour the contents of the tube into a dish of water. After the material is thoroughly washed in water, it may be teased with needles and mounted, or it may be put into a bottle of water and shaken until many of the cells become dissociated.

5 - Sectioning Method or Microtomy: Preparation of large botanical specimens that can not be examined directly under Light Microscope must be cut into small pieces, involve to schedules appropriate time, and use of certain solvents, according to the nature of these samples. It is the first step to prepare a slide of the plant material for microscopic investigation. Fresh or preserved materials are cut into thin sections at suitable plane. It is essential to cut section thin enough to observe the details at the required level. Hand sectioning is carried out with sharp razor. Uniform section of given thickness can be obtained by special Machines called Microtome. Prior to microtome sectioning, material is processed which involves the following steps:

1 – fixation.

2 – dehydration.

3 –Clearing

4 –Sectioning

5 – embedding

6 –Staining

7 - Mounting