Plant Microtechnique

The preparation of the samples includes the selection of necessary equipment, reagents and materials, selection of appropriate dyes, and knowledge in ways that prepared the samples.

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 Herbarium or museums and classrooms. The preparation divides into two types:

1- Dry preparation

2 - Wet preparation

Wet preparation :

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.

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 cannot be seen and examined with the naked eye or manual lenses.

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:

Types of sample preparation:

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

1- Temporary preparation
2 - Semi temporary preparation
3- Permanent preparation

Temporary preparation:

Sample usually mounted with water:

2 - Semi- temporary preparation:

Glycerin may be used as it is in temporary preparation , but also used lactic acid and phenol, particularly when preparing algae, fungi, ferns, thin sections and minute plant specimens.

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 cannot 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.

Preparation of microscopic samples of different plants depend on plant sizes and complexity of their structures. There are several methods, including:

1 - Squash Method
2 - Smear Method
3 - Whole Mount
4 - Maceration Method
5 - Sectioning Method

1- Squash Method

In some cases, a researcher needs to study the internal structures of botanical samples that are not shown by sections. Squash Method shows the internal structures of the samples and their relations.

This method used to study the spores that exist within the sporangia, or study the phases of meiosis occurring within the pollen mother cells in the anther to form pollens or to study mitosis in stems and roots tips.

There are several methods to prepare microscopic slides for phases mitosis division:

1- Acetocarmine Staining Method
2 - Colchicine Method
3 - The karyotype

2- Smear method:

This preparation aims to spread the individual cells and cell organelles suspended in a liquid and make them in a homogeneous single layer(membrane) on a glass slide in order to be killed and fixed at once without showing artifacts. Generally all the cells or cell organelles which present stick on the slide and then no need for adhesives to fix them on the slide, because adhesives may be pigmented and give artifacts. This method used for the preparation of plant specimens-their bodies consist of a single cell, like bacteria , some fungi and algae , isolated cells or cell organelles, e.g. Plastids and mitochondria.

3 - 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.

To prepare a complete samples of higher plants, small leaves or small parts of large leaves or flower parts or stripped leaf and stems epidermises, and other parts. To study the characteristics of the epidermal cells, such as stomata, trichomes and cuticle

4 - 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.

5- Sectioning method or microtomy:

Preparation of large botanical specimens that cannot 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 – Embedding 4 – Sectioning

6 –Staining 5-Mounting