Physical fixation

Physical fixation

- It is accomplished by microwaving and cryopreserving the samples.
- Microwaving rapidly inactivate cellular activities using microwave (MV) energy
- Cryopreserving rapidly inactivate cellular activities using low temperatures.

Chemical vs Physical Fixation

- The application of physical methods in fixing vacuolated plant cells and tissues remains of limited value when compared to animal studies.
- The combined use of chemical and physical fixation is recommended for optimal results, and this can lead to further improvements in fixation and processing of biological specimens.

Chemical vs Physical Fixation

- There is no ideal chemical fixative that can fix and preserve all cellular components.
- Physical fixation minimizes artifact formation, typically, only samples of smaller sizes can be adequately processed, especially for ultrastructural studies.

Cryofixation of Botanical Specimens

- At the LM level, CF and sectioning of tissue are more successful with animal tissues than with plant tissues.
- The presence of vacuoles in plant cells make them prone to ice damage during freezing, and the inherent properties of plant cell walls also make sectioning difficult

Cryofixation

- CF arrests cell activities instantaneously at low temperatures (−180°C).

Cryofixation

- Low temperature vitrifies cell water and prevents ice formation and, together with subsequent freeze substitution (FS) techniques and embedding processes, produce better quality images which can be seen at the ultrastructural level.

Cryofixation for LM

- At the LM level, samples are pretreated and protected by a cryoprotectant, frozen in liquid nitrogen, and sectioned using a cryostat and processed accordingly

Cryofixation for EM

- (1) plunge freezing
- (2) spray freezing
- (3) propane-jet freezing
- (4) high-pressure freezing (HPF)

Microwave Fixation

- Although CF takes only milliseconds to immobilize the tissue, the subsequent processing time can be long, up to several days.
- MF has become more popular in recent years

Microwave Fixation

- MW energy causes excited molecules to rotate, generating a quick and homogeneous heat within a sample, thus greatly speeding up chemical reactions during fixation and subsequent processing steps.

Microwave Fixation

- It also improves the accessibility of fixatives and reagents to cells and maintains antigenicity of cell components

Microwave Fixation

- The inclusion of a vacuum chamber in the MW cavity further enhances penetration of fixatives and other solvents.
- This feature is especially beneficial to the processing of botanical specimens.
- The vacuum-MW combination enhances the diffusion of fixative and improves the quality of the cell morphology

Comments on Practical Issues of Fixation

Fixatives and Fixation of Botanical Specimens at the LM Level

- The methods used in sample collection and fixation vary depending on the tissue of interest.
- For good quality fixation, it is important to collect and fix the tissue immediately and at the same time.

Suggestions on tissue handling

- Avoid physical damages to the specimen during dissecting; the tissues should be dissected or excised using sharp knives or double-edged razor blades.

Suggestions on tissue handling

- At the time of dissection, the specimen needs to be trimmed carefully with the final image and the correct plane of section in mind in order that the specimen can be embedded easily.

Suggestions on tissue handling

- Tissues should be immersed in the fixative or buffer at all times during the excision, if possible.
- The cut surfaces should be in contact with the fixative as soon as possible.

Suggestions on tissue handling

- The final size of specimens for fixation depends on the choice of fixative, the embedding medium, and the objective of the experiment.
- Retrimming of tissue blocks after dehydration or prior to embedding is not recommended, as the tissues will be very brittle and it is difficult to make precise cuts.

Suggestions on tissue handling

- Tissues covered by cuticular materials, the specimens have to be cut open to allow for the penetration of the fixative.
- If necessary, for LM, a small drop of detergent (e.g., Tween 20) can be added to the fixative to disrupt the surface tension of the cuticle.

Suggestions on tissue handling

- All botanical specimens should be subjected to a vacuum treatment to ensure that air is removed from intercellular spaces.

Suggestions on tissue handling

- It is always advisable to vacuum the sample for 5–15 min to extract air from the tissues in order to improve on fixative penetration and subsequent infiltration of the embedding medium.

For large specimens

- For large specimens, a fast penetrating fixative is preferred.
- Hence, FAA or a 4 % buffered PFA solution is sufficient.

For high-resolution LM

- Which involves the use of acrylate resins, in order to improve fixation quality, a combination of PFA (1–2 %) and glutaraldehye (2–3 %) solution is preferred.

For high-resolution LM

- The specimen size is usually smaller than those fixed for paraffin sectioning.
- Furthermore, in order to avoid problems with polymerization of acrylate resins, the tissues need to be sliced thin, to a thickness of 1–3 mm.