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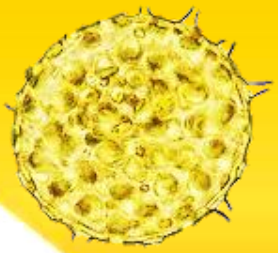

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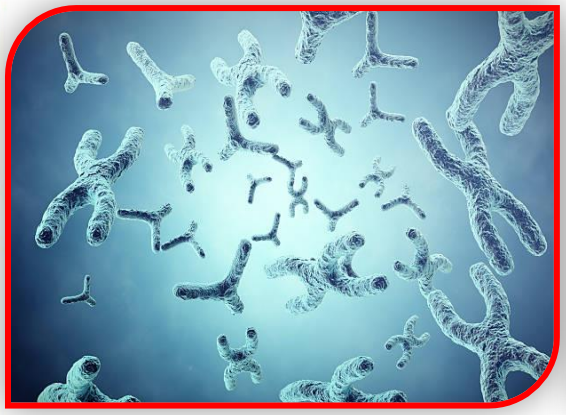

BOT 322
EXPERIMENTAL TAXONOMY

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BOT322 EXPERIMENTAL TAXONOMY

Lab Activity No 01
Title of the Activity: Examination of Essential Tools in Experimental Taxonomy with Emphasis on Safety Considerations

Learning Objectives:
- To identify and understand the use of essential tools in experimental taxonomy.
- To learn the correct handling and safety measures associated with these tools.
- To understand the significance of these tools in taxonomic research.

Aim:
To study and examine important tools used in experimental taxonomy and their safety considerations.

Apparatus:
- Dissecting microscope
- Compound microscope
- Forceps
- Scalpel
- Glass slides and cover slips
- Measuring scale
- Petri dishes
- Hand lens
- Herbarium press
- Microtome
- Safety gloves and goggles

Theory:
Experimental taxonomy involves the use of various instruments for the collection, preservation, and microscopic analysis of plant specimens. These tools aid in studying morphological, anatomical, palynological, and cytological characteristics essential for taxonomic classification. Proper handling and safety measures are crucial to avoid contamination, damage to specimens, and potential hazards while working with sharp instruments or chemicals.

Diagram: (See Figure 1)

Procedure:
1. **Identification of Tools:** Observe and list the tools commonly used in experimental taxonomy.
2. **Understanding Functions:** Study the function of each tool and its relevance in taxonomy.
3. **Demonstration of Usage:** Handle each tool carefully under supervision and practice its correct usage.
4. **Safety Considerations:** Follow safety guidelines while using sharp tools, glassware, and microscopes.
5. **Maintenance:** Learn how to clean, store, and maintain the tools for long-term use.

Observations Table:

Tool Name	Function	Safety Considerations
Dissecting Microscope	Magnification of surface structures	Handle carefully, avoid direct sunlight
Forceps	Handling small specimens	Use with a steady hand to avoid damage
Scalpel	Cutting plant material	Use with caution, store with cover
Herbarium Press	Drying and preserving plant samples	Ensure proper ventilation, avoid moisture
Microtome	Thin sectioning of plant tissues	Wear gloves, operate with supervision

Result:
The tools essential for experimental taxonomy were studied, and their functions and safety precautions were understood.

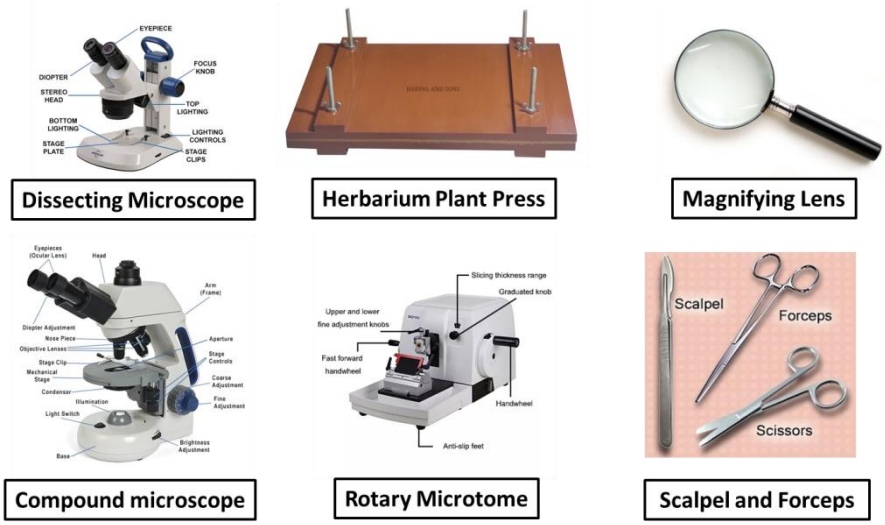


Figure 1. Diagram illustrating important tools in experimental taxonomy

Conclusion:
Understanding the correct usage and safety measures of taxonomic tools is crucial for accurate specimen analysis and laboratory safety.

Precautions:
- Handle sharp tools like scalpels with care to prevent injuries.
- Always wear safety gloves and goggles while working with chemicals or sharp instruments.
- Store tools properly after use to maintain their functionality.
- Clean microscopes and glass slides before and after use to ensure clear observations.
- Follow proper disposal methods for plant material and used slides.

Lab Activity No 02
Title of the Activity: Analysis of Key Chemicals Used in Experimental Taxonomy with Safety Precautions

Learning Objectives:

- To identify and understand the role of key chemicals used in experimental taxonomy.
- To analyze the proper handling, storage, and disposal of these chemicals.
- To emphasize safety precautions while working with chemicals in taxonomic studies.

Aim:
To study and analyze important chemicals used in experimental taxonomy and their associated safety measures.

Apparatus & Chemicals:

- **Chemicals:**
  - FAA (Formalin-Acetic Acid-Alcohol) – for specimen fixation
  - Glycerine – for temporary slide mounting
  - Safranin – for staining plant tissues
  - Fast Green – for counterstaining tissues
  - Acetocarmine – for chromosome staining
  - Iodine Solution – for starch detection in plant cells
  - Ethanol – for dehydration and preservation
  - Xylene – for clearing plant tissues
- **Apparatus:**
  - Glass beakers
  - Droppers
  - Pipettes
  - Microscope slides and cover slips
  - Safety gloves and goggles

Theory:
In experimental taxonomy, chemicals are essential for specimen preservation, staining, clearing, and microscopic examination. Fixatives like FAA help maintain tissue integrity, while stains like Safranin and Acetocarmine enhance structural visibility. Chemicals such as ethanol and xylene facilitate tissue processing for microscopic analysis. Proper safety precautions are necessary to prevent hazardous exposure and ensure safe laboratory practices.

Diagram: see Figure 2.

Procedure:

1. **Identification of Chemicals:** List and categorize the chemicals based on their function (fixatives, stains, clearing agents, etc.).
2. **Observation of Chemical Properties:** Note color, solubility, and reactivity of each chemical.
3. **Demonstration of Usage:** Prepare slides using different chemicals to understand their role in specimen processing.
4. **Safety Guidelines:** Follow safety precautions while handling chemicals, such as wearing protective gear and working in a well-ventilated area.
5. **Storage and Disposal:** Learn proper storage methods (e.g., keeping volatile chemicals sealed) and appropriate disposal techniques to minimize environmental impact.

Observations Table:

Chemical Name	Function in Taxonomy	Safety Precautions

FAA	Fixative for preserving plant tissues	Use in fume hood, avoid direct contact
Glycerine	Temporary mounting medium	Avoid ingestion, store in airtight bottles
Safranin	Stains lignified and cutinized tissues	Wear gloves, avoid skin contact
Fast Green	Counterstain for plant sections	Use in minimal quantities, store safely
Acetocarmine	Staining chromosomes in cytological studies	Use with care, can be a potential irritant
Iodine Solution	Detects starch in plant tissues	Handle with gloves, avoid inhalation
Ethanol	Dehydrating and preserving agent	Highly flammable, keep away from flames
Xylene	Clearing agent for tissue transparency	Use in well-ventilated areas, toxic fumes

Result:
The roles, handling procedures, and safety precautions for key chemicals used in experimental taxonomy were analyzed.

Figure 2. Use of key chemicals in taxonomic procedures

Conclusion:
Proper handling and safety measures are essential when working with chemicals in experimental taxonomy to ensure accurate results and maintain laboratory safety.

Precautions:
- Always wear gloves, lab coats, and safety goggles when handling chemicals.
- Work in a well-ventilated area, especially with volatile substances.
- Store chemicals in labeled containers and away from heat sources.
- Dispose of chemicals according to standard laboratory waste disposal guidelines.

- Avoid direct contact with skin and eyes; wash immediately if accidental exposure occurs.

Lab Activity No 03
Title of the Activity: Preservation and Poisoning Techniques for Herbarium Specimens

Learning Objectives:
- To understand the methods used for preserving herbarium specimens.
- To learn about different poisoning techniques to prevent fungal and insect damage.
- To study the importance of proper preservation in plant taxonomy.

Aim:
To study various preservation and poisoning techniques used in the preparation of herbarium specimens.

Apparatus & Chemicals:
- Plant specimens
- Herbarium press (wooden or metal)
- Blotting paper/newspaper sheets
- Mounting sheets
- Adhesive (Fevicol or PVA glue)
- Forceps and scalpel
- Brushes
- Insect repellents (naphthalene, paradichlorobenzene)
- Fumigants (mercuric chloride solution, alcohol-formalin mixture)
- Labels and writing materials

Theory:
A herbarium is a collection of dried, pressed, and preserved plant specimens systematically arranged for taxonomic study. Proper preservation ensures long-term storage without fungal growth or insect infestation. Poisoning techniques are employed to protect specimens from decay and pests. Traditional methods use chemicals like mercuric chloride, while modern approaches favor safer alternatives like freezing or microwave treatment.

Diagram: see Figure 3.

Procedure:
A. Preservation Techniques:
1. **Collection of Specimens:** Collect plant samples with essential parts (flowers, leaves, stems, roots).
2. **Pressing and Drying:**
    - Place specimens between blotting paper in a herbarium press.
    - Change papers regularly to speed up drying.
3. **Mounting on Herbarium Sheets:**
    - Arrange dried specimens aesthetically on a mounting sheet.
    - Secure them using glue, thread, or adhesive tape.
4. **Labeling:**
    - Attach a label containing botanical name, family, collection location, collector's name, and date.

B. Poisoning Techniques:

1. **Chemical Poisoning:**
    - Apply **mercuric chloride** (traditional, but toxic) or safer alternatives like alcohol-formalin mixture to prevent decay.
    - Use **naphthalene balls** or **paradichlorobenzene** to repel insects.
2. **Fumigation:**
    - Expose specimens to **formaldehyde vapors** or **alcohol-formalin solution** in a closed chamber.
3. **Freezing Method:**
    - Store specimens at –20°C for 48 hours to kill insects and fungi without chemicals.
4. **Microwave Treatment:**
    - Brief exposure (30-60 seconds) in a microwave eliminates pests.

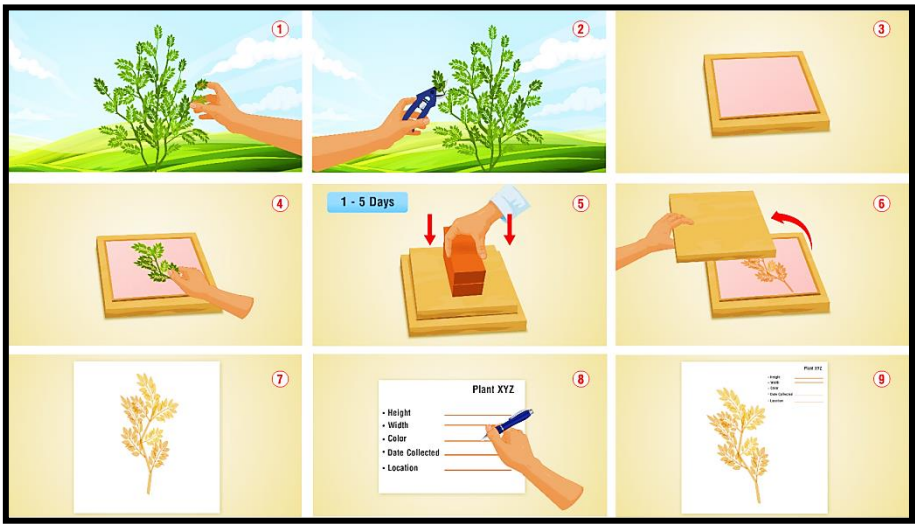


Figure 3. Diagram illustrating the herbarium preparation

Observations Table:

Method	Purpose	Advantages	Disadvantages
Chemical Poisoning	Prevents fungal and insect damage	Long-lasting protection	Some chemicals are toxic
Fumigation	Kills insects and prevents decay	Effective for large batches	Requires special setup
Freezing	Kills pests without chemicals	Eco-friendly and safe	May not prevent long-term fungal growth
Microwave Treatment	Quick insect removal	Safe, easy, and effective	May alter specimen structure

Result:

Different preservation and poisoning techniques were studied, and their effectiveness in herbarium preparation was analyzed.

Conclusion:

Proper preservation and poisoning techniques are essential to maintaining herbarium specimens for long-term study and reference in plant taxonomy.

Precautions:

- Use chemical poisons with caution; avoid inhalation and direct skin contact.
- Always label preserved specimens correctly for future reference.
- Ensure proper ventilation when using fumigants.
- Regularly inspect herbarium specimens for signs of insect or fungal infestation.
- Use eco-friendly methods like freezing when possible.

Lab Activity No 04

Title of the Activity: Investigation of Stomatal Types in Representative Dicot and Monocot Species Using Light Microscopy

Learning Objectives:

- To study the structure and types of stomata in dicot and monocot plants.
- To compare stomatal distribution and arrangement in dicot and monocot leaves.
- To understand the taxonomic significance of stomatal characteristics.

Aim:

To investigate and compare the stomatal types in representative dicot and monocot plant species using light microscopy.

Apparatus & Chemicals:

- Fresh leaves of a dicot plant (e.g., *Hibiscus*, *Sunflower*)
- Fresh leaves of a monocot plant (e.g., *Maize*, *Grass*)
- Light microscope
- Forceps and scalpel
- Glass slides and cover slips
- Watch glass
- Safranin stain
- Glycerine
- Needle and brush
- Dropper
- Distilled water

Theory:

Stomata are microscopic pores present in the epidermis of leaves and stems, playing a crucial role in gas exchange and transpiration. The type, distribution, and arrangement of stomata differ between monocots and dicots.

Types of Stomata:

1. **Anisocytic (Cruciferous Type)** – Three unequal subsidiary cells (e.g., *Brassica*).
2. **Paracytic (Rubiaceous Type)** – Two subsidiary cells parallel to the guard cells (e.g., *Rubia*).
3. **Diacytic (Caryophyllaceous Type)** – Two subsidiary cells perpendicular to the guard cells (e.g., *Dianthus*).
4. **Actinocytic** – Subsidiary cells arranged radially (e.g., *Mollugo*).
5. **Gramineous (Dumbbell-shaped)** – Guard cells are dumbbell-shaped (common in monocots, e.g., *Zea mays*).

Diagram: See Figure 4.

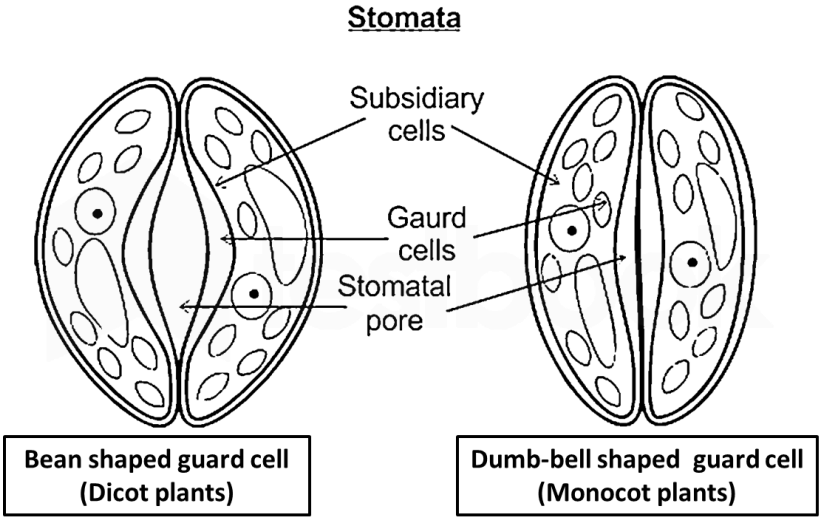


Figure 4. Stomatal types in dicots and monocots

Procedure:

1. **Sample Collection:**
   - Collect fresh leaves of a dicot and a monocot plant.
2. **Peel Preparation:**
   - Carefully remove the lower epidermis from the leaf using a scalpel.
   - Place the peel in a watch glass containing distilled water.
3. **Staining:**
   - Transfer the peel to a glass slide and add a few drops of safranin stain.
   - Let it sit for 1-2 minutes, then rinse with distilled water.
4. **Mounting:**
   - Place the stained peel on a clean slide and add a drop of glycerine.
   - Cover with a cover slip and remove excess liquid using blotting paper.
5. **Microscopic Examination:**
   - Observe the slide under a light microscope.
   - Identify the type of stomata and note differences between monocot and dicot leaves.

Observations Table:

Plant Type	**Example**	**Stomatal Type**	**Guard Cell Shape**	**Distribution**
Dicot	*Hibiscus*	Anisocytic	Kidney-shaped	Lower epidermis
Dicot	*Sunflower*	Diacytic	Kidney-shaped	Lower epidermis
Monocot	*Zea mays*	Gramineous (Dumbbell-shaped)	Dumbbell-shaped	Both epidermises
Monocot	*Grass species*	Paracytic	Dumbbell-shaped	Both epidermises

Result:

Different stomatal types were observed in monocot and dicot plants using light microscopy. Dicots generally exhibit kidney-shaped guard cells with anisocytic or diacytic stomata, while monocots have dumbbell-shaped guard cells with gramineous stomata.

Conclusion:
Stomatal characteristics differ between monocots and dicots and provide significant taxonomic information.

Precautions:
- Handle leaves carefully to avoid damaging the epidermis.
- Use fresh plant material for better clarity under the microscope.
- Avoid over-staining to prevent excessive coloration of the specimen.
- Ensure proper focusing while observing under the microscope.
- Clean slides and microscope lenses properly after use.

Lab Activity No 05
Title of the Activity: Identification of Trichome Types in Representative Dicot and Monocot Species Using Light Microscopy

Learning Objectives:
- To study the morphology and classification of trichomes in dicot and monocot plants.
- To compare trichome types and distribution patterns in dicots and monocots.
- To understand the taxonomic significance of trichome characteristics.

Aim:
To identify and compare the types of trichomes in representative dicot and monocot plant species using light microscopy.

Apparatus & Chemicals:
- Fresh leaves of a dicot plant (e.g., *Hibiscus*, *Sunflower*)
- Fresh leaves of a monocot plant (e.g., *Maize*, *Grass*)
- Light microscope
- Forceps and scalpel
- Glass slides and cover slips
- Watch glass
- Safranin stain
- Glycerine
- Needle and brush
- Dropper
- Distilled water

Theory:
Trichomes are epidermal outgrowths found on plant surfaces, including leaves, stems, and flowers. They play a role in protection, water retention, and herbivore deterrence. Trichome types vary between monocots and dicots and can be classified into two major categories:

Types of Trichomes:
1. **Glandular Trichomes** – Secrete substances like oils and resins (e.g., *Ocimum*).
2. **Non-Glandular Trichomes** – Provide physical protection; further classified as:
    - **Unicellular** – Single elongated cell (e.g., *Zea mays*).
    - **Multicellular Unbranched** – Several cells arranged in a linear fashion (e.g., *Hibiscus*).
    - **Multicellular Branched** – Trichomes with branched structures (e.g., *Cotton*).

- **Peltate Trichomes** – Shield-like structures (e.g., *Brassicaceae*).
- **Stellate Trichomes** – Star-shaped (e.g., *Solanum*).

Diagram:

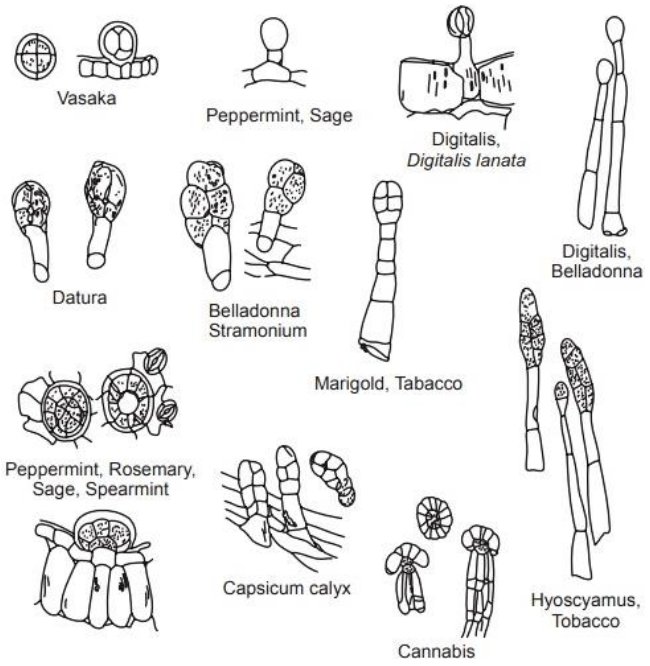


Figure 5. Diagram illustrating different trichome types in dicots and monocots

Procedure:

1. **Sample Collection:**
   - Collect fresh leaves of a dicot and a monocot plant.
2. **Peel Preparation:**
   - Using a scalpel, carefully peel off the epidermal layer from the lower surface of the leaf.
   - Place the peel in a watch glass containing distilled water.
3. **Staining:**
   - Transfer the peel onto a glass slide and add a few drops of safranin stain.
   - Let it sit for 1-2 minutes, then rinse with distilled water.
4. **Mounting:**
   - Place the stained peel on a clean slide and add a drop of glycerine.
   - Cover with a cover slip and remove excess liquid using blotting paper.
5. **Microscopic Examination:**
   - Observe the prepared slide under a light microscope.
   - Identify the trichome type and note the differences between monocot and dicot leaves.

Observations Table:

Plant Type	Example	Trichome Type	Function
Dicot	*Hibiscus*	Multicellular unbranched	Protection and water retention
Dicot	*Sunflower*	Stellate	Defense against herbivores
Monocot	*Zea mays*	Unicellular	Reduces water loss
Monocot	*Grass species*	Glandular	Secretion of essential oils

Result:
Different trichome types were observed in monocot and dicot plants using light microscopy. Dicots exhibited multicellular and branched trichomes, while monocots primarily had unicellular or glandular trichomes.

Conclusion:
Trichome characteristics vary between monocots and dicots, playing an essential role in plant protection, water conservation, and taxonomy.

Precautions:
- Handle leaves gently to avoid damaging trichomes.
- Use fresh plant material for clearer microscopic observation.
- Avoid overstaining to maintain structural visibility.
- Properly clean the microscope lenses and slides after use.

Lab Activity No 06
Title of the Activity: Observation and Analysis of a Typical Embryo through Photomicrography

Learning Objectives:
- To observe and analyze the structural components of a typical plant embryo.
- To understand the differences between monocot and dicot embryos.
- To study the role of photomicrography in documenting embryological features.

Aim:
To examine and analyze a typical embryo using photomicrography.

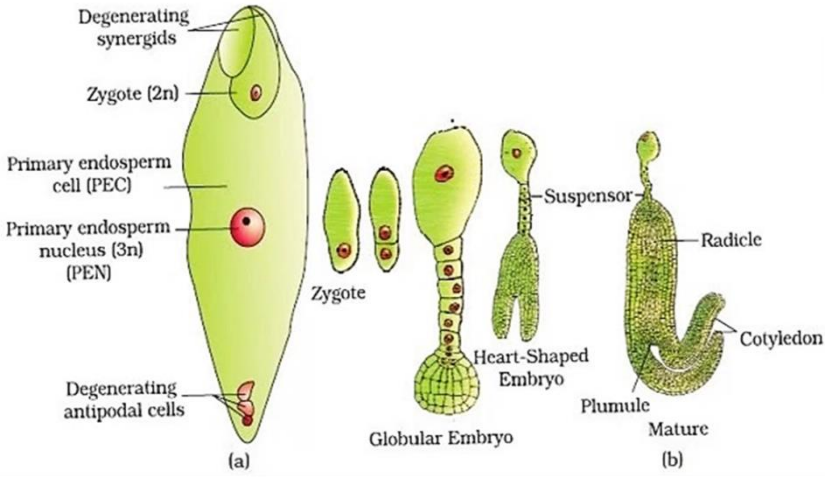
Apparatus & Materials:
- Prepared slides of plant embryos (e.g., dicot embryo of *Capsella bursa-pastoris*, monocot embryo of *Maize*)
- Light microscope with photomicrography attachment
- Camera or mobile device with microscopic adapter
- Distilled water
- Glycerine
- Glass slides and cover slips
- Forceps and scalpel

Theory:
The embryo is the developing plant structure within the seed, consisting of key components essential for germination. The two main types of plant embryos are:

1. **Dicot Embryo**
    - Consists of two cotyledons.
    - Has a well-defined embryonic axis with a radicle, hypocotyl, and plumule.
    - Example: *Capsella bursa-pastoris* (Shepherd's Purse).
2. **Monocot Embryo**
    - Contains a single cotyledon (scutellum).
    - The radicle and plumule are protected by coleorhiza and coleoptile.
    - Example: *Zea mays* (Maize).

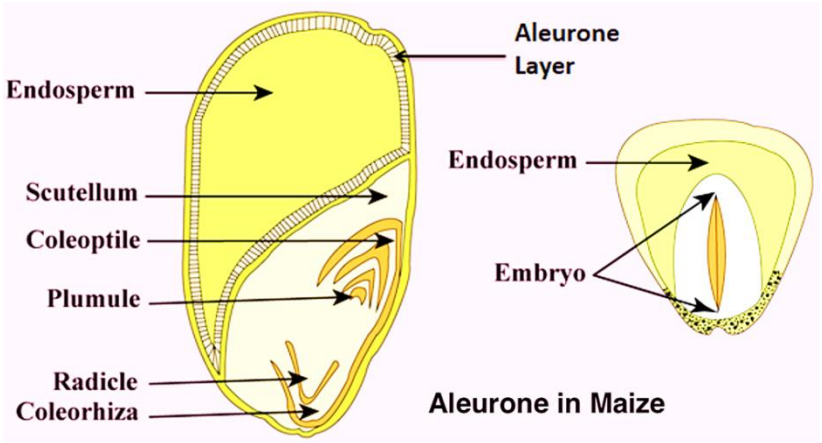
Photomicrography in Embryology:
Photomicrography involves capturing microscopic images of plant structures, aiding in the detailed study of embryology. It provides high-resolution images for comparison and documentation.

Diagram: see Figure 6 A and B
(A labeled diagram showing a dicot and monocot embryo)



A) Dicot embryo of *Capsella bursa-pastoris*

Figure 6.A. Dicot embryo



B) Monocot embryo of Maize

Figure 6.B. Monocot embryo

Procedure:

1. **Preparation of Slides:**
    - Obtain prepared slides of monocot and dicot embryos.
    - Alternatively, dissect seeds carefully to extract embryos and mount them on a glass slide in a drop of glycerine.
2. **Microscopic Observation:**
    - Place the slide under the light microscope.

- Focus at low power and then shift to higher magnification for detailed observation.

3. **Photomicrography:**
   - Adjust the microscope settings for optimal clarity.
   - Capture images using a camera or microscope-attached imaging system.
4. **Analysis and Documentation:**
   - Compare embryo structure between monocots and dicots.
   - Identify key features such as cotyledons, radicle, plumule, and protective layers.

Observations Table:

Embryo Type	**Example**	**Cotyledons**	**Key Features**	**Photomicrography Analysis**
Dicot	*Capsella bursa-pastoris*	Two	Clear embryonic axis, radicle, and plumule	Well-defined, symmetrical structure
Monocot	*Zea mays*	One	Scutellum, coleoptile, coleorhiza	Compact, elongated structure

Result:
Photomicrographs of dicot and monocot embryos were successfully captured, highlighting structural differences.

Conclusion:
Dicot embryos have two cotyledons with a distinct embryonic axis, while monocot embryos possess a single cotyledon with specialized protective layers. Photomicrography provides an effective means for analyzing embryonic structures.

Precautions:
- Handle microscope lenses carefully to prevent damage.
- Ensure proper lighting and focus for clear images.
- Use clean slides and cover slips for accurate observation.
- Store captured images properly for future reference.

Lab Activity No 07
Title of the Activity: Examination of Pollen Characteristics Using Light Microscopy in Relation to Taxonomy

Learning Objectives:
- To study the morphological features of pollen grains using light microscopy.
- To compare pollen characteristics between different plant taxa.
- To understand the significance of palynology in plant taxonomy and classification.

Aim:
To examine the structural characteristics of pollen grains from different plant species using light microscopy and analyze their taxonomic importance.

Apparatus & Materials:
- Fresh or preserved pollen samples (e.g., *Hibiscus, Lilium, Solanum*)
- Light microscope

- Glass slides and cover slips
- Acetocarmine stain or safranin
- Glycerine
- Needle and brush
- Dropper
- Distilled water
- Pollen collection tools (e.g., forceps, scalpel)

Theory:
Pollen grains are the male reproductive units of flowering plants, enclosed within a resistant exine wall. The morphological features of pollen grains, including size, shape, surface ornamentation, and aperture type, are significant in plant taxonomy.

Key Pollen Characteristics:
1. **Pollen Shape** – Spherical, oval, triangular, or boat-shaped.
2. **Aperture Type** –
    - **Monocolpate** – Single longitudinal groove (e.g., monocots).
    - **Tricolpate** – Three furrows (e.g., dicots).
    - **Porate** – Circular pores (e.g., *Solanum*).
3. **Exine Surface Pattern** –
    - Reticulate (net-like)
    - Spinulose (spiny)
    - Psilate (smooth)

Palynology is crucial in taxonomy as pollen features are often conserved within plant families and can help in species identification.

Diagram: see Figure 7

Procedure:
1. **Pollen Collection:**
    - Collect pollen from mature anthers of different plant species.
    - Use a brush or forceps to transfer pollen onto a glass slide.
2. **Staining and Mounting:**
    - Add a drop of acetocarmine or safranin stain to the slide.
    - Mix with a drop of glycerine to preserve hydration.
    - Carefully place a cover slip over the sample, avoiding air bubbles.
3. **Microscopic Observation:**
    - Examine under a light microscope at low power, then increase magnification.
    - Observe pollen size, shape, aperture type, and exine ornamentation.
4. **Data Recording:**
    - Sketch or capture photomicrographs of observed pollen grains.
    - Compare morphological features among different species.

Observations Table:

Plant Species	**Pollen Shape**	**Aperture Type**	**Exine Pattern**	**Taxonomic Significance**
Hibiscus	Spherical	Tricolpate	Reticulate	Common in dicots
Lilium	Oval	Monocolpate	Psilate	Typical of monocots

Result:

Different pollen types were observed using light microscopy, confirming their taxonomic relevance. Dicots generally had tricolpate pollen, while monocots exhibited monocolpate types.

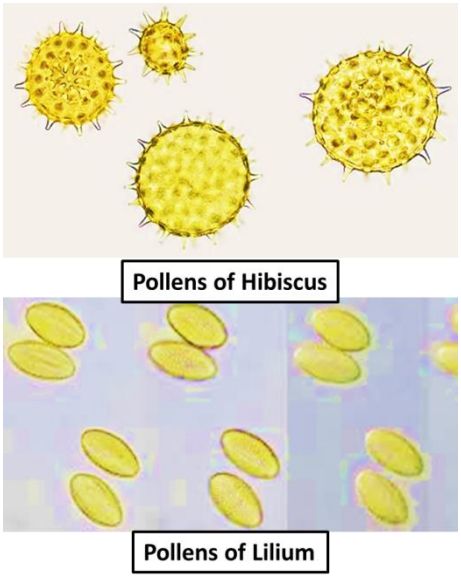


Figure 7. Pollens of Hibiscus and Lilium

Conclusion:
Pollen morphology is a valuable taxonomic tool, aiding in plant classification. Features such as aperture type and exine structure are conserved within families, supporting the role of palynology in systematics.

Precautions:
- Handle pollen samples carefully to prevent contamination.
- Use fresh stains for clear microscopic visibility.
- Avoid over-staining, which may obscure fine structural details.
- Properly clean the microscope lenses after observation.

Lab Activity No 08
Title of the Activity: Analysis of Karyotypes of Two Closely Related Species through Photomicrography for Taxonomic Insights

Learning Objectives:
- To study and compare the karyotypes of two closely related plant species.
- To understand the significance of chromosome number, structure, and morphology in taxonomy.
- To analyze karyotypic differences using photomicrography as a tool in plant systematics.

Aim:
To examine and compare the karyotypes of two closely related species using photomicrography for taxonomic classification.

Apparatus & Materials:
- Root tips of selected plant species (*Allium cepa*, *Allium sativum*, etc.)

- Light microscope with photomicrography attachment
- Glass slides and cover slips
- Forceps and scalpel
- Aceto-orcein or Feulgen stain
- Hydrochloric acid (HCl)
- Distilled water
- Glycerine
- Watch glass
- Droppers
- Filter paper

Theory:

Karyotype and Its Taxonomic Importance

A karyotype is the complete set of chromosomes of an organism, including their number, size, shape, and banding pattern. It provides important taxonomic insights by revealing:

- **Chromosome number** – Helps in distinguishing species and detecting polyploidy.
- **Chromosome morphology** – Variation in size, position of centromeres, and banding patterns aids in classification.
- **Karyotypic asymmetry** – More asymmetrical karyotypes suggest evolutionary advancements.

Comparison of Karyotypes in Closely Related Species

Closely related species may have subtle differences in chromosome structure, indicating evolutionary divergence.

- Example: *Allium cepa* (onion) vs. *Allium sativum* (garlic)
- Example: *Brassica oleracea* vs. *Brassica rapa*

Diagram: See Figure 8

Procedure:

1. Root Tip Collection and Pretreatment

- Collect actively growing root tips from plant samples.
- Treat with 0.002 M 8-hydroxyquinoline for 3 hours at 4°C to arrest metaphase.

2. Fixation

- Transfer root tips to fixative (Carnoy's fluid: 3:1 ethanol-acetic acid) for 24 hours.
- Rinse in distilled water.

3. Hydrolysis

- Treat root tips with 1N HCl at 60°C for 10 minutes to soften tissues.
- Rinse again with distilled water.

4. Staining and Slide Preparation

- Stain with aceto-orcein or Feulgen stain for 20 minutes.
- Place stained root tips on a slide, add a drop of glycerine, and gently squash with a cover slip.

5. Microscopic Observation and Photomicrography

- Examine under a light microscope at high magnification.
- Capture photomicrographs of metaphase chromosomes.
- Analyze chromosome number, morphology, and karyotypic features.

Observations Table:

Species	Chromosome Number	Chromosome Morphology	Karyotypic Asymmetry	Taxonomic Significance

Allium cepa	2n = 16	Mostly metacentric chromosomes	Symmetrical karyotype	Basic chromosome number in *Allium*
Allium sativum	2n = 16	Some submetacentric chromosomes	Slightly asymmetrical	Karyotypic variations suggest divergence

Result:
Photomicrographs of karyotypes revealed structural variations between the two closely related species, indicating chromosomal differentiation.

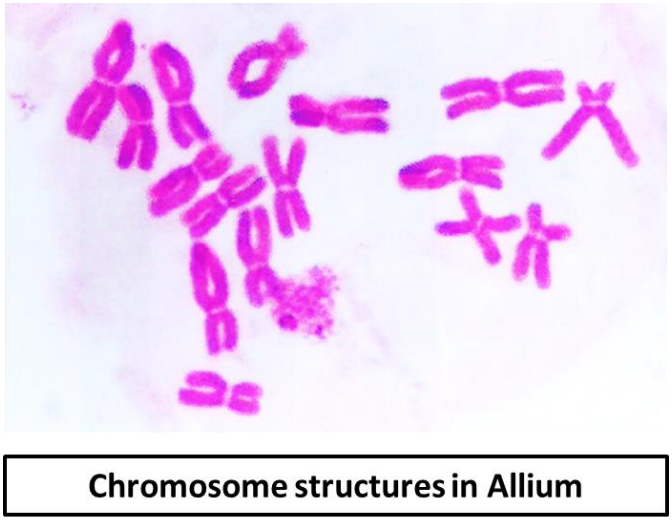


Figure 8. Karyotype in Allium

Conclusion:
Karyotypic analysis provides significant taxonomic insights by identifying chromosome number, morphology, and asymmetry. Small differences in karyotypes can indicate evolutionary divergence in closely related species.

Precautions:
- Handle root tips carefully to prevent chromosomal damage.
- Avoid over staining, which may obscure chromosome details.
- Ensure even squashing of the root tip to obtain clear metaphase spreads.
- Clean microscope lenses before and after observation.

Lab Activity No 09
Title of the Activity: Utilization of the Tropicos.org Database to Determine the Correct Name and Synonyms of a Common Species

Learning Objectives:
- To understand the role of Tropicos.org in plant taxonomy.
- To learn how to use the database to find the correct scientific name and synonyms of a plant species.
- To analyze taxonomic changes and nomenclatural updates using an online resource.

Aim:

To explore the Tropicos.org database and retrieve the correct scientific name and synonyms of a selected plant species.

Apparatus & Materials:

- Computer or smartphone with internet access
- Access to the **Tropicos.org** website
- List of plant species for investigation
- Notepad or digital document for recording observations

Theory:

Tropicos.org and Its Role in Taxonomy

Tropicos.org is an online botanical database maintained by the **Missouri Botanical Garden**, providing taxonomic information, synonymy, references, and distribution data for plant species. It is widely used by taxonomists to verify plant names and their historical nomenclature.

Why Taxonomic Databases Are Important:

- Plant names undergo changes due to reclassification based on new research.
- Synonyms help trace the naming history of a species.
- Validating a plant's scientific name ensures consistency in botanical studies.

Diagram: see Figure 9.

Procedure:

1. Accessing Tropicos.org

- Open a web browser and go to **www.tropicos.org**.

2. Searching for a Plant Species

- Locate the search bar and enter the scientific name of the plant (e.g., *Mangifera indica*).
- Click the search button to retrieve results.

3. Analyzing the Search Results

- Identify the **accepted scientific name** listed at the top.
- Scroll through the results to find **synonyms**, authorities, and references.

4. Recording the Taxonomic Details

- Note the accepted name, synonyms, and classification details.
- If the name has changed, record the previous names and their respective authors.

5. Interpreting the Taxonomic Data

- Compare results with other taxonomic sources (e.g., The Plant List, POWO).
- Understand why a species name may have been revised or reclassified.

Observations Table:

Common Name	**Searched Name**	**Accepted Name**	**Synonyms**	**Family**
Mango	*Mangifera indica*	*Mangifera indica L.*	*Mangifera domestica* (invalid)	Anacardiaceae
Neem	*Azadirachta indica*	*Azadirachta indica A. Juss.*	*Melia indica* (synonym)	Meliaceae

Result:

The correct scientific name and synonyms of the selected plant species were successfully retrieved using Tropicos.org.

Conclusion:
Tropicos.org is a valuable resource for verifying plant names, understanding synonymy, and ensuring taxonomic accuracy. The database helps standardize botanical nomenclature and track historical changes in plant classification.

Precautions:

- Ensure correct spelling of scientific names for accurate search results.
- Verify results with multiple sources if necessary.
- Be aware that taxonomic changes may occur over time with new research.

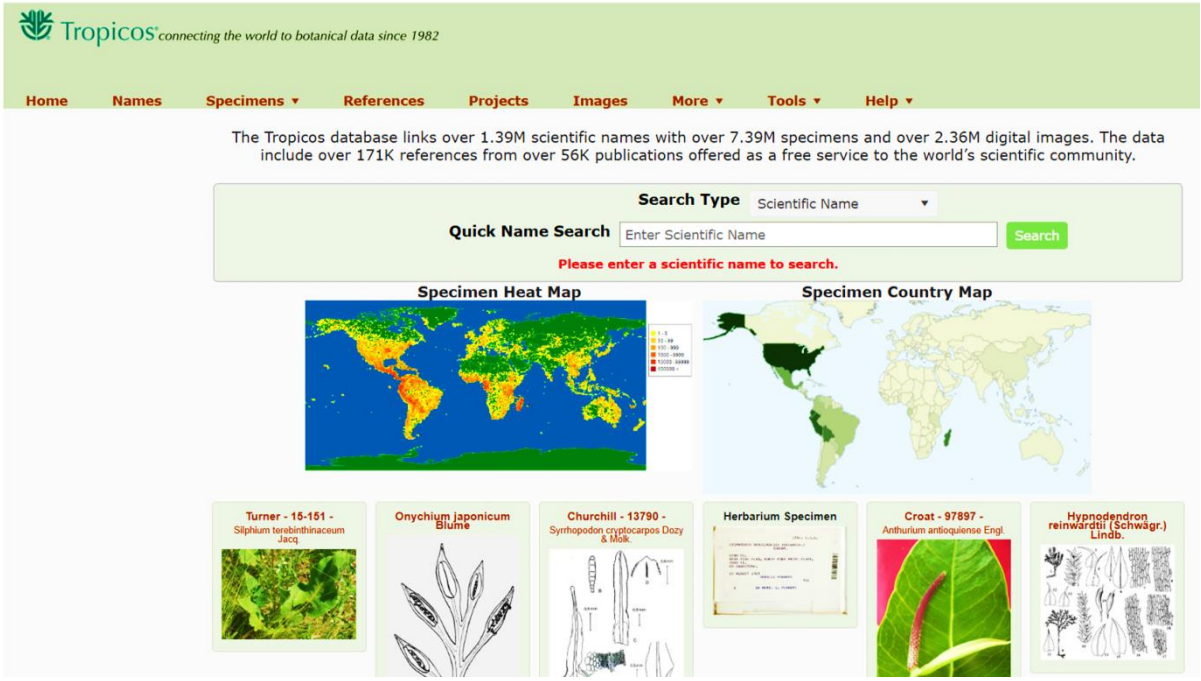


Figure 9. Tropicos.org Database website screen

Lab Activity No 10
Title of the Activity: Collection and Preservation of Leaf Tissue in Silica Gel for Molecular Taxonomic Studies

Learning Objectives:

- To understand the importance of molecular taxonomy in plant classification.
- To learn the method of collecting and preserving leaf tissue for DNA extraction.
- To explore the role of silica gel in rapid dehydration and long-term preservation of plant samples.

Aim:
To collect and preserve leaf tissue in silica gel for molecular taxonomic studies by ensuring proper sample dehydration and DNA integrity.

Apparatus & Materials:

- Fresh leaf samples from selected plant species
- Silica gel (anhydrous)
- Zip-lock bags or airtight containers
- Forceps and scissors

- Labeling materials (tags, markers)
- Gloves
- Notebook for recording details

Theory:

Molecular Taxonomy and DNA Preservation

Molecular taxonomy involves analyzing DNA sequences to determine evolutionary relationships among species. High-quality DNA extraction requires well-preserved plant tissue.

Why Use Silica Gel?

- Silica gel rapidly dehydrates leaf tissue, preventing enzymatic degradation.
- Drying preserves DNA integrity for later extraction.
- It is an efficient alternative to liquid nitrogen storage, especially in fieldwork.

Diagram:

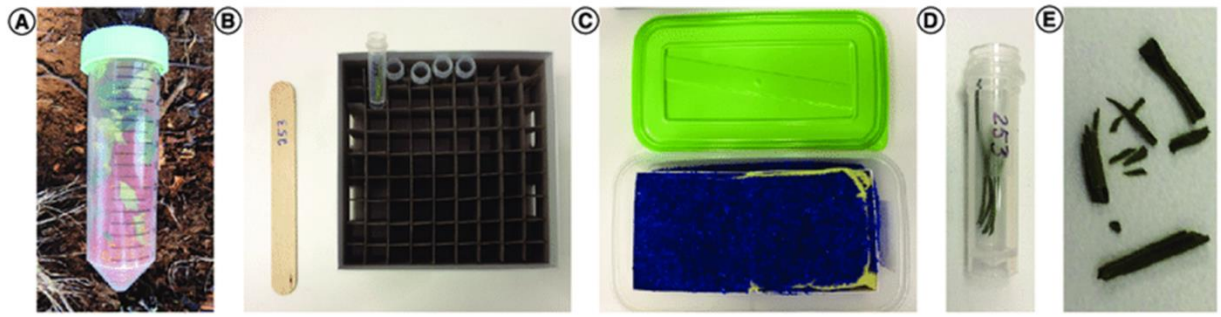


Figure 10. Diagram illustrating leaf tissue drying using silica gel

Procedure:

1. Leaf Sample Collection

- Select fresh, healthy leaves from the target plant species.
- Use forceps or scissors to collect leaf samples, avoiding contamination.
- Prefer young leaves, as they contain higher amounts of DNA.

2. Initial Processing

- Remove excess moisture or debris from the leaves.
- Cut leaves into small pieces (1–2 cm) for efficient drying.

3. Preservation Using Silica Gel

- Place leaf fragments in a zip-lock bag or airtight container.
- Completely cover the leaf tissue with silica gel to ensure rapid dehydration.
- Label the bag with species name, collection date, and location.

4. Storage and Monitoring

- Keep the sample in a dry, cool place for at least 24–48 hours.
- Check for complete dehydration (leaves become brittle and paper-like).
- Replace silica gel if it becomes saturated (color change in indicator gels).

5. Long-Term Storage and Use

- Once dried, store samples at room temperature or in a freezer for DNA extraction.
- Ensure proper documentation for future molecular taxonomic analysis.

Observations Table:

Plant Species	**Leaf Condition (Before**	**Leaf Condition (After**	**Storage**

	Drying)	Drying)	Status
Solanum lycopersicum	Fresh, green, flexible	Brittle, light green	Stored in silica gel
Mangifera indica	Moist, thick	Dry, crispy	Stored in silica gel

Result:
Leaf samples were successfully dried and preserved using silica gel, ensuring high-quality DNA for molecular taxonomic studies.

Conclusion:
Silica gel provides an efficient method for preserving plant tissue for molecular analysis. The technique ensures DNA stability for future taxonomic and phylogenetic studies.

Precautions:
- Use only fresh, healthy leaves for collection.
- Ensure complete leaf coverage with silica gel for rapid drying.
- Label samples correctly to avoid misidentification.
- Store in airtight conditions to prevent moisture contamination.