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E-Content for of B.Sc. Ist sem Botany

Archegoniate and Plant Architecture Course Code: B040201T Unit-VII and VIII

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Pollen studies with reference to Morphology, Viability and Pollination in Plants

Pollen grains consist of three substances

1. Cytoplasm. The inside of the cell filled with living substance. this also degrades rapidly during fossilisation.
2. Intine: The inner layer of the cell wall, the intine, consists mainly of cellulose and pectin, this also degrades rapidly during fossilisation.
3. Exine: The outer cell wall, the exine, consists mainly of sporopollenin, an N-free polymeric substance belonging to the terpenes. Its chemical formula is: C90 H130-158 024-44. Sporopollenin is chemically unsaturated and is corroded by oxygen (oxidation), but is otherwise resistant even to strongly alkaline substances and organic acids. Sporopollenin is thus one of the most resistant substances in the plant world

Palynology

Palynology - study of all aspects and future prospects of pollen grains. The word '**Palynology'** was coined by Hyde and Williams in 1944. (*Greek: Palynein* = powder*, Logos* = to study)

Pollen Viability

- Such studies are carried out for breeding experiments to obtain percentage pollen viability.
- Only viable pollen germinates on the stigma to affect in fertilization.

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- Pollen viability can be tested by Staining pollen taken directly from the anthers with acetocarmine. The viable pollen take colour.
- For pollen tube studies, pollen are taken from anthers and placed on agar medium in a drop of acetocarmine, in cavity slides. These are left overnight and observed under LM. Pollen tube growth can be seen.

Emasculation

- Such studies are required for observing various reproductive processes of plants – autogamy, geitonogamy and zenogamy. The plants are emasculated followed by bagging and hand pollination.
- **Autogamy** : self pollen is applied to the stigma of same flower and then emasculated and bagged to check fertilization.
- **Geitonogamy:** Pollen of same plant is applied to its stigma, emasculated and bagged.
- **Xenogamy**: Pollen from different plant is applied and bagged as above.
- These studies establish the pathway of reproduction in plants.

Methodology for studying Pollen grains

Pollen are studied with the aid of LM and SEM for taxonomic derivations.

Light Microscope: Helps in making observations on Primary, secondary as well as tertiary characters, but in a 2-D View.

Scanning Electron Microscope: Helps in making detailed observations on ultrastructure of the Primary, secondary and tertiary characters in a 3-D View.

Transmission Electron Microscope: This is used for making observation on pollen ontogeny, cellular inclusions and developmental stages of the exine.

POLLEN MORPHOLOGICAL STUDIES

- Pollen morphological studies are based only on wall characters.
- The cytoplasmic contents therefore need to be washed off I in such a manner that only the wall remains intact.
- Pollen is therefore chemically treated by a process called ACETOLYSIS.

After acetolysis the exine details become comprehensible to show distinct endoexine, foot layer, Columella and tectum. All surface patterns and details of exine strata can be studied only after acetolysis.

A C E T O L Y S I S

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EQUIPMENTS & GLASSWARE:

1. CENTRIFUGE MACHINE
2. WATER BATH
3. CENTRIFUGE TUBES 15 ML CAPACITY
4. BEAKERS
5. Measuring cylinder
6. TEST TUBE HOLDERS
7. BLOTTING PAPER
8. SLIDES
9. COVER GLASS RECTANGULAR 4 X 2 CM OR ANY OTHER SIMPLE COVER SLIP
10. FORCEPS, NEEDLES, GLASS RODS

(CORRESPONDING TO THE NUMBER OF SAMPLES PROCESSED AT ONE TIME TO AVOID CONTAMINATION).

CHEMICALS AND REAGENTS

1. GLACIAL ACETIC ACID
2. ACETIC ANYHYDRIDE
3. CONC. SULPHURIC ACID
4. DISTILLED WATER
5. XYLENE
6. PHENOLIC GLYCERINE JELLY
7. PARAFFIN WAX.
8. CAVITY BLOCKS (in special cases)

Steps for Acetolysis

1. Fresh anthers are dissected and placed in a centrifuge tube in 70% alcohol and teased gently with a glass rod.

In case of anthers extracted from herbarium specimen the same are first soaked for 24 hrs. in 70% alcohol. Then placed in centrifuge tubes.

Note: For preparing clean slides the pollen may are dissected out from anthers on slides in a drop of distilled water. These are carefully transferred to the centrifuge tubes to which alcohol is added. The method varies with the quantity of pollen available

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The tubes are gently fixed in the centrifuge machine and centrifuged at 2000 – 3000 rpm for 5-6 min. Pollen mass gets settled at the bottom and the supernatant is gently decanted.

Note: Last drop should not be decanted as this might also carry the residual mass of pollen.

Glacial acetic acid is added to this, centrifuged and decanted as before.

4. To this is added the acetolysis mixture (9:1 acetic anyhydride + conc. H_2SO_4 prepared separately in a measuring cylinder).

Note: Plastic centrifuge tubes should not be used as acetolysis mixture is highly corrosive.

5. The tubes containing pollen in acetolysis mixture are placed in a water bath and heated to boiling for about 5 min. and then cooled, centrifuged and decanted as before.

6. Glacial acetic acid is again added to this, followed by 2-3 washes with distilled water.

Note: The heating times needs to be adjusted with the type of pollen, less for thin walled and more for thick-walled grains. Literature can be referred to get an idea.

At this stage the sample is divided into two parts –each part is processed separately:

A. For Light microscope (LM) studies

B. For Scanning electron microscope (SEM) studies

A. For Light microscope (LM) studies

8. About 10 drops of glycerine (50*%)* is added to the sediment and left for 15 minutes.

9. After centrifugation and decanting, the tubes are very gently inverted on filter paper and left overnight.

10.A small piece of solid phenolic glycerine jelly is picked on a needle tip, lowered into the tube and brought in contact with pollen sediment at bottom of centrifuge tube.

Note: Glycerine should be lightly touched with pollen residue, do not dip.

11.The pollen in glycerine jelly is transferred to slide,

covered with 18 mm cover slip, gently warmed to

disperse pollen and sealed with molten paraffin.

12.Excess wax is cleaned with cotton dipped in xylene.

13.A label containing details of the sample is affixed on

right hand side of the slide.

Precautions

- **While preparing pollen slides, use of separate needles/forceps and glass rods is suggested**

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for each sample as pollen can easily get contaminated by mere touch of the needles from other sample.

- **Each centrifuge tube must be kept at a distance from another to avoid contact at the rim.**

Pollen Herbarium

- Duplicate slides are prepared for each specimen.
- One set (≡ voucher slide/Type) is deposited in a pollen herbarium.
- A Pollen Herbarium is a pollen reference slide bank.
- It is of immense use in Palynological studies as it is a repositry of representative pollen types of different families, and needs to be 'matched with' during pollen identification

B. For Scanning electron microscope (SEM) studies

15. Tubes containing pollen in distilled water are centrifuged and decanted.

16. The pollen residue is sequentially treated with 70% alcohol followed by absolute alcohol, centrifuged, decanted and shaken well in few drops of absolute alcohol.

17. A small drop of this is transferred to specimen stub affixed with double sided tape or a piece of cover glass.

18. Excess alcohol is allowed to evaporate.

19. Pollen are then coated with this film of gold (*c.* 200 Å) in Sputter Coater These are then viewed in SEM under accelerating voltage of 10 KV and photographs are taken in different views and at different magnifications (2000 – 10,000 x).

- ***each species produces a unique type of pollen or spore***

Some plants are wind-pollinated & disperse millions of pollen grains or spores, most of which (~90%) fall very close to the plant; a few might travel great distances

Within a genus: (pollen will often look similar) Acacia sp.

At the species level: (often only small differences)

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POLLEN & SPORES ARE EVERYWHERE

- Found in the air over the middle of the oceans.
- Found in the air all over the world including the air over the
- North and South Poles
- Found in rivers, lakes, seas, and at the bottom of the oceans
- Found inside buildings, in cars, on and inside animals, on
- and inside people, in soils, and rocks up to 2.2 billion
- years old
- The vast majority of dispersed pollen grains are
- unlucky because they end up as particulate matter
- and become part of soil, dirt, and dust.
- Those that fall on the stigmatic surface event in fertilization.

Enormity of Pollen and Spores –Some interesting facts

During an average spring or summer day in most areas each cubic meter of air contains about 1,000-20,000 pollen & spores

The average adult inhales about 10 m3 of air a day, more if doing physical exercise, pollen is trapped in nasal passages, can be a key to the season of year

- During peak pollination periods there can be up to 100,000
- pollen grains/m3 of air
- Some bracket fungi such as: ***Ganoderma can discharge ~30***
- billion spores daily for months (May-September)

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