Palynology
Part-V

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BSc. Botany SEMESTER V

Paper VIII

Plant Resource Utilization, **Palynology and Biostatistics**

Unit-IV

- An introductory knowledge to palynology, morphology, viability and germination of pollen.

Pollen viability

- The viability a pollen grains greatly affect the fertilization and development of an embryo.
- Viability of pollen varies greatly after being shed from anthers.
- Pollen viability is affected by temperature and humidity.
- Viability of pollen grain can be tested by:
  - In vitro pollen germination.
  - Metabolic or enzyme activity
  - The presence of cytoplasm
- In vitro pollen germination is simplest most accurate.

Methods of testing pollen viability

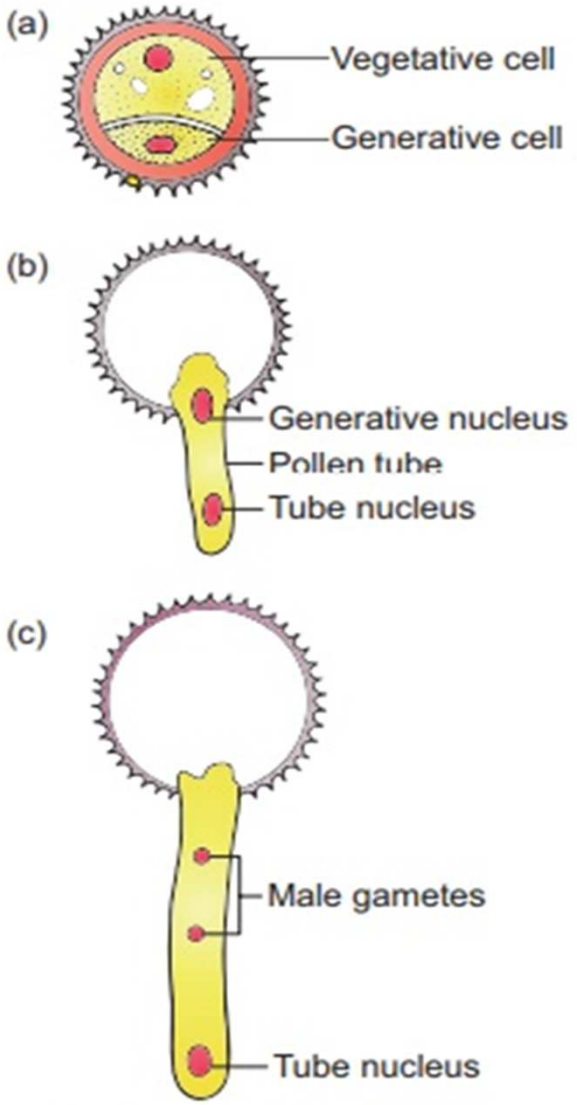
- In vitro pollen germination can be performed by hanging drop method.
- The hanging drop containing pollen grains are consist of 15-20% sucrose, 0.01°% and 0.03% of H_3BO_3 and $Ca(NO_3)_2$ respectively.
- In vitro germination could be performed using a drop of above said solution along with pollen and hang it in a cavity slide to see pollen germination under microscope.
- Pollen germination of some plants start within 15 min of incubation in sucrose, boron and $Ca(NO_3)_2$ solution.

Pollen germination

- Pollen grains are considered to be geminated when pollen tube length become greater than or equal to pollen diameter.
- The pollen grains are mostly shed at 2 celled stage and in few cases at 3 celled stage.
- Further development of male gametophytes (pollen grain) takes place at the stigmatic surface.
- The pollen present on stigma are usually germinated and produce pollen tube.
- Pollen grain grow through style and reaches to ovule which are deep seated away form stigma into ovary.

Pollen germination

- Initially pollen grain contain a tube nucleus (larger) and a generative nucleus (small).
- Tube nucleus move into pollen tube and generative cell nucleus divides and produce two sperms (male gametes), which follows tube nucleus.



In vitro pollen germination

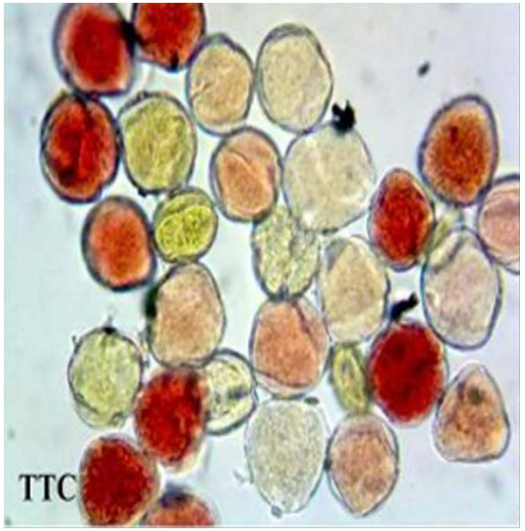
- In vitro studies on pollen tube growth shows that pollen germination and pollen tube growth require the followings:
  - High relative humidity,
  - Adequate temperature condition.
  - Nutrient such as carbohydrate (sucrose), boron, calcium & flavonols which facilitate pollen germination,
  - Enzymes like cellulase, pectinase, callase etc. are release after pollen germination and help in pollen tube elongation.
  - Therefore, medium used for in vitro pollen germination contain following components: sucrose. H_3BO_3, $Ca(NO_3)_2$, $MgSO_4$, KNO_3 and pH 7-8
  - Sometimes, extracts of stigma and style are useful for pollen grain germination.

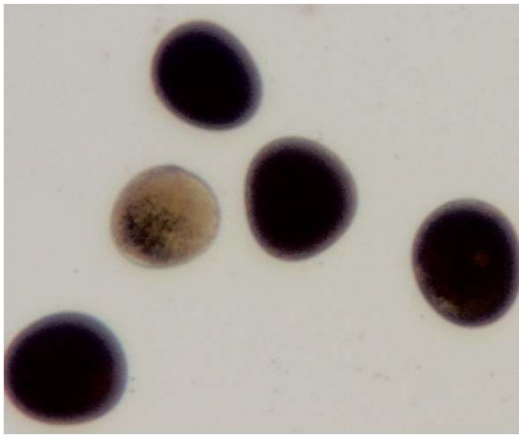
Germinating pollen grain

Biochemical test for pollen viability

Pollen viability could be tested by certain biochemical methods as given below:

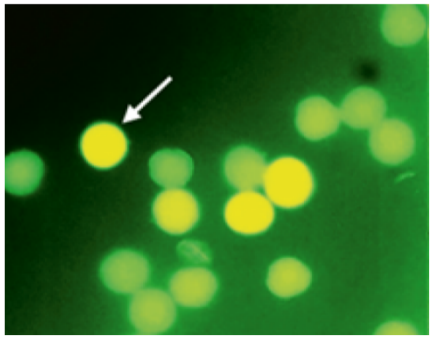
- Dehydrogenase activity can be detected in the pollen grains using 1% solution 2,3, 5-triphenyl tetrazolium chloride (TTC) or 2,5-diphenyl monotetrazolium bromide (MTT) in a 5% sucrose solution. Viable pollen grain turn deep pink in colour.
- Peroxidase activity can be detected in the pollen grains using H_2O_2 and p-phenylenediamine reagent. Viable pollen grains turn deep brown in colour.

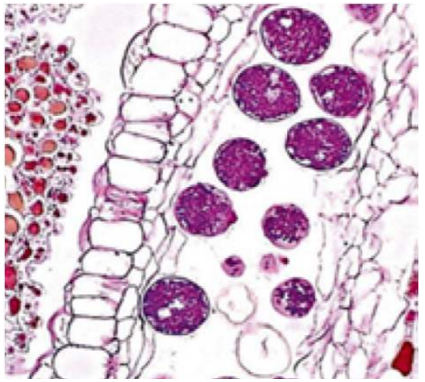


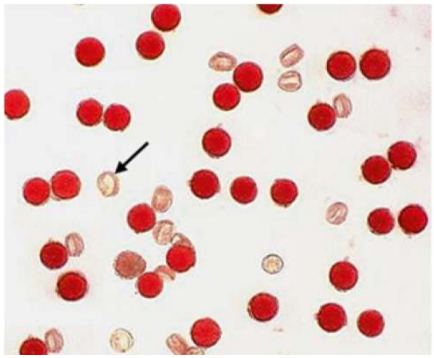


Biochemical test for pollen viability

- Esterase activity using FDA solution: Viable pollen fluoresces green in under UV light when they show activity.
- Lugol's Iodine test: Viable pollen grain develop deep blue colour in lugol's iodine & which is a test for presence of starch in pollen grains.
- Acetocarmine test: Viable pollen grains are stained deep red by acetocarmine, which suggest intensive RNA / protein synthesis in the viable pollen grains.







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