Reproductive Ecology: Pollen-Pistil Interaction

III MSc Botany

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What is Pollination biology?

Why is it important?

Only Angiosperms?

By what means?

Animals pollinates for about 75% of all the crops and about 90% of all the flowering plants.

Plant

Vegetative and Reproductive phase

Annual

Biennial

Perennial

Semelparous/monocarpic

Types of Reproduction

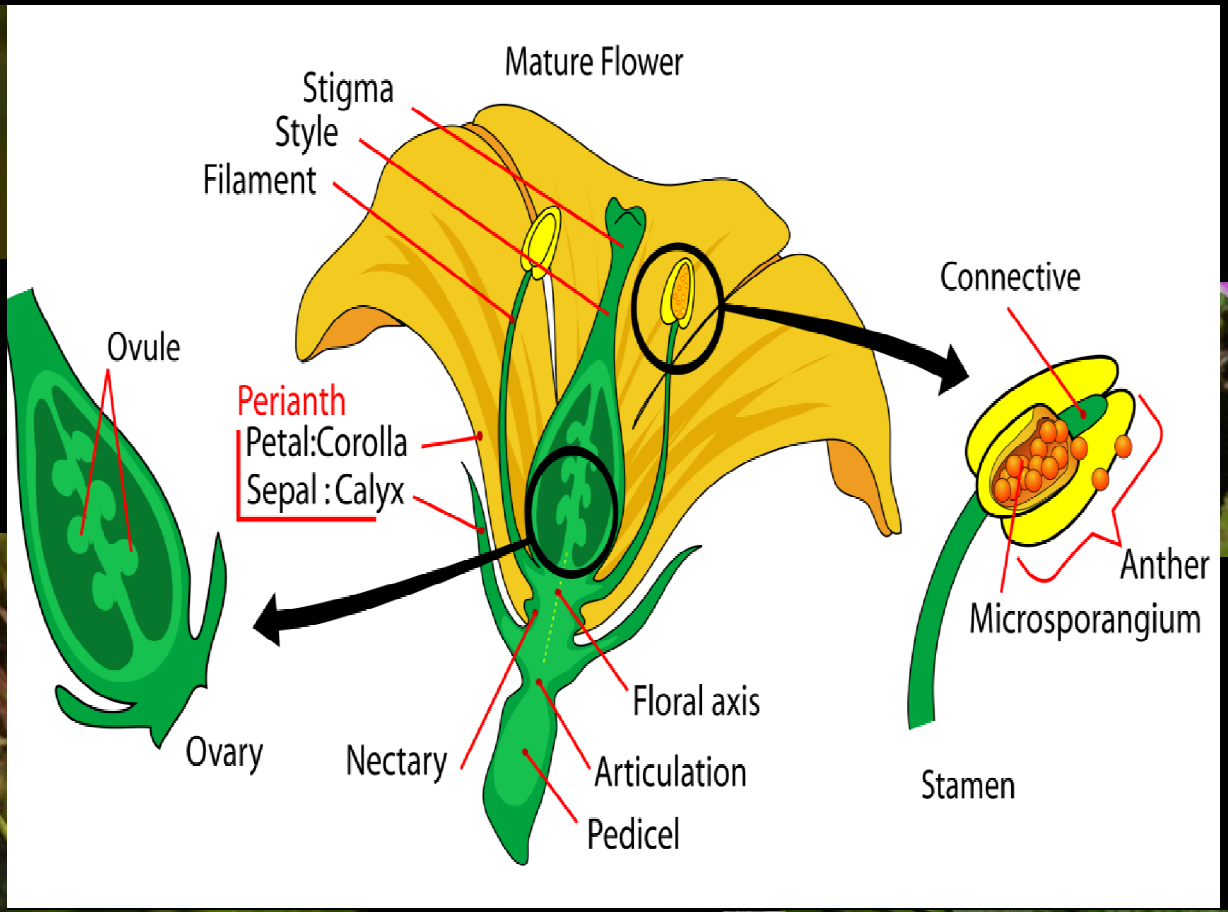
- Apomixis (clones)
- Vegetative propagation (clones)
- Sexual reproduction – only method that permits genetic recombination

Major events in sexual reproduction

- Flower initiation and development
- Development of functional ovules and pollen grains
- Pollination
- Breeding system
- Development of fruit and seeds
- Seed dispersal, seed germination and seedling establishment

G1

Flower; the basic unit of pollination biology



Slide 6

G1 flower

Giby, 17-06-2011


St Bernard's Lily (*Anthericum liliago*)


Bermuda Buttercup (*Oxalis pes-caprae*)


Oleander (*Nerium oleander*)


Lantana (*Lantana camara*)


Scarlet Pimpernel (*Anagallis arvensis*)


Verbascum (Verbascum sinuatum)


Common Mallow (Malva sylvestris)


Spanish Oyster (Scolymus hispanicum)


Stork's bill (*Erodium malacoides*)


Bindweed (*Convolvulus arvensis*)


Blue Gem (*Hebes x franciscana*)


Calla Lily (*Zantedeschia aethiopica*)

Sexual Systems

Hermaphrodite: Individual plants bear only bisexual flowers

Monoecious: Individual plants bear male and female flowers separately

Andromonoecious: Individual plants bear bisexual and male flowers

Gynomonoecious: Individual plants bear bisexual and female flowers

Polygamomonoecious: Individual plants bear bisexual, male and female flowers

Sexual System (cont)

Dioecious: Individual plants bear either male or female flowers

Androdioecious: Individual plants bear bisexual and male flowers

Gynodioecious: Individual plants bear bisexual and female flowers

Pollination Types

Autogamy – Transfer of pollen grains from the anther to the stigma of the *same* flower

Geitonogamy – Transfer of pollen grains from the anther to the stigma of *another flower* of the *same plant or another plant* of *the same clone*

Xenogamy – Transfer of pollen grains from the anther to the stigma of a *different* individual plant (not clonal material)

Allogamy – Transfer of pollen grains from anther to the stigma of *another* flower of the *same or another* plant (includes both geitonogamy and xenogamy)

Methods to Study Sexual Reproduction

- Traditional Methods: Field studies and light microscopy
- Advanced microscopy with better fixatives
- Integration of other disciplines - physiology, biochemistry, tissue culture, cell biology, genetics and molecular biology

Field studies are the most important approaches for collecting data relevant to conservation biology

Essential Structures Involved in Sexual Reproduction

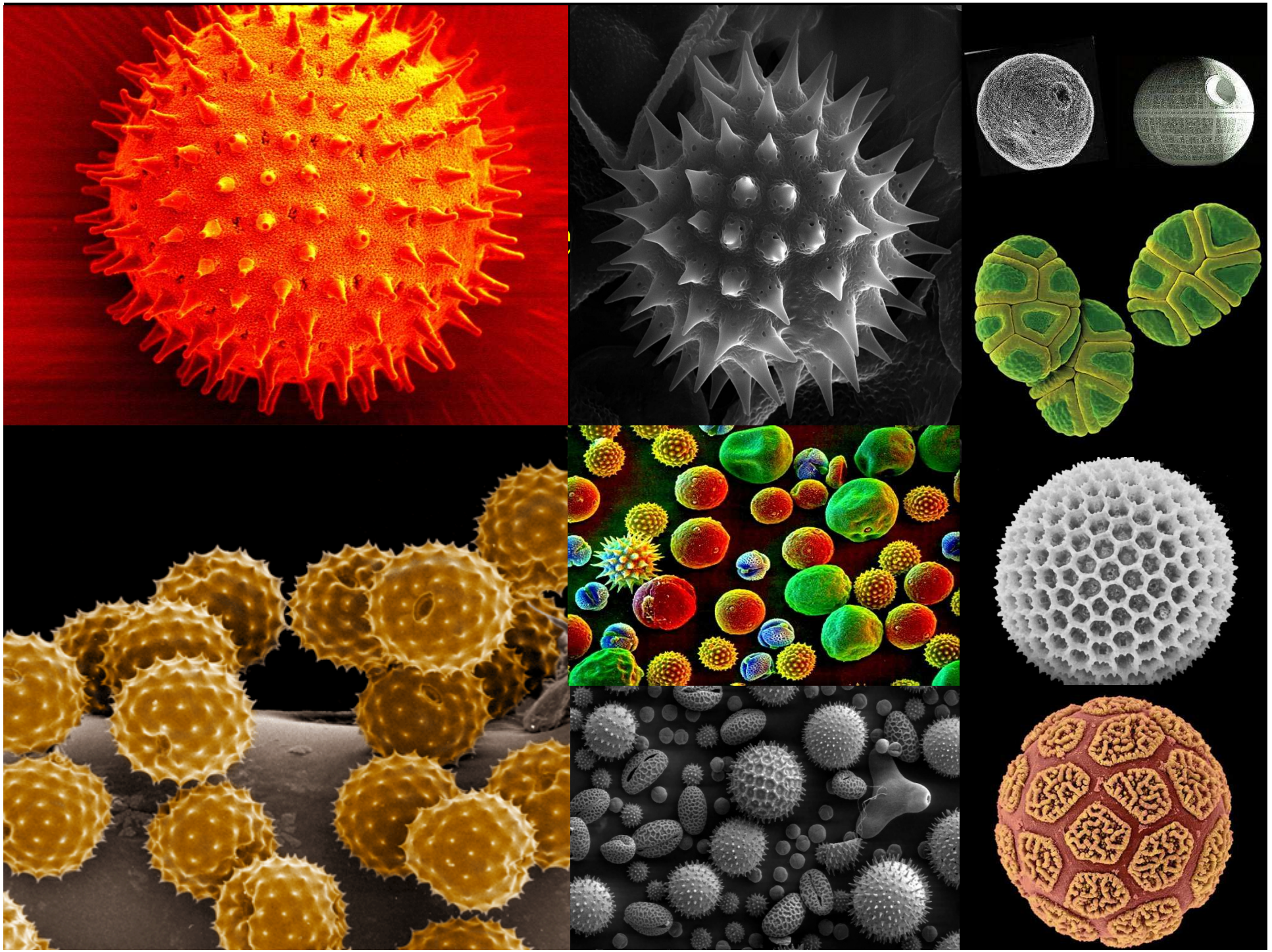
Pollen grains

- Development
- Free-dispersed phase
- Pollination
- Pollen germination and pollen tube growth

Pistil

- Morphological details
- Stigma receptivity
- Pollen-pistil interaction and fertilization
- Fruit and seed set

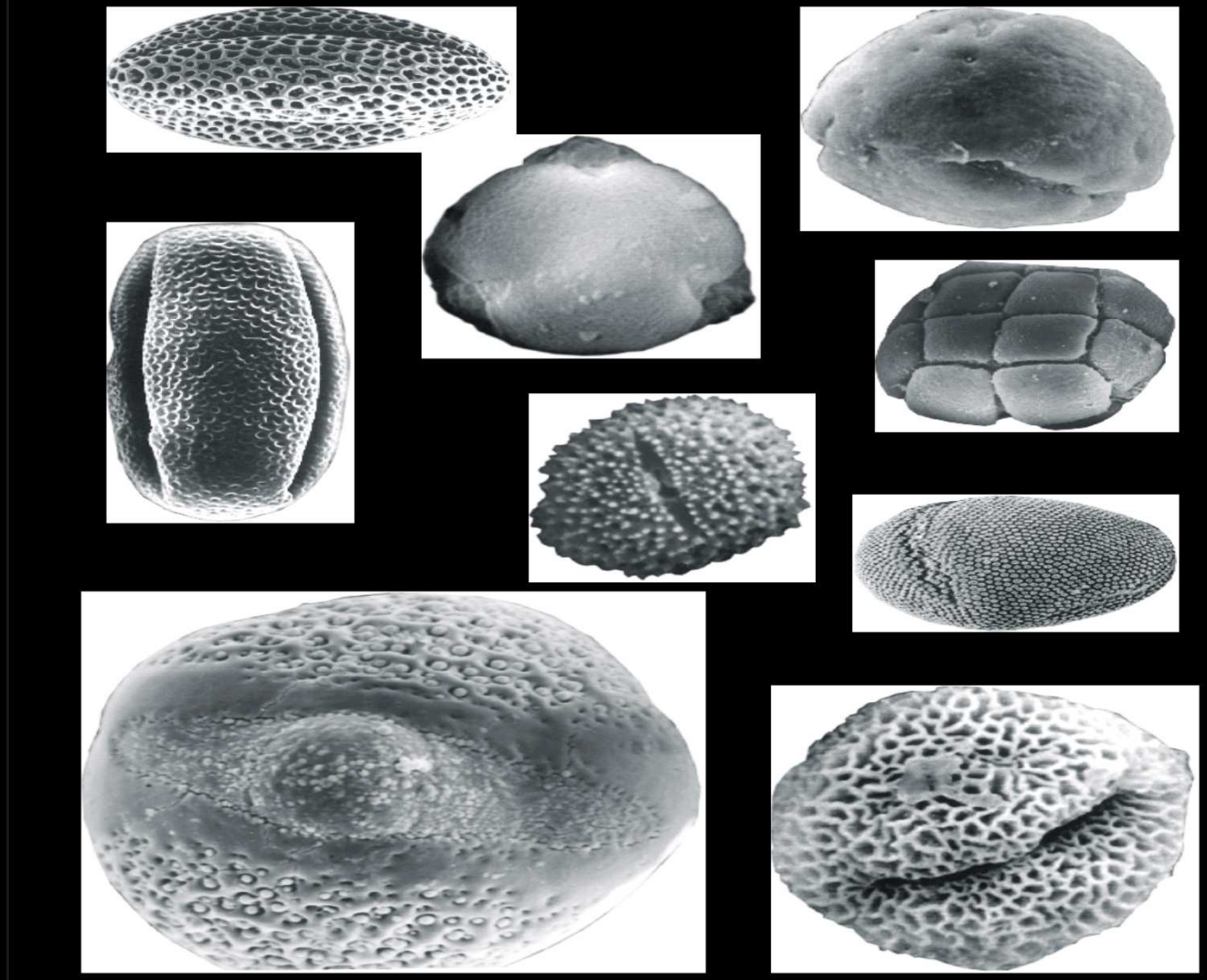
Pollen-microsporangium



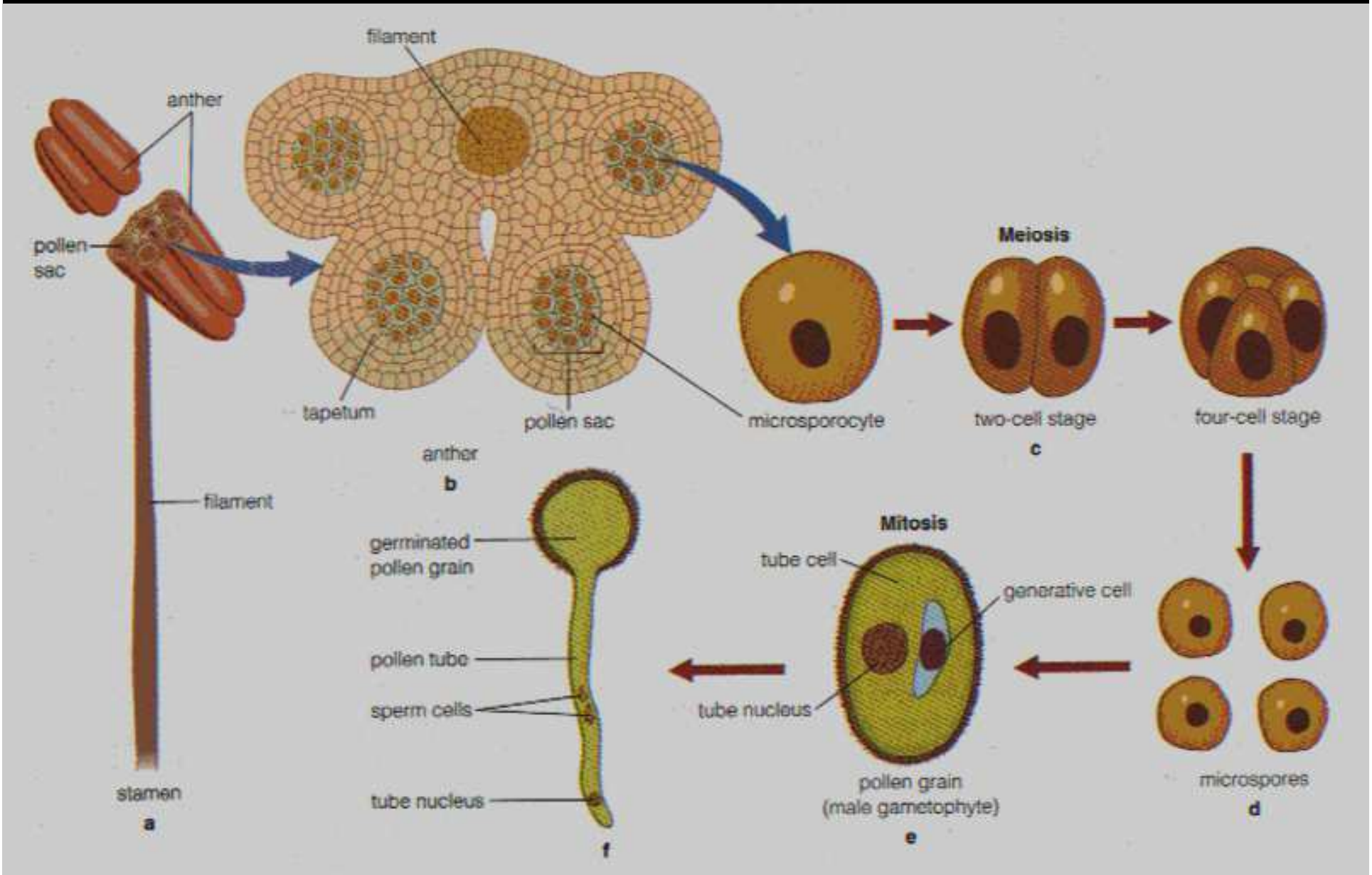
Pollen

- Moisture content- less than 20%: metabolic activity is low
- Extracellular Components- Important in pollen-pistil interaction
- Pollen can withstand high temperature, high acidity and high alkalinity

SEM of some pollen grains



Pollen Development



Free-dispersed Phase of Pollen

- Permits gene flow
- Depending on the period and prevailing conditions of free-dispersed phase, quality of pollen may be affected
- Quality of pollen is assessed largely on the basis of viability
- For effective seed-set, pollen grains have to land on a compatible and receptive stigma before they lose viability

Pollen

- Pollen Collection
  - Anthesis and Anther Dehiscence
  - Collect flower bud just before anthesis; Petri dish, desiccator
  - Bag the flowers in the previous evening
  - avoid contamination

Pollen

- Pollen storage
  - Short term- desiccator, Glass Vials, Aluminum foil cups, Gelatin Capsules kept over Silica/saturated solution of suitable salts
  - Cold storage
  - But repeated freezing and thawing decreases viability
  - Long term- Cryopreservation and Lyphilization

Pollen

- *in vitro* Pollen Germination
  - Controlled hydration
  - Culture Medium;
    - sucrose 10%
    - Boric Acid
    - Calcium Nitrate
    - Magnesium Sulfate
    - Potassium Nitrate
- Fixation of pollen culture
- Scoring pollen germination (% germination)

Pollen Viability

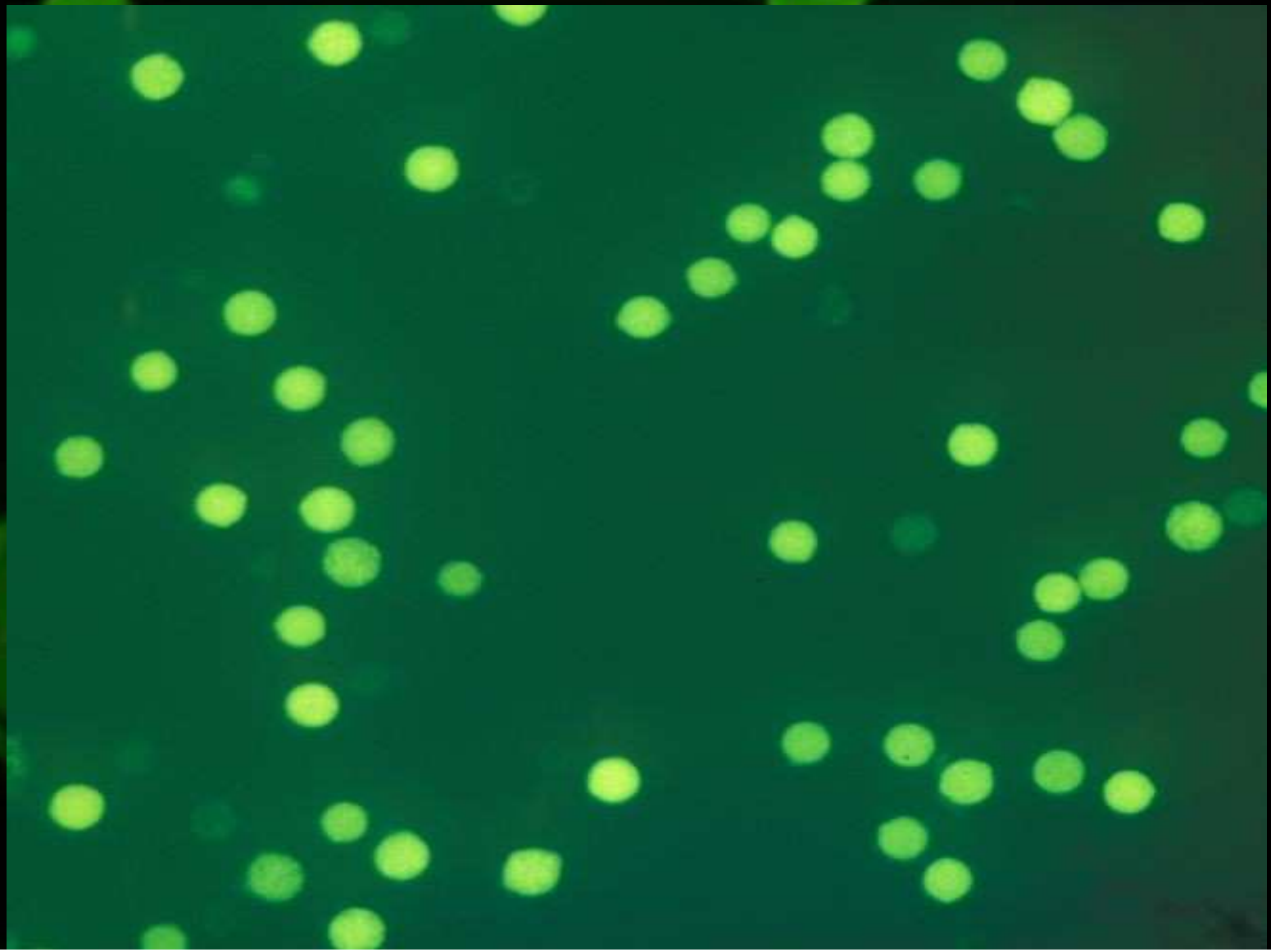
- Pollen viability after release from anther varies from species to species
- Pollen of some tree species may retain for several months whereas, in grasses (Poaceae) viability would lost within few minutes after pollen release (Shivanna and Johri, 1985)
- Deficiency of respiratory substrates, irreversible loss of membrane permeability and inactivation of enzymes and growth hormones would cause the pollen viability (Shivanna and Johri, 1985)

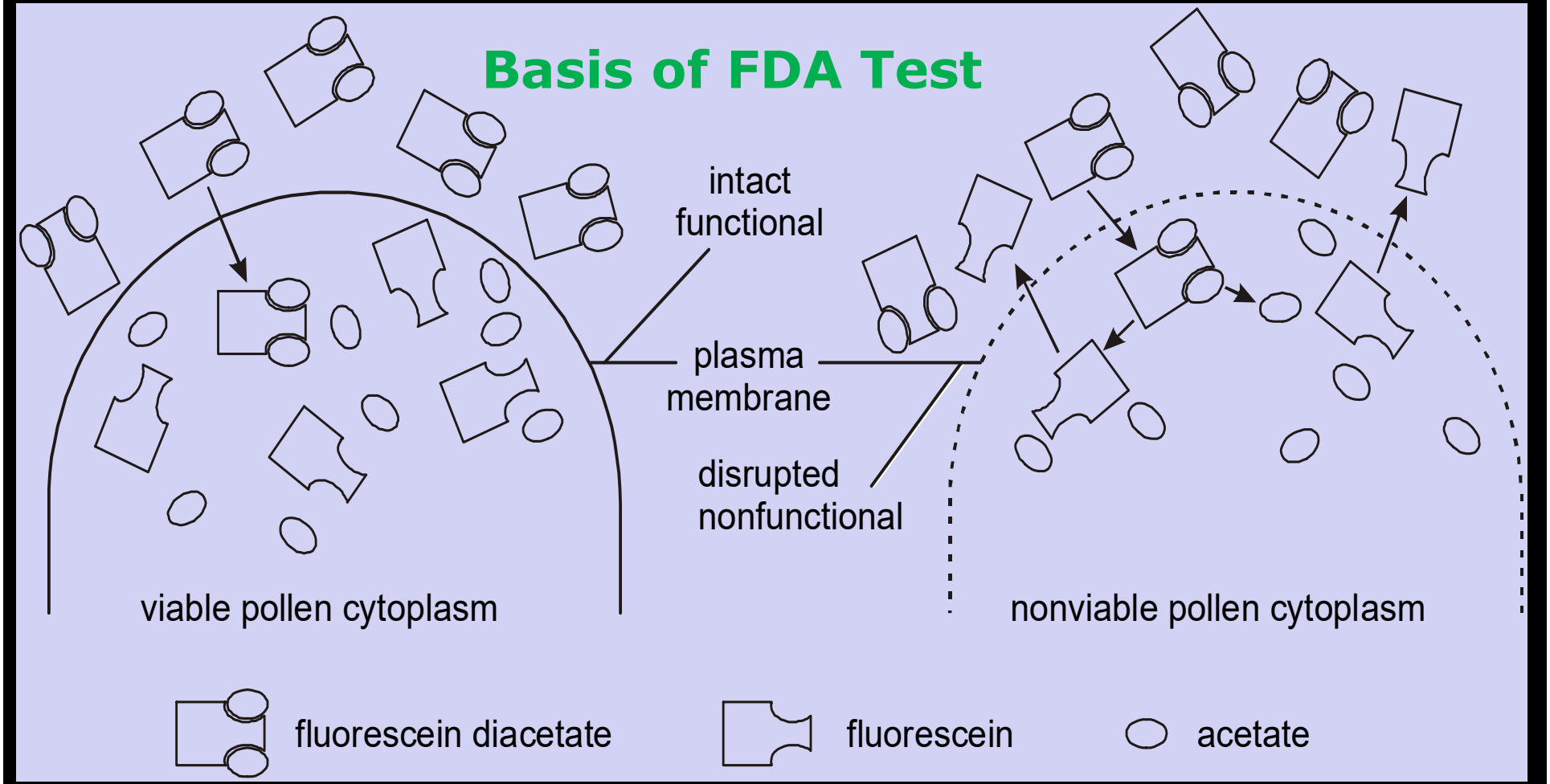
Tests for Pollen Viability

- *in vivo* fruit and seed set (at different intervals)
- Pollen germination and pollen tube growth in pollinated pistils
- Tetrazolium test
- *in vitro* germination test- Sucrose solution
- Fluorescein Di Acetate (FDA) test

Tests for Pollen Viability

Fluorescein Diacetate (FDA) Test





FDA (non-fluorescent)-Hydrolysis by esterases= Fluorescein (Fluorescent)

Florescence get accumulated in the cytoplasm of viable pollen and give Florescence

Plasma membrane; if active Fluoresce If not active no fluorescence

Differential Staining

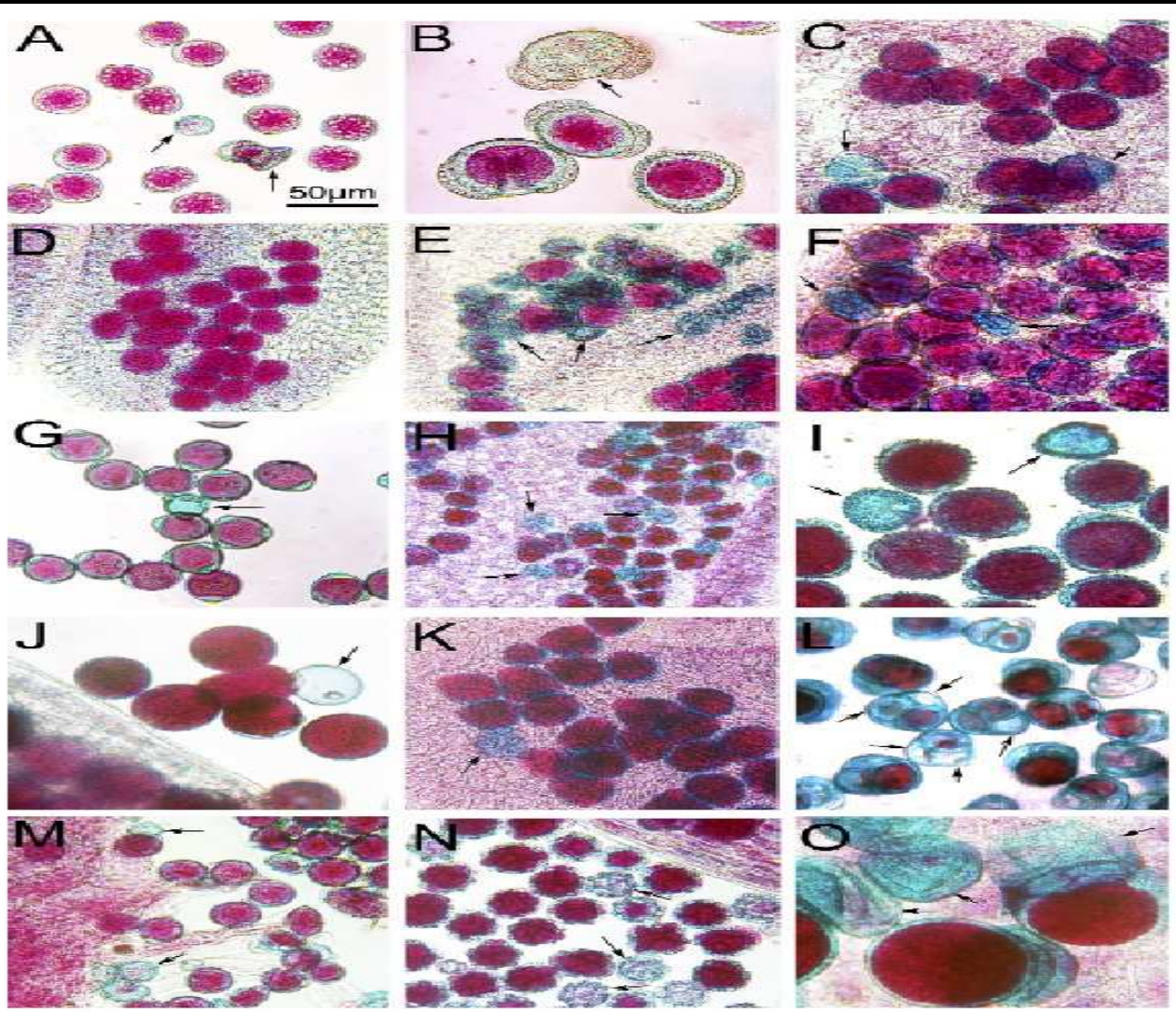


Figure 1. Differential staining of aborted and non-aborted pollen grains. (A) *Ginkgo biloba*. (B) *Pinus resinosa*. (C) *Acer rubrum*, stained without phenol. (D) *Arabidopsis thaliana* Ler (diploid). (E) *Arabidopsis thaliana* Ler (autotetraploid). (F) *Acer rubrum*, stained with phenol. (G) *Betula populifolia*. (H) *Fragaria versca* (Rugen). (I) *Lonicera tatarica*. (J) *Oryza sativa*. (K) *Prunus padus*. (L) *Rhododendron mucronulatum*. (M) *Solanum lycopersicum* (Microtom). (N) *Taraxacum officinale*. (O) *Tulipa spp*. Bar=50 µm, all images are at the same magnification. Arrows indicate aborted pollen grains that stained blue-green. Non-aborted pollen grains are stained magenta-red.

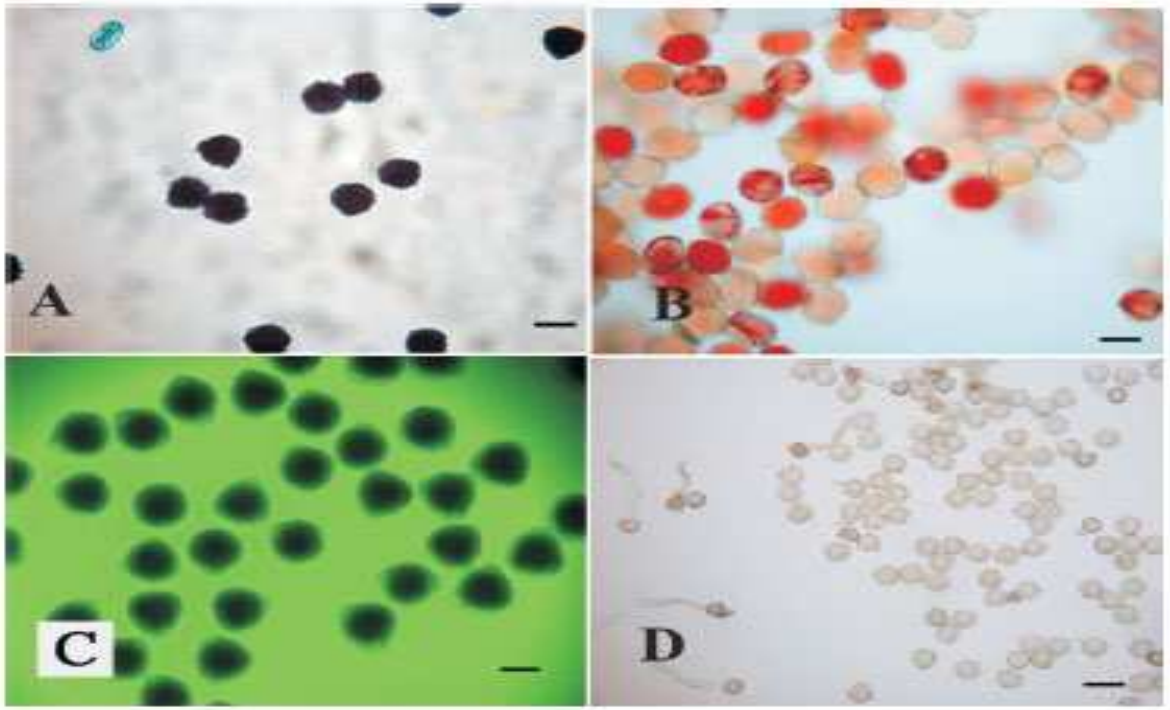
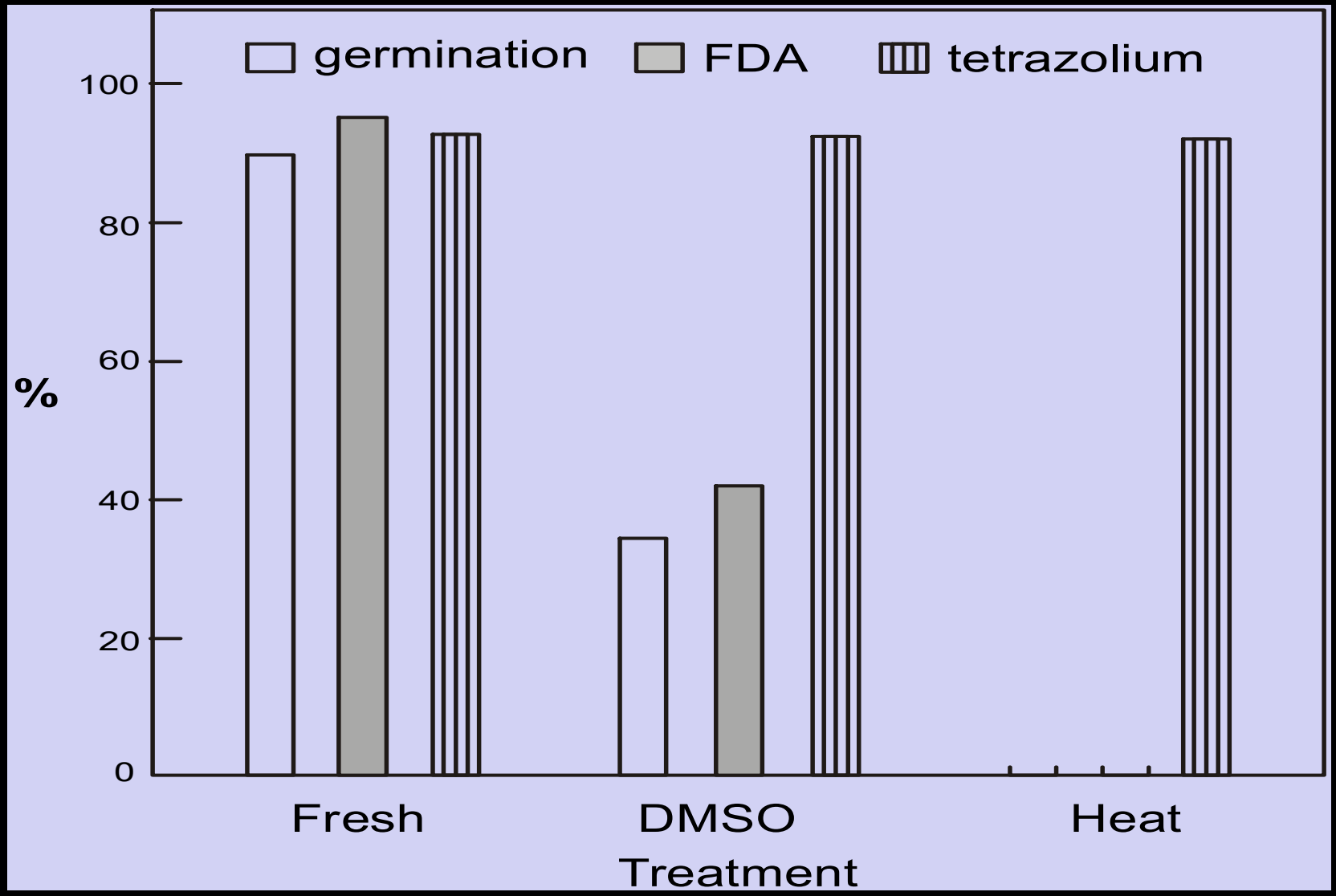
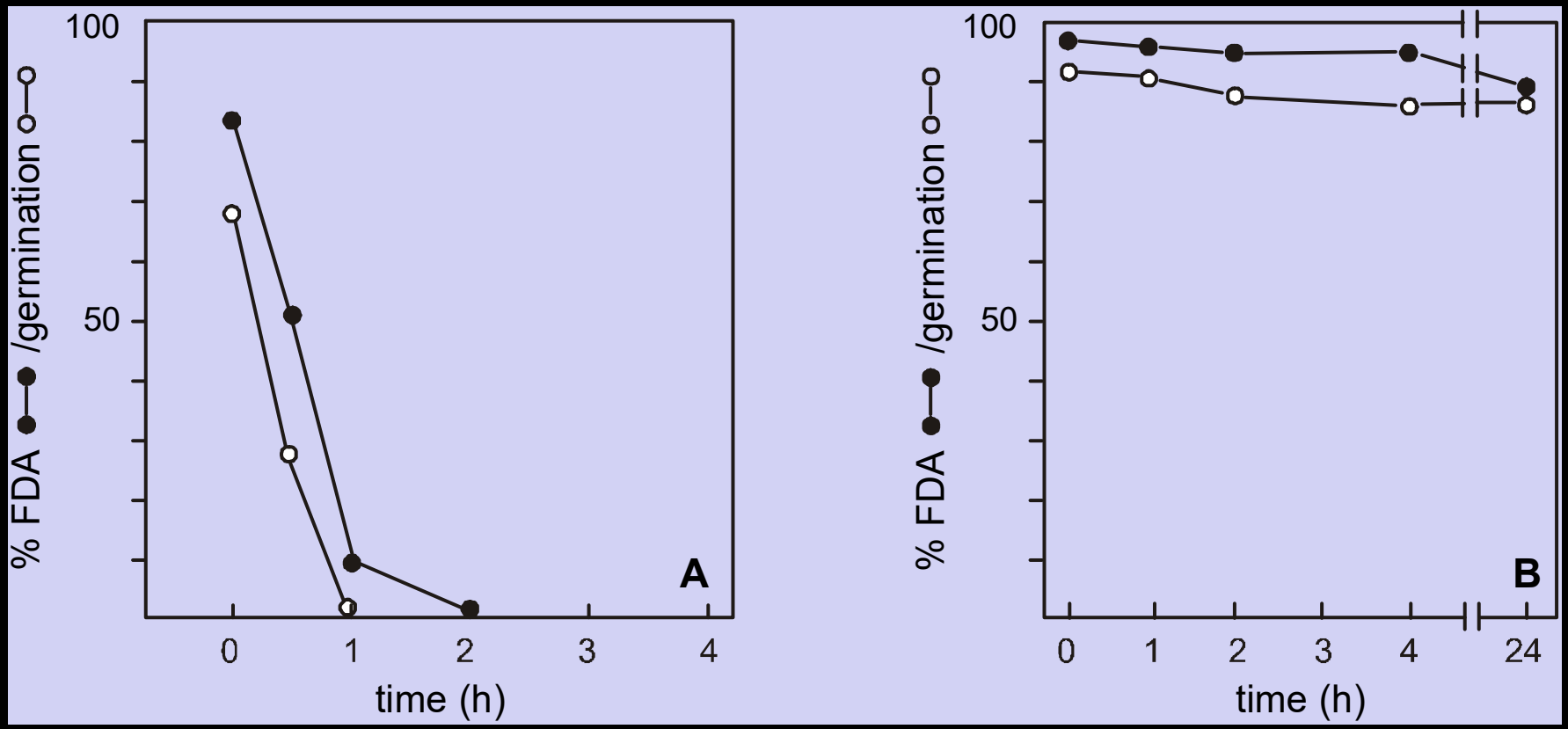


Figure 5. Viability of *Crotalaria* sp. pollen grains revealed by staining and germination tests. (A) Fresh pollen grains of *C. spectabilis* stained with Alexander's solution; intact microsporocytes with deep purple cytoplasm are viable, while the fragmented ones with weak green cytoplasm are inviable. Bar: 20 µm. (B) Fresh pollen grains of *C. micans* in 5% 2,3,5-triphenyltetrazolium chloride in 50% sucrose solution; deep red microsporocytes are viable, and colorless ones, inviable. Bar: 20 µm. (C) Fresh pollen grains of *C. spectabilis* stained with fluorescein diacetate (FDA); nonfluorescent microsporocytes are not viable. Bar: 20 µm. (D) *C. spectabilis* pollen grains in germination inducing liquid medium. Bar: 50 µm.

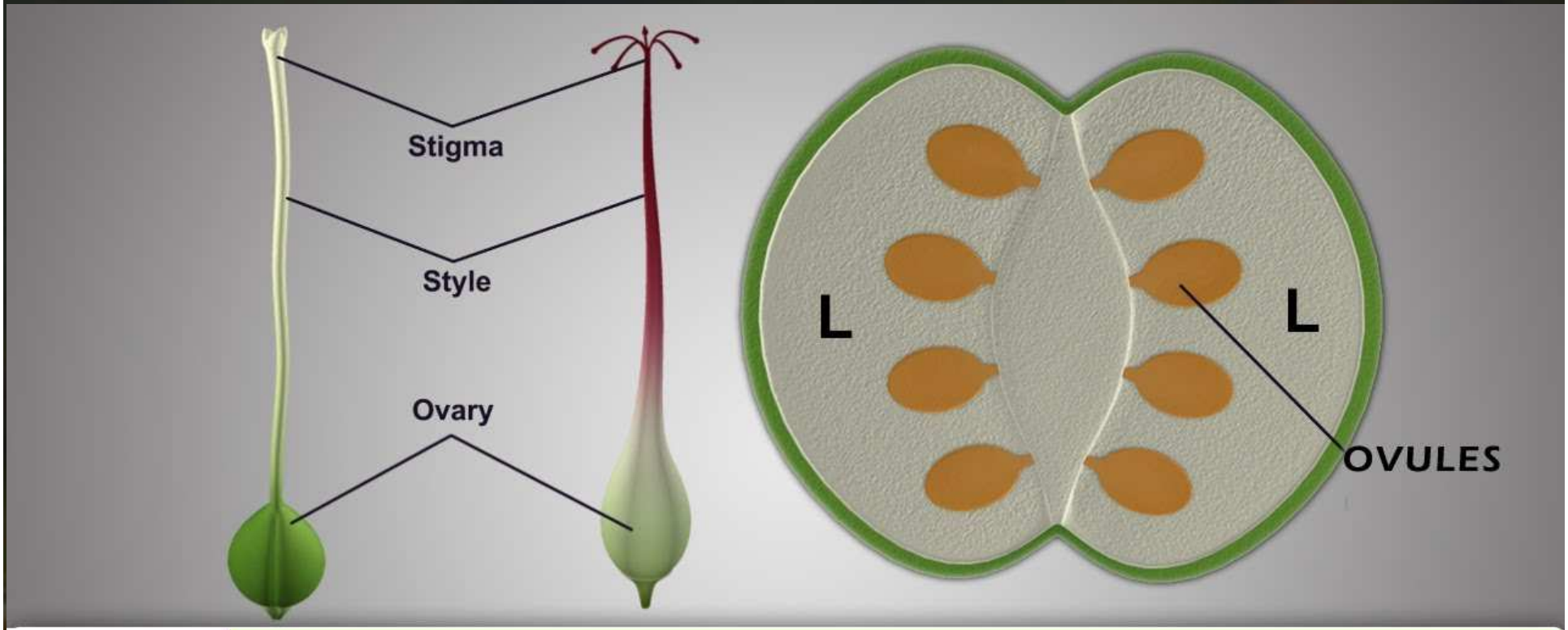
Comparison of Tests for Pollen Viability



Pollen viability in *Secale* (A) and *Cytisus* (B)



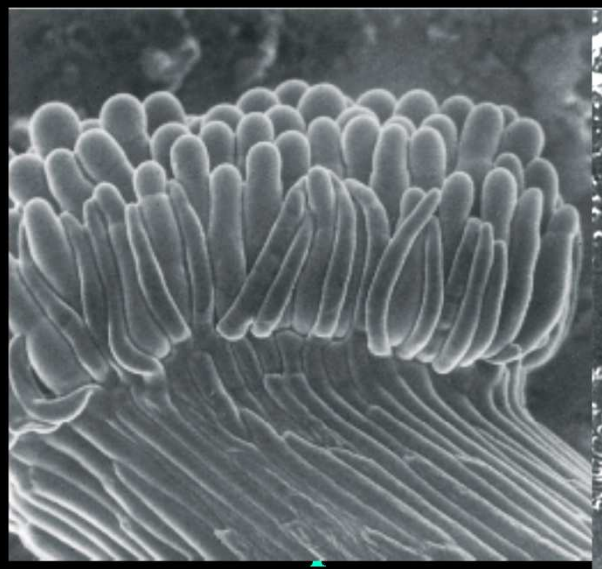
Gynoecium (stigma, style and ovary)

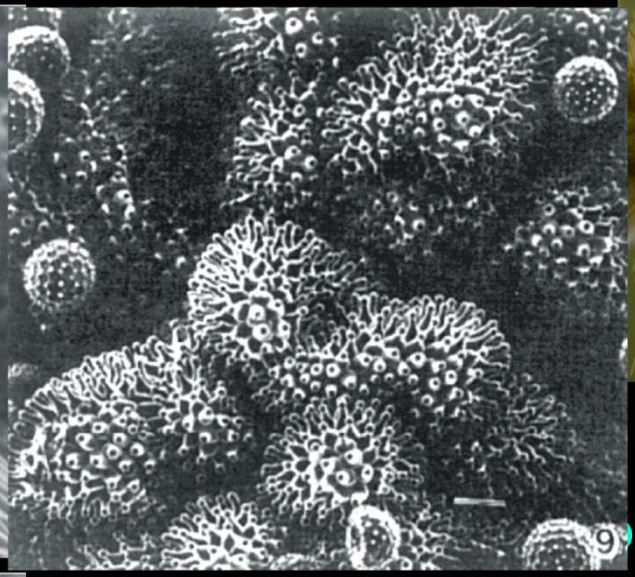


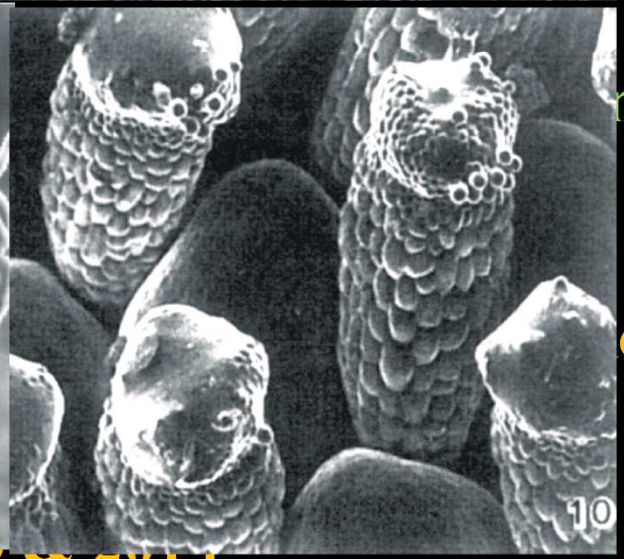
ACTIVE
The Joy Of Happy Learning

GYNOECIUM (PISTIL)

Types of stigma







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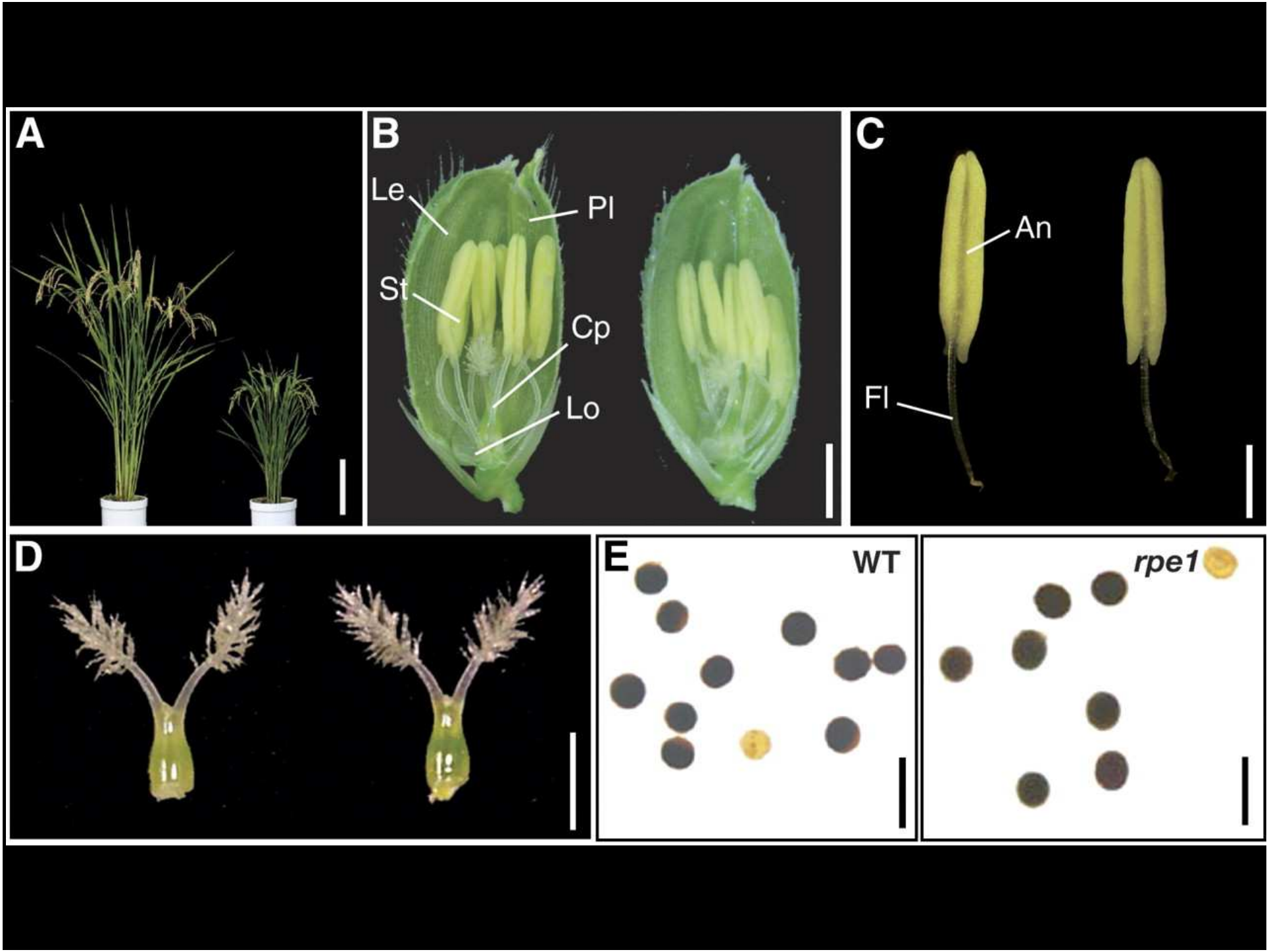
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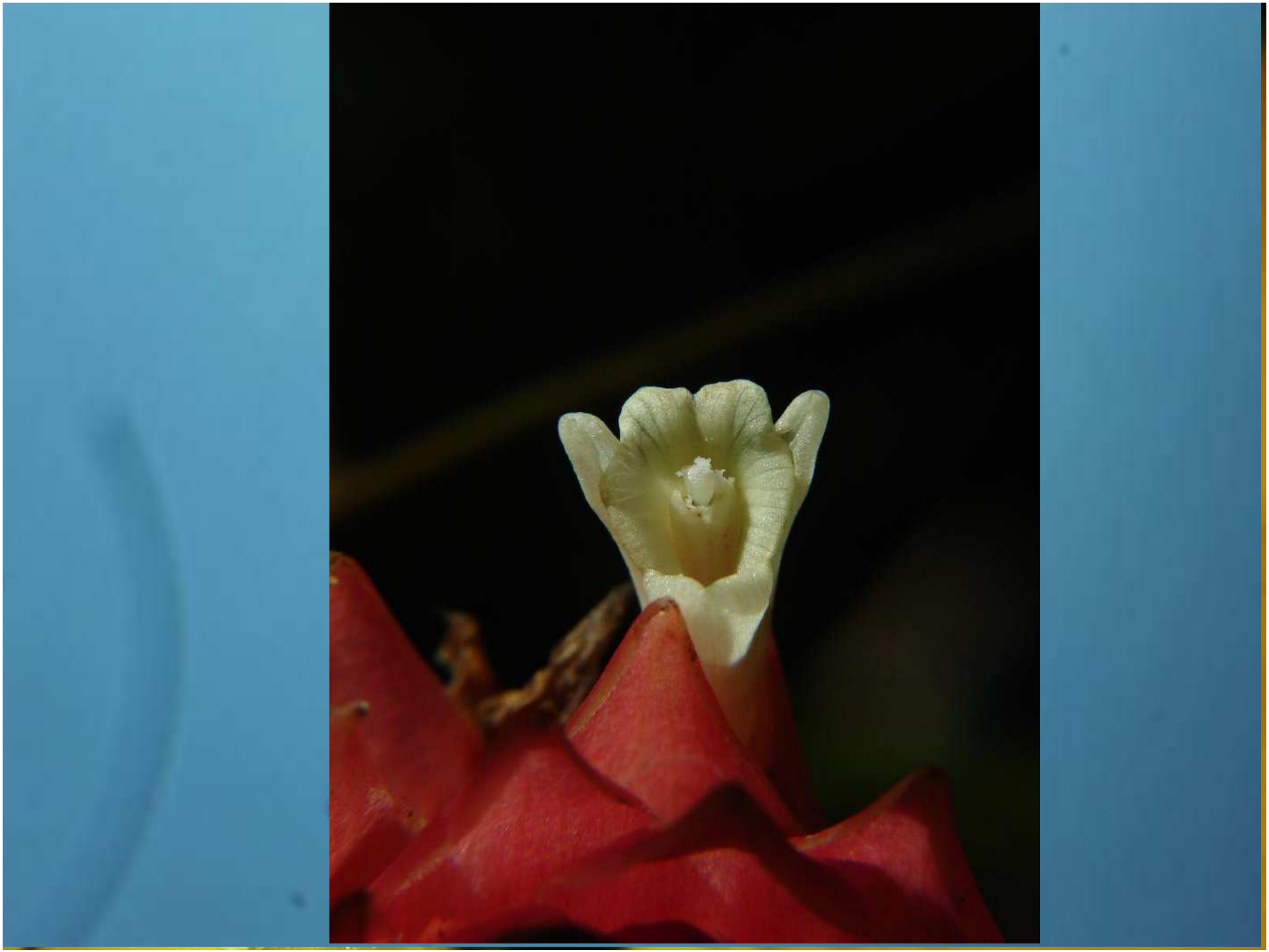
Stigma receptivity

- critical for successful post-pollination events
- maximum soon after anthesis
- varies from species to species
- influenced by temperature and humidity (varies from species to species)
- although stigma can support pollen germination at bud stage (Solanaceae and Brasicaceae), the pollen tube may not grow through the style to reach the ovule (Shivanna and Sastri, 1981)



Events on the stigmatic surface

- Adhesion- important for dry stigma- includes enzymatic digestion of stigmatic cuticle layer
- Imbibition/hydration
- Carbohydrate sources
  - Maintains required osmotic potential of the medium (imp)
  - Serves as a substrate for pollen metabolism
  - Pollen contain certain endogenous sugars for germination and early tube growth
  - Exogenous growth-sucrose/fructose/glucose/raffinose
  - 2 celled pollen- low levels of sucrose (10-15%)
  - 3 celled pollen (>20%)
- OP of tube is lower than that of pollen

- Pollen germination and Pollen tube growth
- Poly Ethylene Glycole of different mol W (4000-10000 D) with low conc. of sucrose shown to improve *in vitro* pollen germination and pollen tube growth.

- It also enhanced when certain amino acids are also provided with PEG

- Boron and Calcium – stimulatory effect
- Boron is not in pollen but in stigma
- Plays an important role in the carbohydrate metabolism and induce pollen tube elongation.

Pollen germination and Pollen tube growth

- Calcium

- Larger population than smaller
- Heat stable water soluble substance termed Pollen Growth Factor (PGF)
- Effective conc. of PGF is attained in larger population
- CALCIUM ION
- effects of Ca is mediated through Ca^{2+} binding protein, calmodulin

- Ca gives rigidity
- Controlling permeability
- Absence of Ca in the germination medium increases permeability that leads in loosing internal metabolites

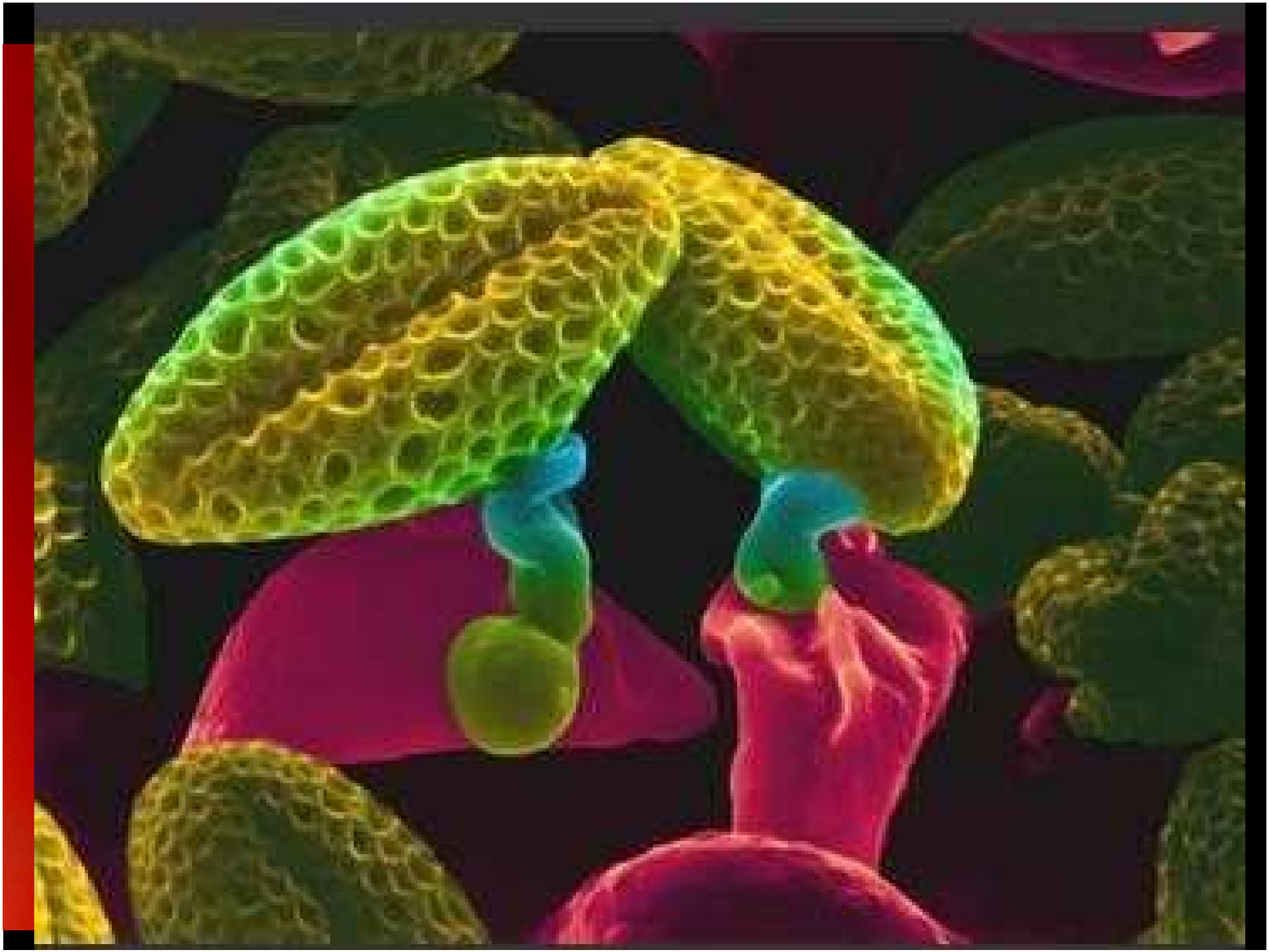
Events on the stigmatic surface

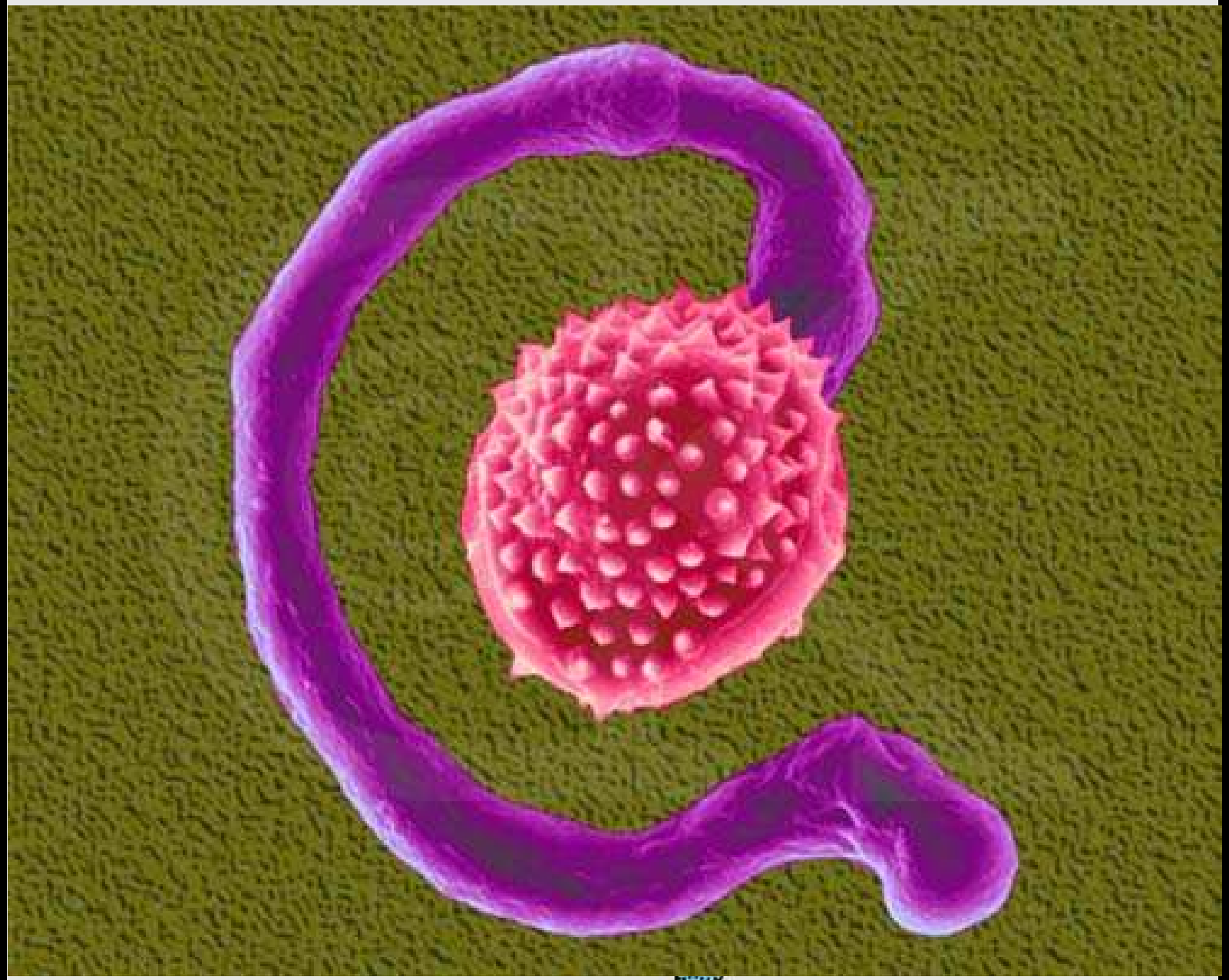
- Pollen should be Viable and Compatible
- Stigma should be receptive at the time of pollination
- Enzymes and Proteins recognises the right pollen
- Role of **Arabinogalactan Proteins (**AGP)-along the path of pollen tube through stigma and style to ovule/s
- Presence of AGP as a marker for Stigma receptivity

Stigmatic surface

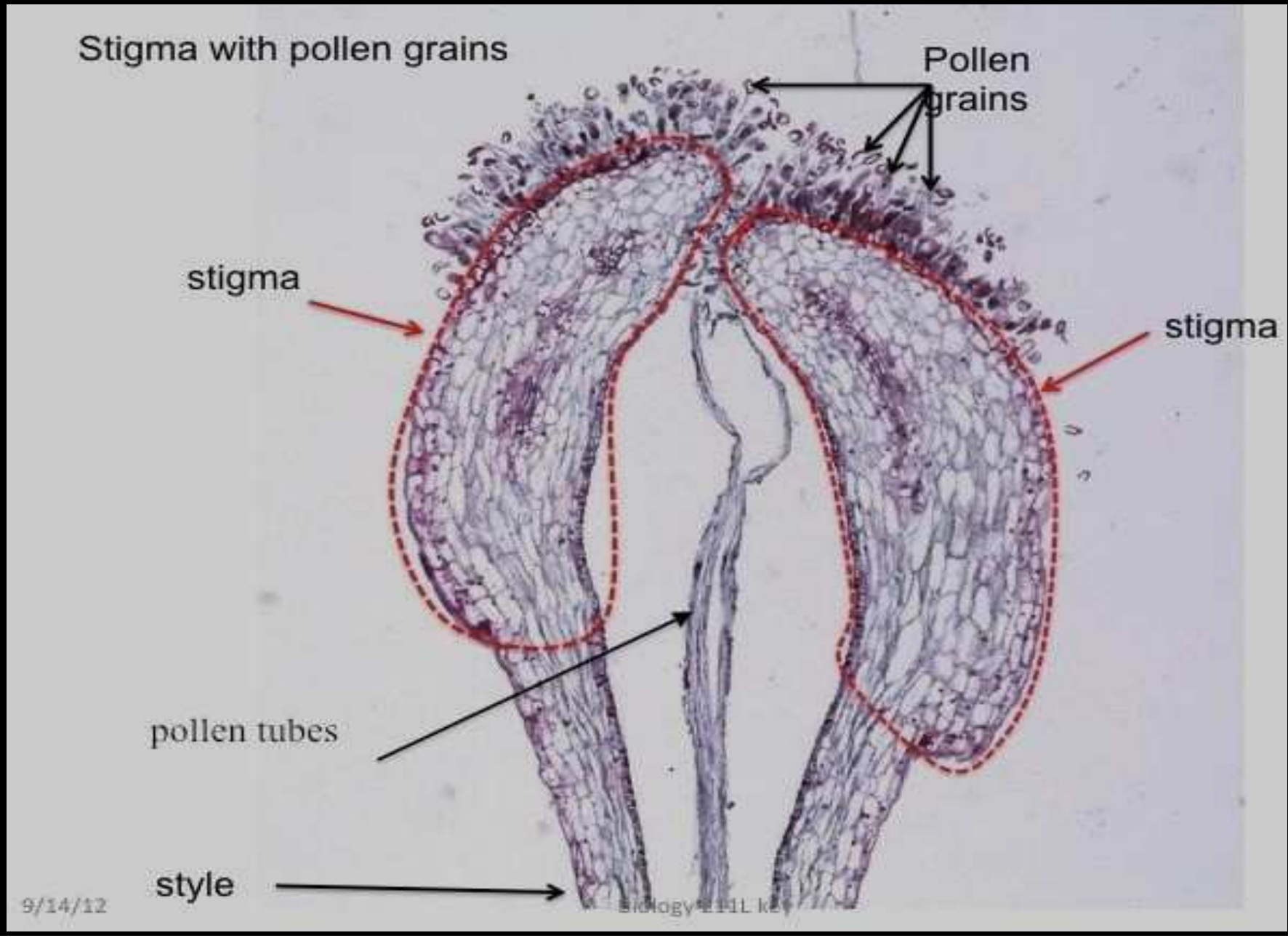
- Stigmatic exudates
- Adhesive Carbohydrates, Lipids and Proteins
- Extracellular proteins
- Esterases and Peroxidases
- Arabinogalactan Proteins (AGPs) -heavily glycosylated, hydroxyproline-rich protein
- Extracellular Matrix (ECM) also present in the longitudinal space of cells in the stigma and style (leads to ovules)

In dry stigma some components of pellicle are involved





Stigma Surface



Events in pollen tube growth

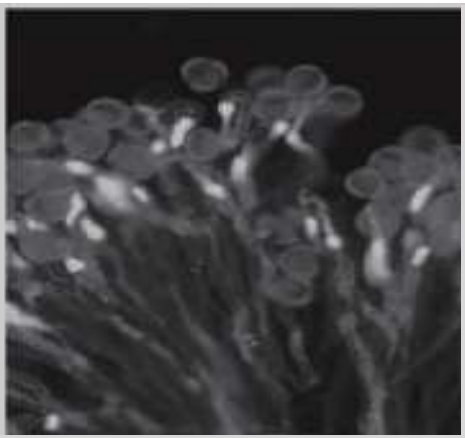
- Tube grows through the intercellular space of the style- directional
- ECM in the intercellular space
    - contain Polysaccharides, Amino Acids, Proteins, Glycoprotiens, Proteoglycans and phenolic compounds- which are heavily glycosylated
- requires synthesis of large amount of wall compounds for pollen tube growth and respiration-energy

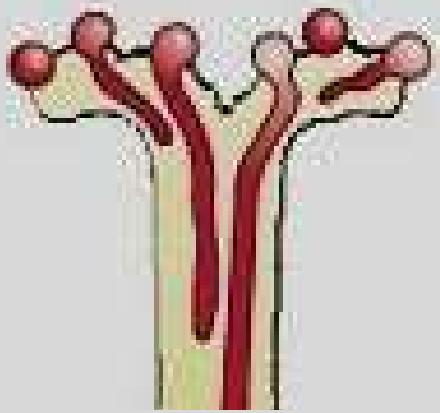
Events in pollen tube growth

- Transmitting Tissue specific (TTS) Protein- in the stylar transmitting tissue that is glycosylated and get deglycosylated by the growing pollen tube
- The resulting carbohydrates provide nutrients for pollen tube growth
- Other proteins such as Stigma specific Protein-1 SSP-1 also play a role in tube growth

Events in pollen tube growth

- Pollen tube should be guided to grow inside rather than outward or on the surface
- γ –Amino Butyric Acid (GABA) and heat stable Cystine rich Peptide Molecule (SCPM) plays the role of the "guide" to the growing pollen tube through the stigma and style to ovules





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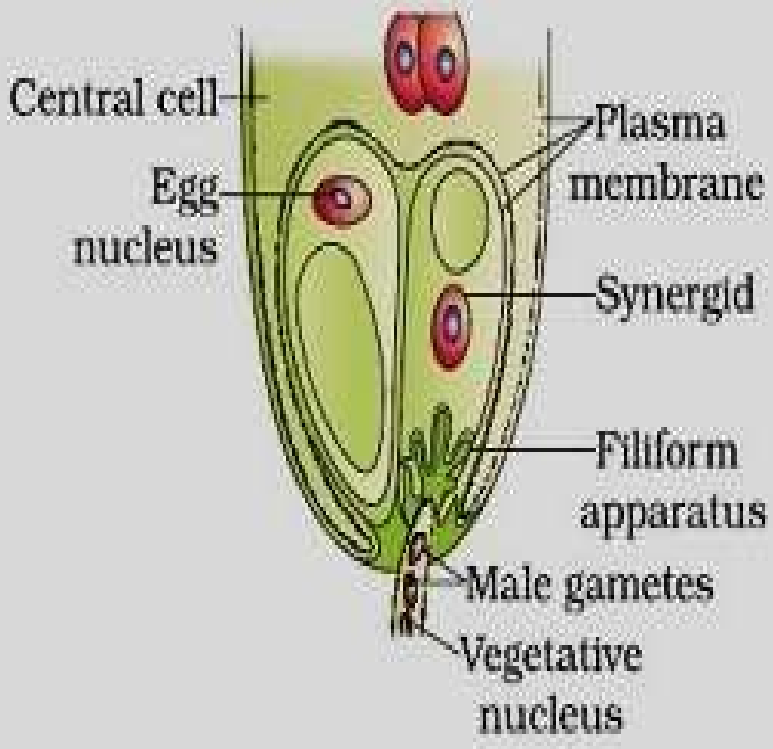

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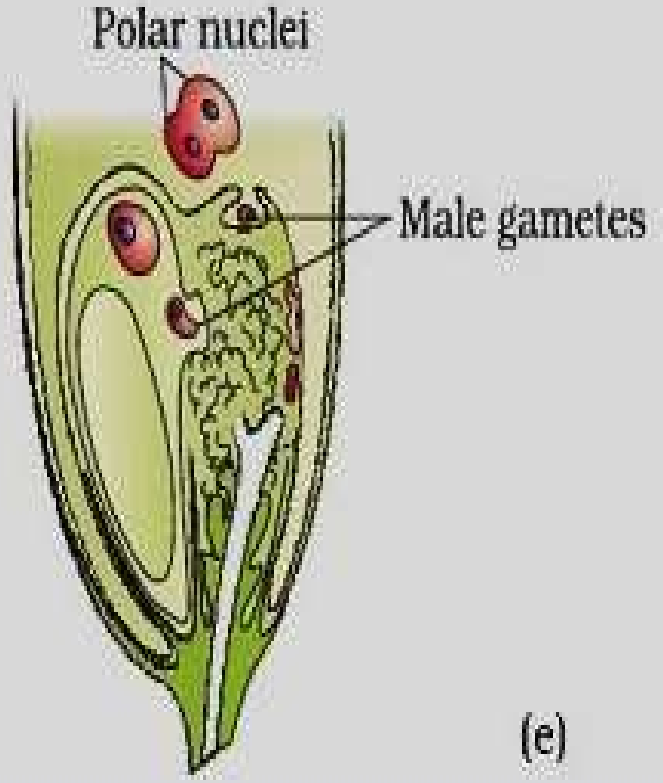

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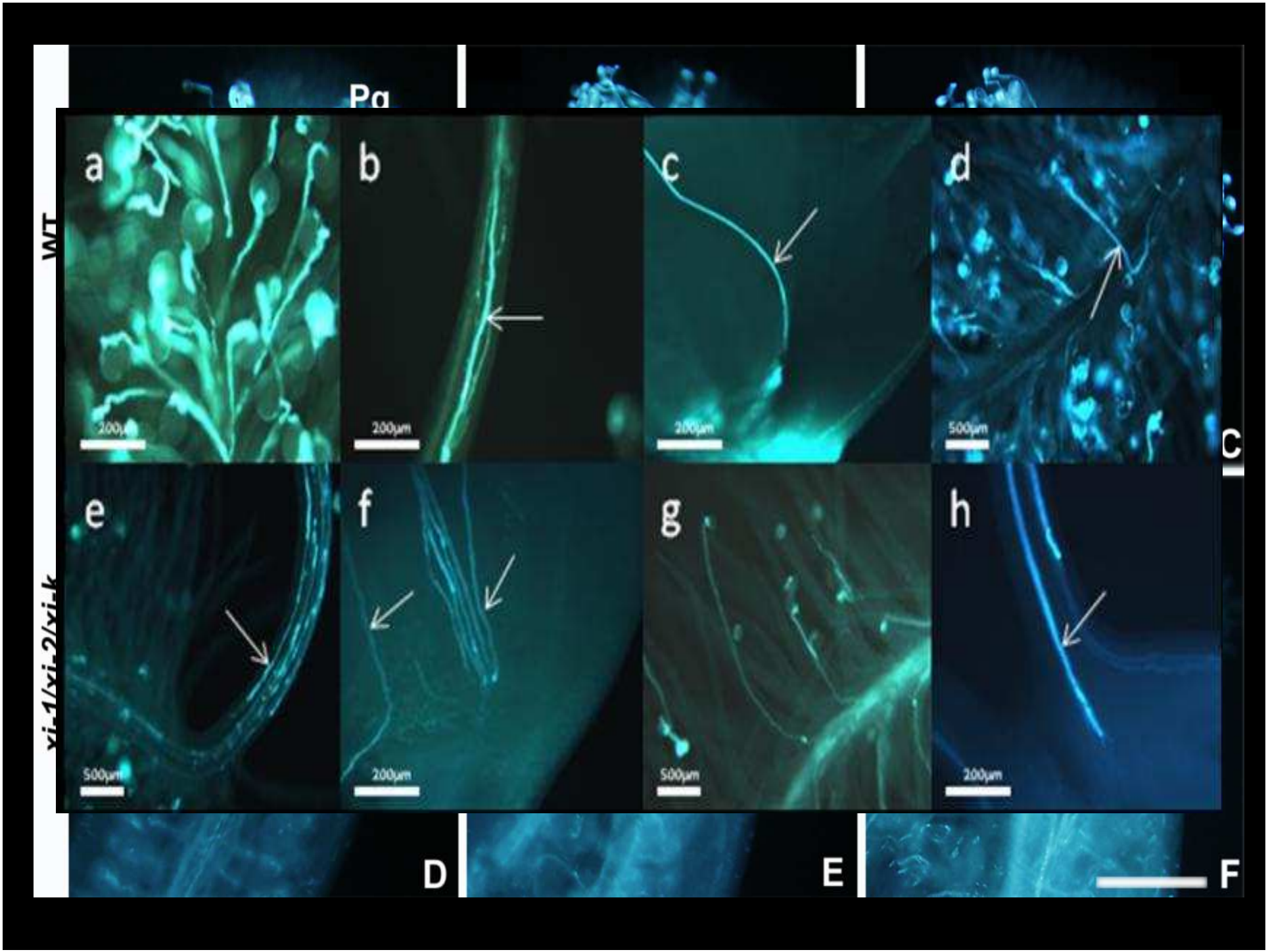
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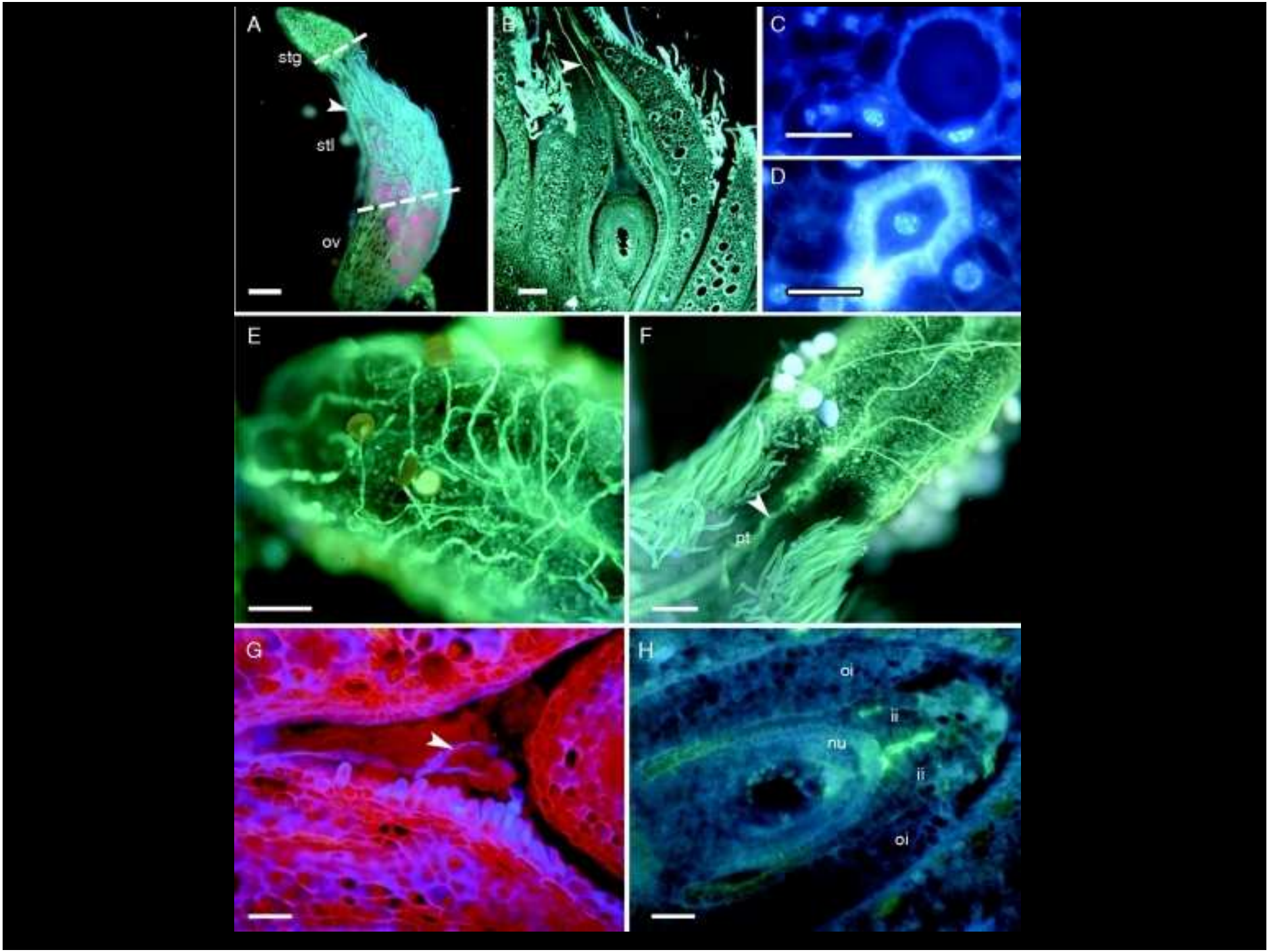
Events in pollen tube growth to the ovary

- Synergids play a key role in tube growth in to the ovary

Double Fertilization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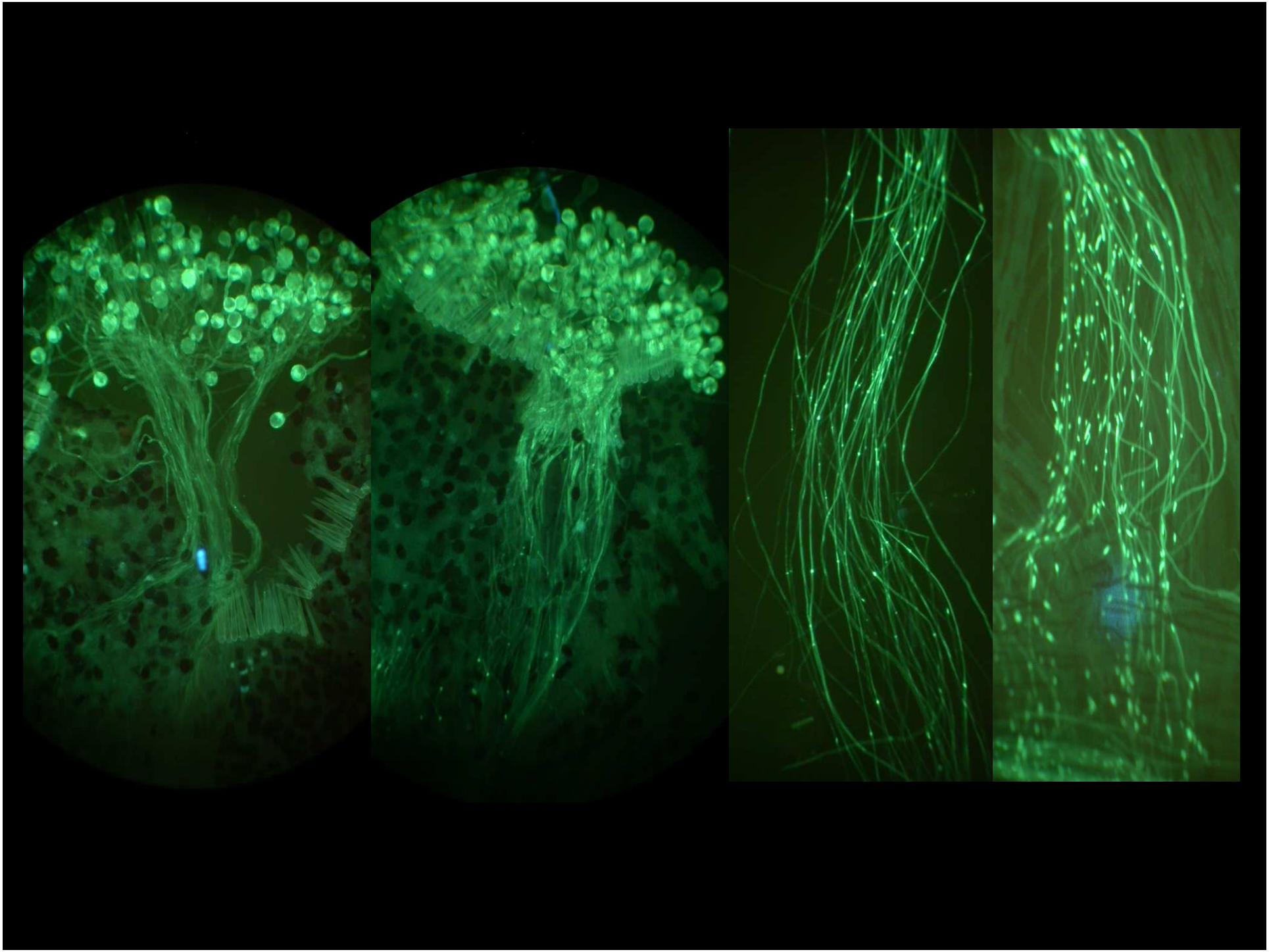
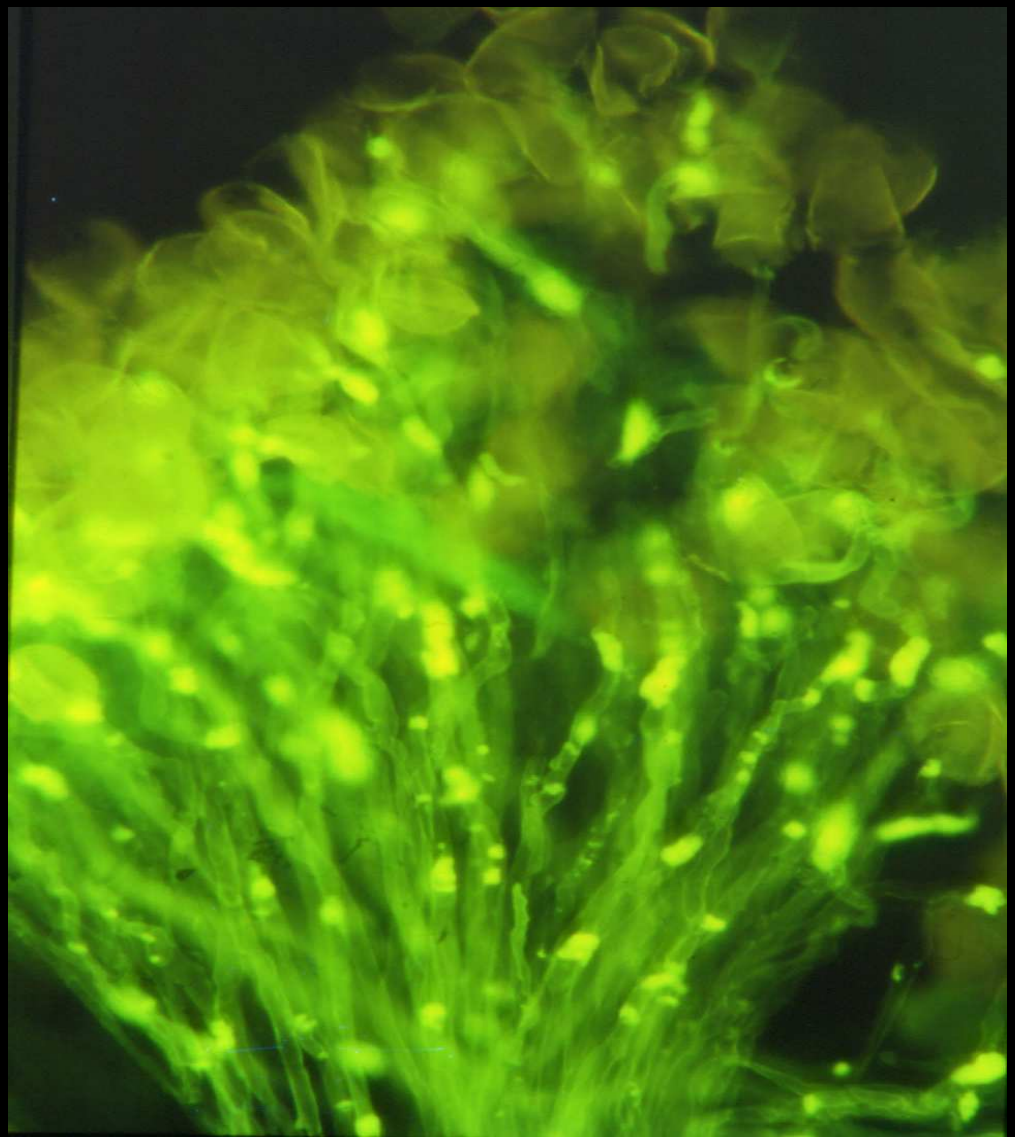
Tests for Pollen Pistil Interaction

- Cyto-chemical Localiization of esterases on stigma surface (N- Naphthyl Acetate)
- Aniline Blue Fluorescence Method
- Multiple Staining Method
- Controlled Pollinations

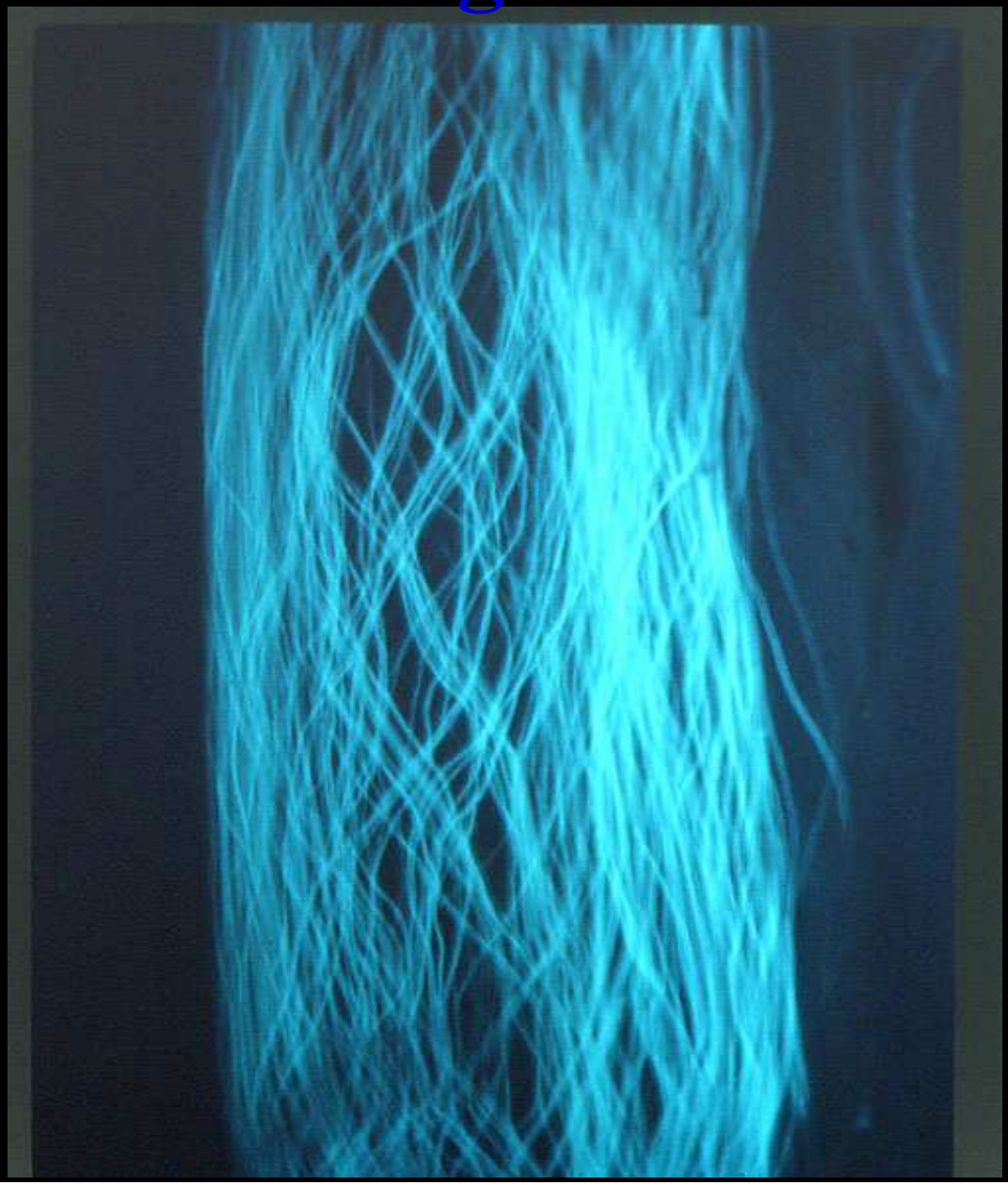


Self-incompatibility

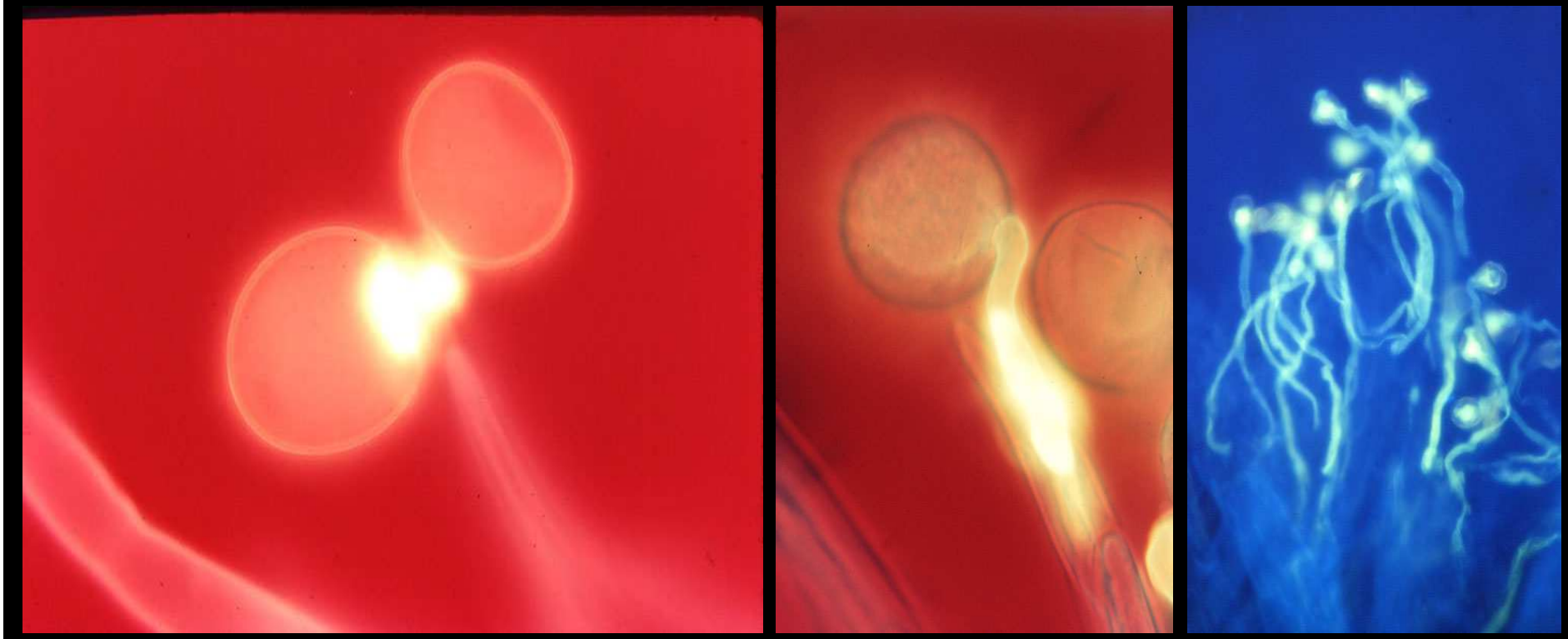
Pollen germination following compatible pollination



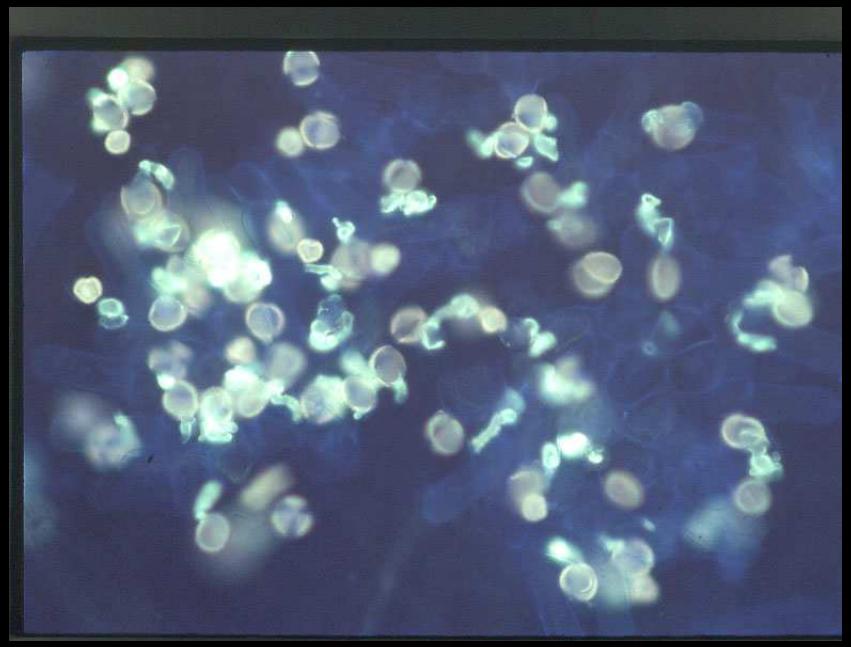
Pollen tube growth in the style



Inhibition of pollen germination/pollen tube growth



Inhibition of pollen tube entry



Important aspects of pollination biology

- Phenology
- Floral morphology
- Floral advertisement and rewards
- Pollination agent
- Floral visitors and pollinators
- Extent of specialization of flowers and pollinators
- Pollination efficiency
  - Pollinators' density
  - Presence of competing crops

Acknowledgements

Prof. K R Shivanna
Dr. P A Sinu

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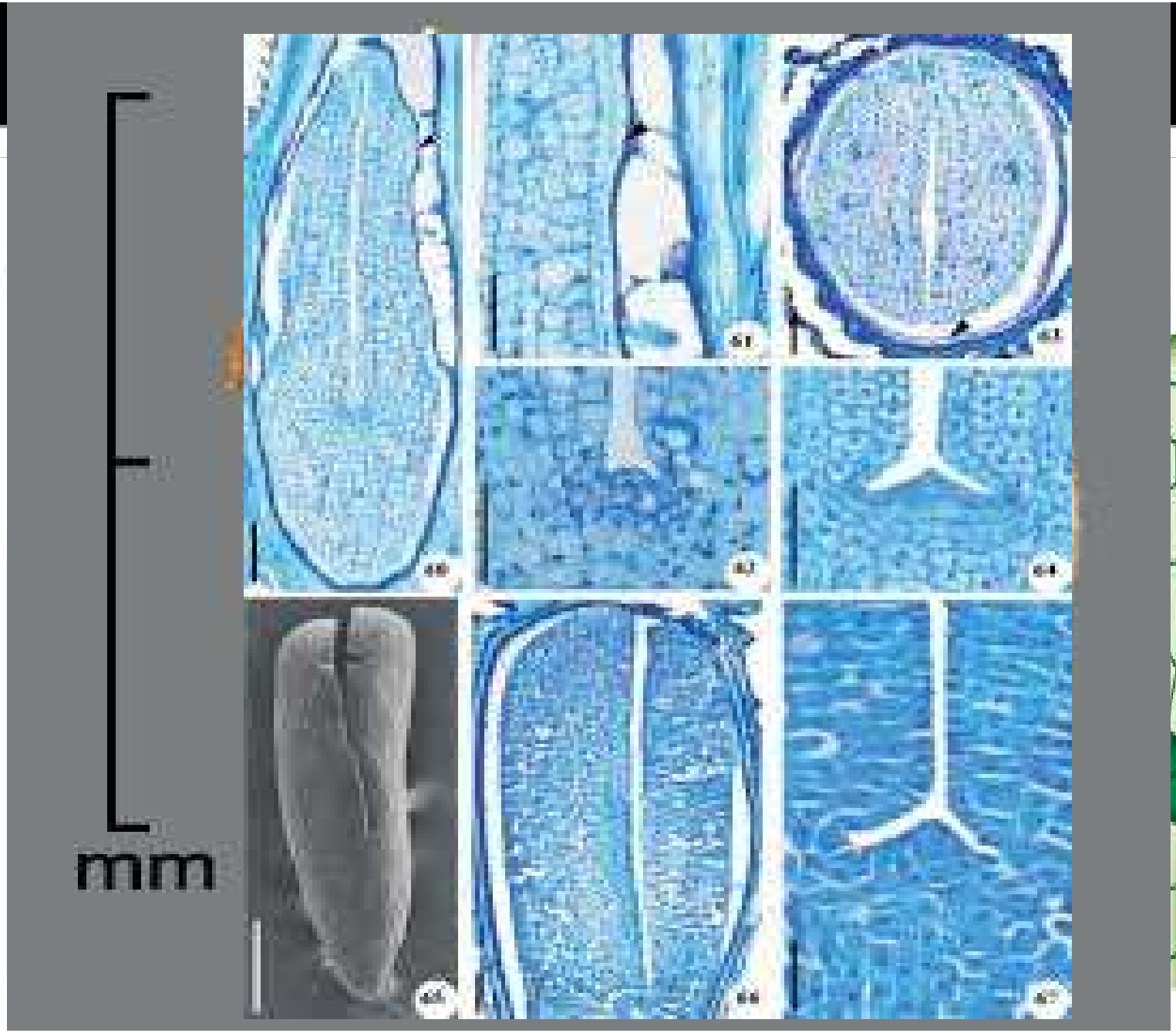

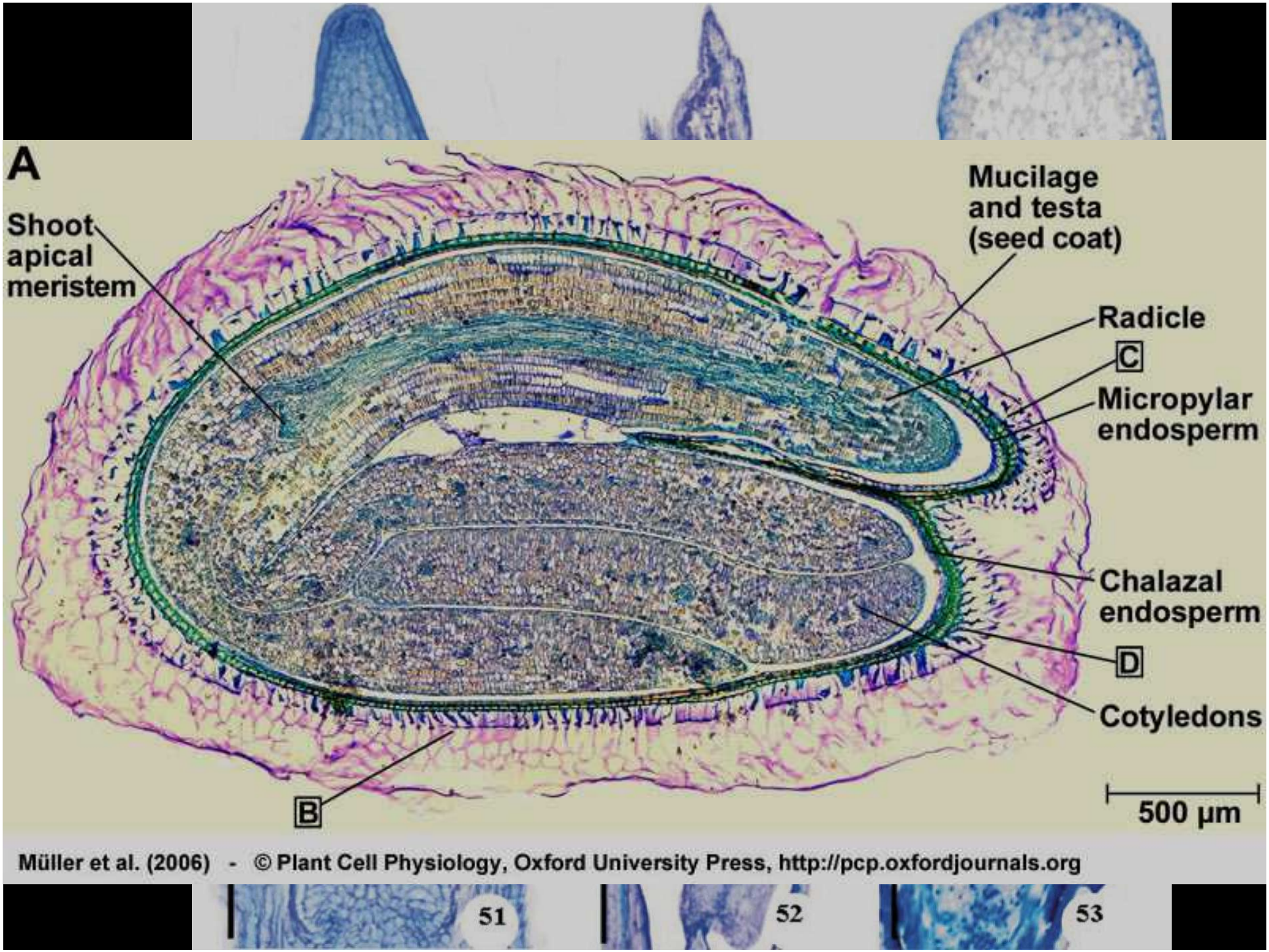

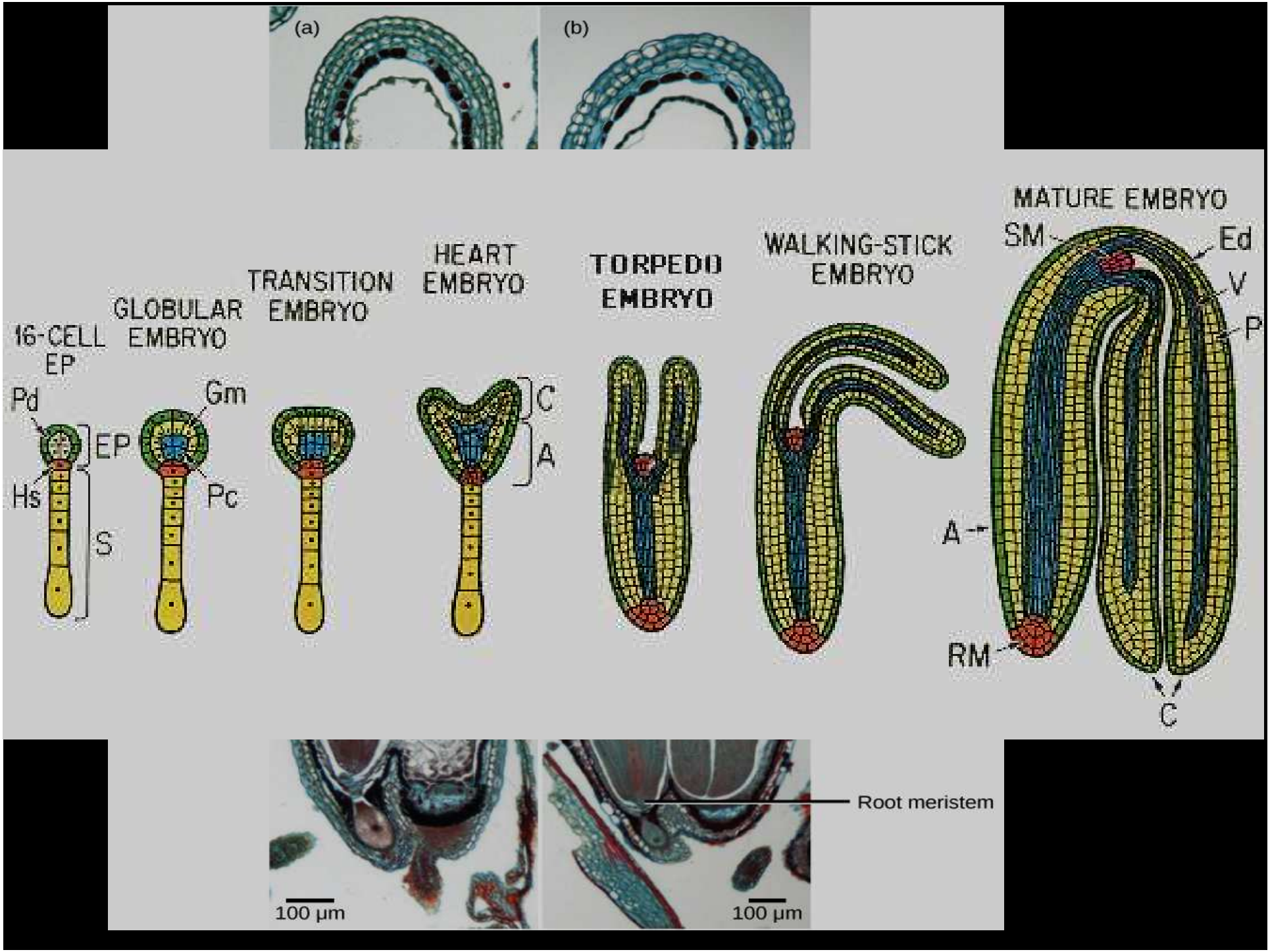
(a)
(b)
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HEART EMBRYO
TORPEDO EMBRYO
WALKING-STICK EMBRYO
MATURE EMBRYO
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A
RM
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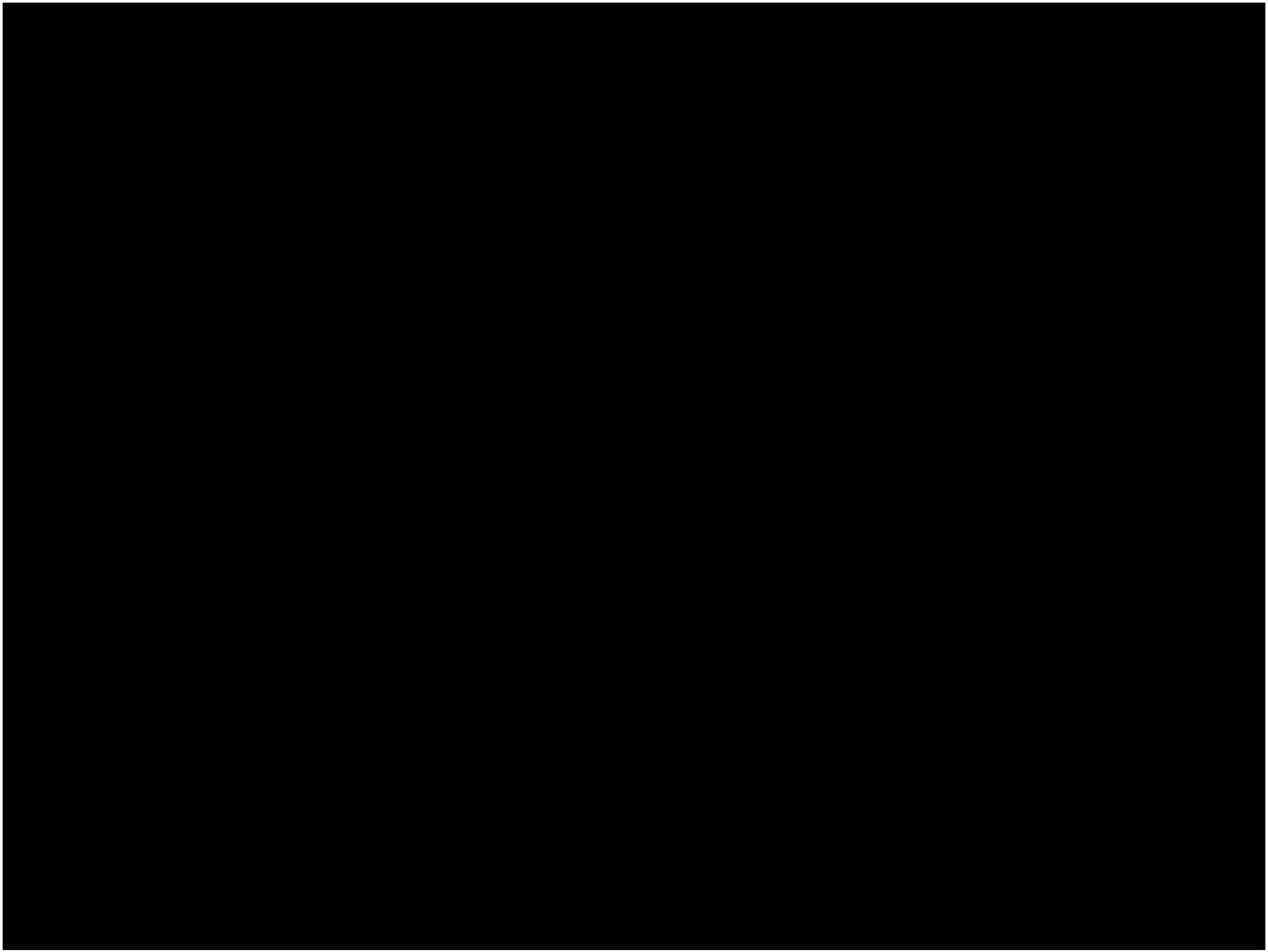
Root meristem
100 µm
100 µm

Figs. 46-50. Ovules of *Crepis japonica* (46, 49), *Porophyllum ruderale* (47) and *Tridax procumbens* (48, 50). Longitudinal section showing outer epidermis (oe), mesophyll (me), inner epidermis (ie) and vascular bundle (vb) (46-48); ovules under scanning electron microscopy (SEM) (49, 50). Scale bars= 50 µm (46), 100 µm (47, 49), 150 µm (48), 200 µm (50).



Pollination syndromes and vectors

Wind – Anemophily

Water – Hydrophily

Animals – Zoophily

- Insects – Entemophily
  - Beetles – Cantherophily
  - Flies – Myophily
  - Moths – Phalaenophily
  - Butterflies – Psychophily
  - Bees – Melittophily
  - Ants – Myrmecophily
- Birds – Ornithophily

Flower traits and Pollinators

Floral trait	Pollinator
Reduced sepals and petals	Wind
Flowers reduced, pollen grains are lighter with thick exine	Water
Large sepals or petals	Animal
Petals white	Moth or bat (nocturnal)
Petals coloured	
Flower tubular	
Sweet odour	Butterfly
No odour	Bird
Flower not tubular	Bee or beetle

Estimation of nectar

Depends on the amount of nectar present in the flower

If the amount is small:

Use calibrated microcapillaries (0.5 µl – 50 µl)

If the amount is large:

Use micropipettes

Estimation of the concentration of sugars

Use hand refractometer

For finer analyses biochemical methods can be used

Stigma receptivity

The most authentic method:
Manual pollination on flower buds/flowers of different stages and studies on pollen germination and pollen tube growth.

Receptive stigmas support good pollen germination and pollen tube growth.

Floral visitors, pollen/nectar robbers and pollinators

Floral visitor: Any animal that visits the flower

Pollen/nectar robber: Any visitor that forage the nectar/pollen without bringing about pollination

Pollinators:
Come in contact with the pollen grains and stigma
Carry pollen load on their body
Deposit pollen grains on the stigma

Breeding System

All aspects of sex expression in plants which effect the relative genetic contributions to the next generation of individuals within a species (Wyatt, 1983)

(basically it indicates the extent of inbreeding and/or outbreeding)

Controlled pollinations/Manual pollinations

Controlled pollinations in which pollination is brought about manually with the pollen of the known type

Adequate precautions have to be taken to make sure that only the pollen of the desired type land on the stigma and to prevent contamination by any unwanted pollen

Bagging of individual flower
Bagging of inflorescence
Isolation of individual plant
Raising plants in insect-free green house/glass house

Bags of different mesh sizes to allow passage of airborne pollen but prevent entry of insects

Outbreeding devices

Dichogamy: **Temporal separation of anther dehiscence and stigma receptivity**

Herkogamy: **Spatial separation of the anthers and the stigma**

Self-incompatibility: **Self-pollinations do not result in fertilization because of inhibition of pollen germination or pollen tube growth in the pistil**

Dicliny: **Flowers are unisexual**

- ***Monoecious***: **Male and female flowers are borne on the same plant**
- ***Dioecious***: **Male and female flowers are borne on different plants**