ANTHER AND POLLEN CULTURE

Haploids and Agricultural applications for haploids -

- **Haploid -** Gametic number of chromosomes, n which may not be equivalent to x.

Application:

- Rapid generation of homozygous genotypes after chromosome doubling
- Reduce time for variety development, e.g. 10 to 6 years or less
- Homozygous recombinant line can be developed in one generation instead of after numerous backcross generations
- Selection for recessive traits in recombinant lines is more efficient since these are not masked by the effects of dominant alleles

Haploids and Agricultural applications for haploids -

- **Haploids** are very valuable in plant breeding for several reasons
- Since they carry only one allele of each gene, mutations and recessive characteristics are expressed in the plant.
- Plants with lethal genes are eliminated from the gene pool.
- Can produce homozygous diploid or polyploid plants - valuable in breeding
- Shorten the time for inbreeding for production of superior hybrids genotypes.

Processes Leading to Production of Haploid Plants

Formation *in vivo*

–Spontaneous occurrence in low frequency

–Induction by physical and/or chemical treatment

–Chromosome elimination following interspecific hybridization. Specific for some plants such as barley. Not widespread.

Parthenogenesis - from unfertilized egg

Apogamy - from other cells of the mega-gametophyte, **example**

Chromosome elimination - chromosome elimination in somatic cells, most common method used with plant breeding.

Processes Leading to Production of Haploid Plants

- •*In vitro* methods:
- –**Anther culture** (androgenesis) -production of haploid
- plants from microspores
- •Anther culture for production of haploids reported in about 250
- species
- •Solanaceae, Cruciferae, Gramineae, Ranunculaceae most commo
- –**Ovule culture** (gynogenesis) -production of haploid
- plants from unfertilized egg cell
- Haploid

Production of Haploids *In Vitro* through Anther and Microspore Culture

HISTORY

- W.TULECKE(1953)

First observed that mature pollen grains of *Ginkgo biloba* (a gymnosperm) can be induced to proliferate in culture to form haploid callus.

S.GUHA AND S.C MAHESWARI (1964)

- First reported the direct development of embryos from microspores of *Datura innoxia* by the culture of excised anther.

J.P. BOURGIN AND J.P.NITSCH (1967)

- Obtained complete haploid plantlets from anther culture of *Nicotiana tabacum*.

ANTHER CULTURE

- Anther culture is a technique by which the developing anthers at a precise and critical stage are excised aseptically from unopened flower bud and are cultured on a nutrient medium where the microspores within the cultured anther develop into callus tissue or embryoids that give rise to haploid plantlets either though organogenesis or embryogenesis.

POLLEN CULTURE

- Pollen or microspore culture is an in vitro technique by which the pollen grains preferably at the uninucleated stage ,are squeezed out aseptically from the intact anther and then cultured on nutrient medium where the microspores, without producing male gametes , develop into haploid embryoids or callus tissue that give rise to haploid plantlets by embryogenesis or organogenesis.

ANDROGENESIS

- Androgenesis is the *in vitro* development of haploid plants originating from totipotent pollen grains through a series of cell division and differentiation.
- It is of two types.

ANDROGENESIS

1) **Direct androgeneis:-**

   The microspores behaves like a zygote and undergoes chance to form embryoid which ultimately give rise to a plantlet.

2) **Indirect Androgenesis:-**

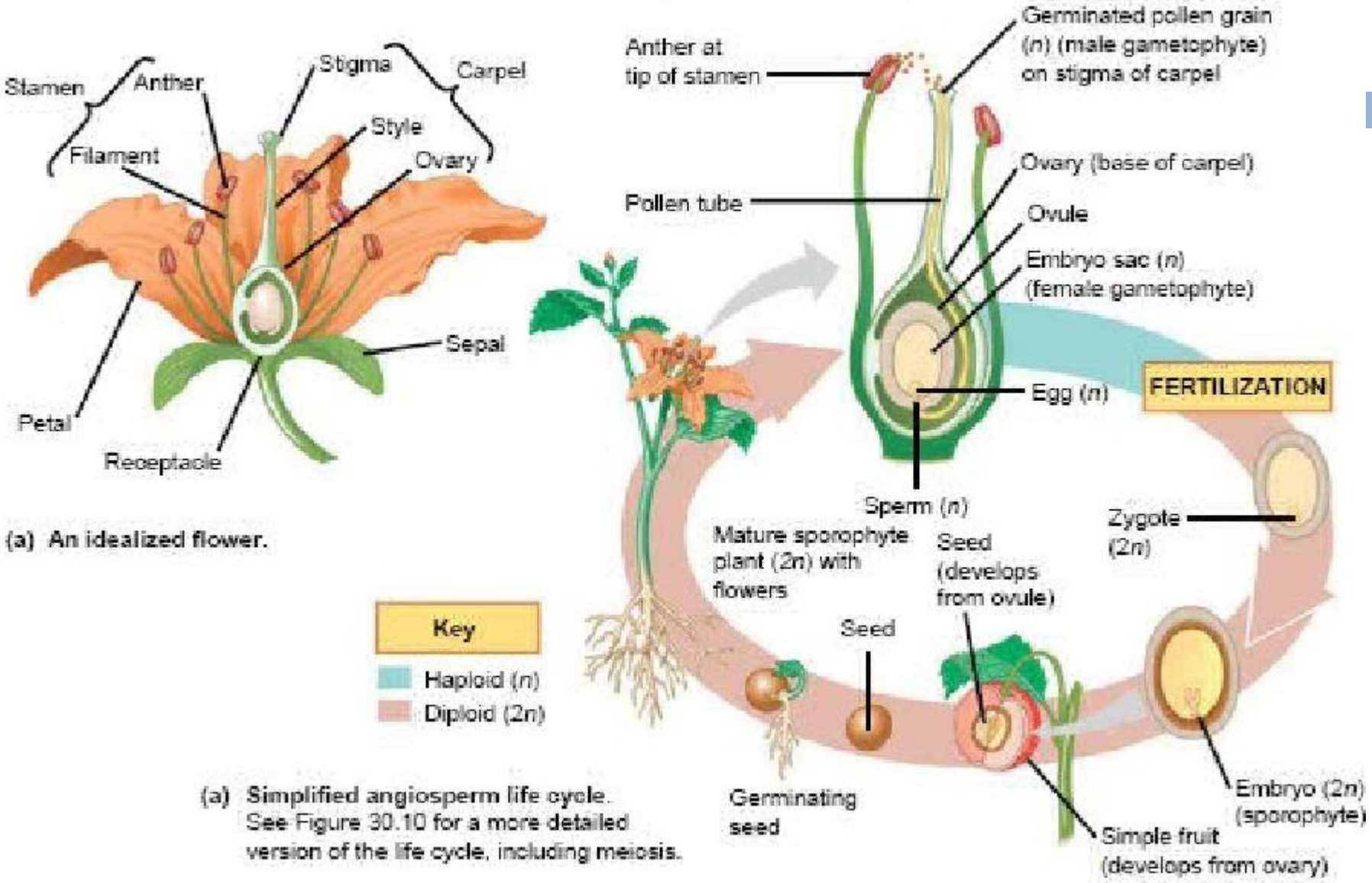
   The microspores divide repeatedly to form a callus tissue which differentiates into haploid plantlets.

Normal pollen development

Pollen mother cells are in anther primordia

- First phase - meiosis - pollen mother cell (PMC) A tetrad froms from each PMC
- Second phase - microspores released from tetrads
- Third phase - microspores mature into pollen grains - first pollen mitosis
- Generative and vegetative cells formed
- Second pollen mitosis, maybe after germination

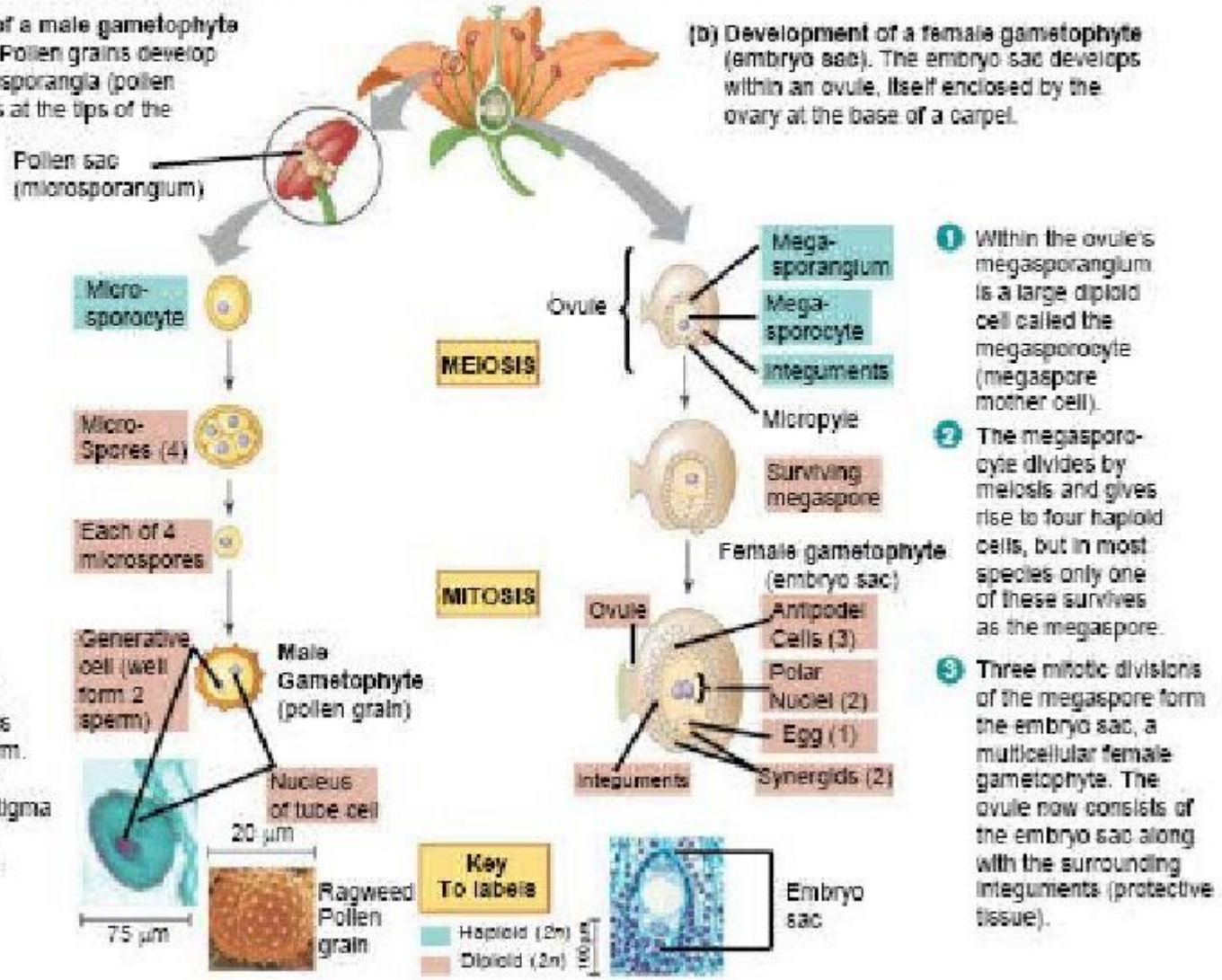
An overview of angiosperm reproduction

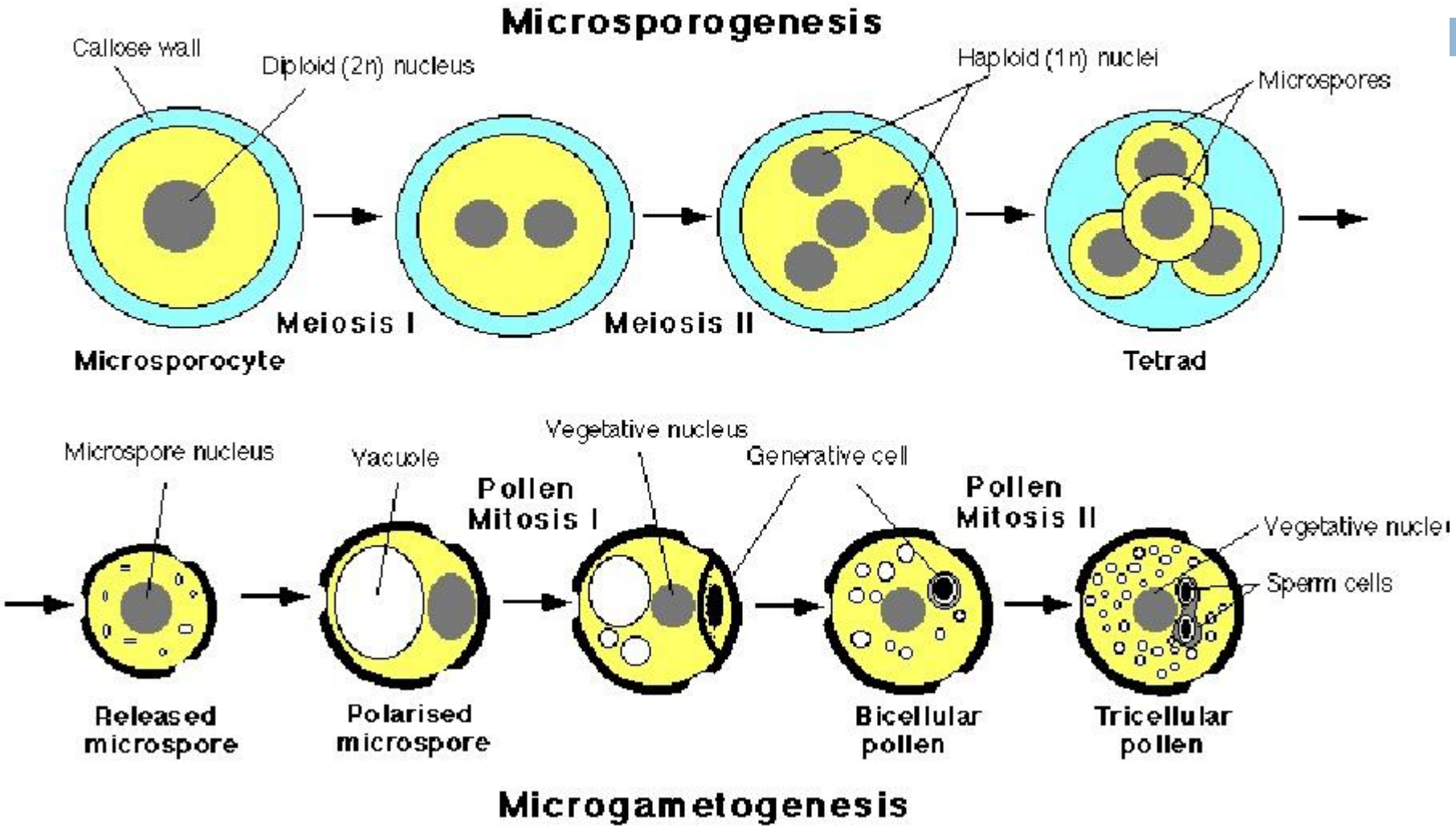


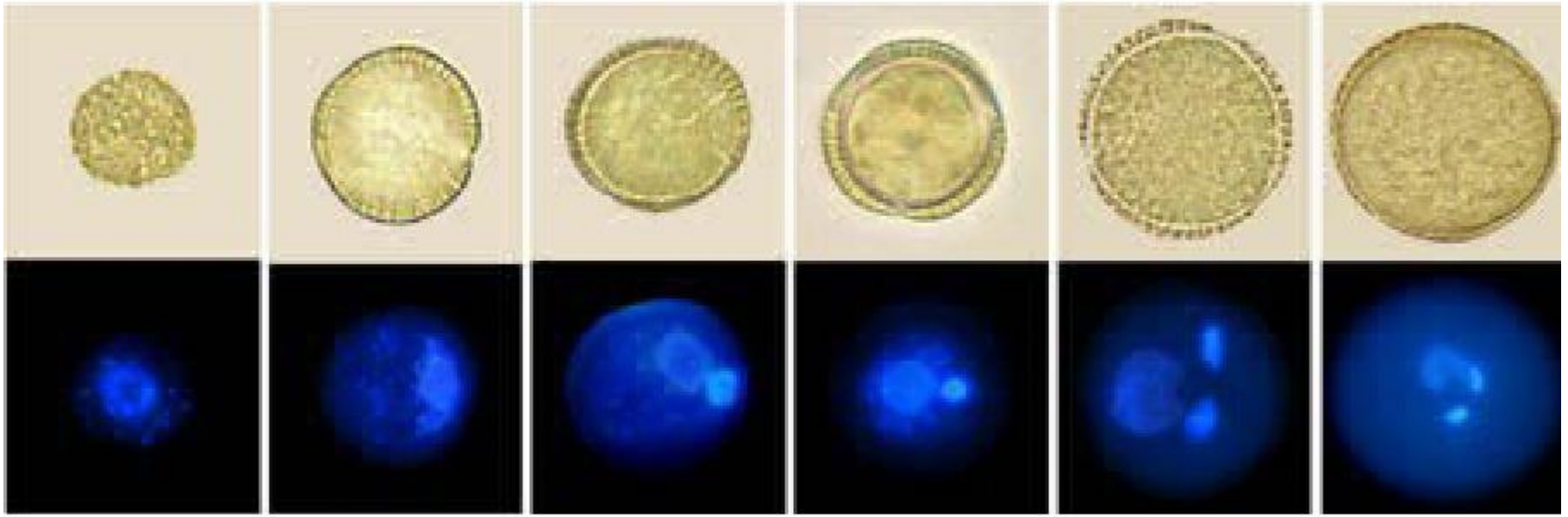
(a) An idealized flower.

(a) Simplified angiosperm life cycle. See Figure 30.10 for a more detailed version of the life cycle, including meiosis.

The development of angiosperm gametophytes (pollen grains and embryo sacs)




Microsporogenesis
Callose wall
Diploid (2n) nucleus
Haploid (1n) nuclei
Microspores
Meiosis I
Meiosis II
Microsporocyte
Tetrad
Microspore nucleus
Vacuole
Vegetative nucleus
Generative cell
Pollen Mitosis I
Pollen Mitosis II
Vegetative nucleus
Sperm cells
Released microspore
Polarised microspore
Bicellular pollen
Tricellular pollen
Microgametogenesis



PRINCIPLE OF ANTHER AND POLLEN CULTURE

- The production of haploid plants exploiting the totipotency of microspore .

- In this process the normal development and function of the pollen cell to become a male gamete is stopped and is diverted forcedly to a new metabolic pathway for vegetative cell division .

DEVELOPMENT OF ANDROGENIC HAPLOIDS

Pathway -1:-

The microspores divide by an equal division and identical daughter cells contribute to the saprophyte development.

Vegetative and generative cells are not distinctly formed in this pathway .

Example:-Datura innoxia.

Pathway:II:-

The division of uninucleate microspores is unequal resulting in the formation of a vegetative and generative cell.

The saprophyte arise through further divisions in the vegetative cell while the generative cell does not divide.

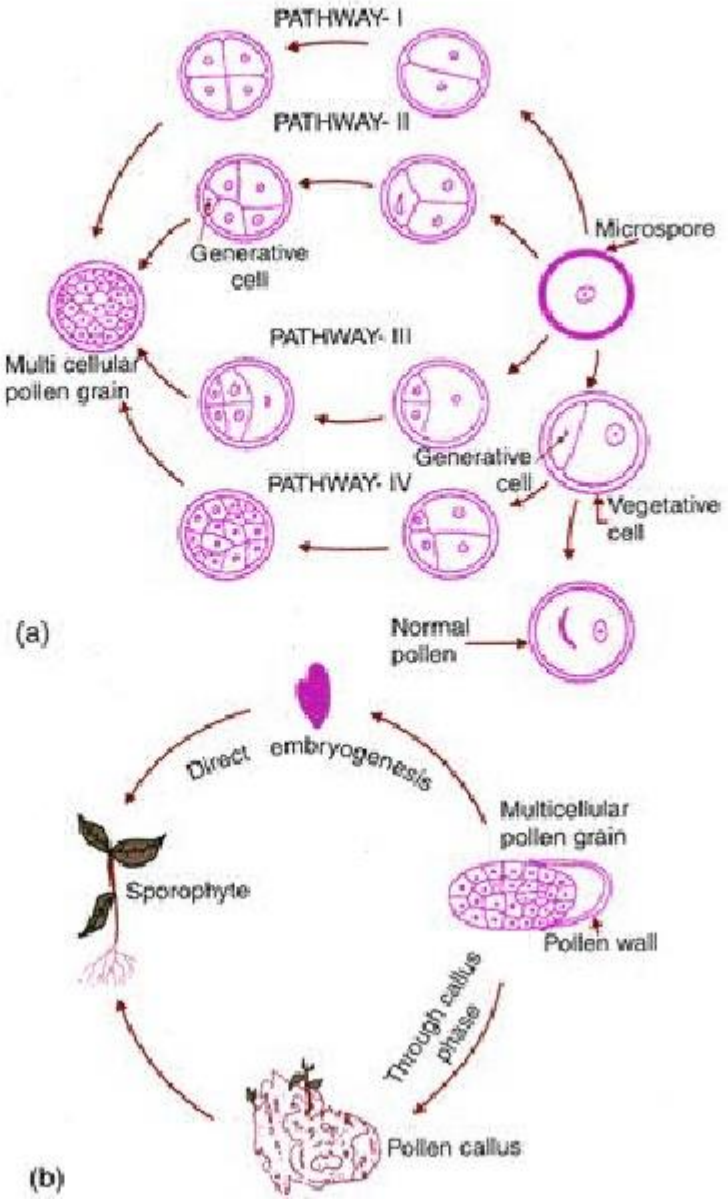
Example:-Nicotina tabacum

Pathway III:-

- The uninucleate Microspores undergoes a normal unequal division
- The pollen embryo are formed from generative cell alone.
- Example ;- *Hyoscyamus niger*.

Pathway IV ;-

- The division of microspore is asymetrical.
- Both vegetative and generative cell divide further and contribute to the development of the sporophyte. Example:- *Atropa belladona*.


PATHWAY- I
PATHWAY- II
Microspore
Generative cell
Multi cellular pollen grain
PATHWAY- III
Generative cell
Vegetative cell
PATHWAY- IV
(a)
Normal pollen
Direct embryogenesis
Multicellular pollen grain
Sporophyte
Pollen wall
Through callus phase
Pollen callus
(b)

FACTORS INFLUENCING ANTHER CULTLRE

1) GENOTYPE OF DONOR PLANTS:-

The genotype of the donor plant plays a significant role in determining the frequency of pollen production.

- Example :- Horedum of each genotype differs with respect to androgenic response in anther culture.

2) ANTHER WALL FACTOR:-

The anther wall provide the nourishment in the development of isolated pollen of a number of species.

- There are reports that glutamine alone or in combination with serine and myoinositol could replace the anther wall factor for isolated cultures.

FACTOR INFLUENCING ANTHER CULTURE

3) CULTURE MEDIUM:-

The anther culture medium requirements vary with genotype and probably the age of the anther as well as condition under which donor plants are grown.

- In corporation of activated charcol into the medium has stimulated the induction of androgenesis.
- The iron in the medium plays a very important role for the induction of haploids .
- Potato extracts ,coconut milk and growth regulators like auxin and cytokininare used for anther and pollen culture.

FACTOR INFLUENCING ANTHER CULTURE

3) CULTURE MEDIUM:-

- Two hormone groups
- Without hormones - mostly dicots. Most success with solanaceous species. Do not want the anther wall to form callus.
- With hormones - most non-solanaceous species. Many monocots. Require hormones or complex organics such as coconut milk.
- Medium particularly important in cereals and rice to be able to produce green plants. A major difficulty was large number of albino plants that resulted.
- Sucrose - ranges from 2% (Nicotiana) to 10% (Brassica)

FACTOR INFLUENCING ANTHER CULTURE

4) ANTHER STAGE –

- Microspore or pollen must shift from gametic to sporophytic pattern of development
- Most responsive cells for haploid embryo formation are those between the tetrad stage of microsporogenesis to just past the first pollen mitosis.
- In most of the cases anthers are more responsive when cultured at uninucleate microspore stage
- Ex: Wheat, Barley, Rice
- Anther of some species give the best response if the pollen is cultured at first mitosis or later stage.
- Ex: Datura, Tobacco

FACTOR INFUENCING ANTHER CULTURE

5) **Effect of temperature:-**

Temperature enhance the induction frequency of microspore androgensis.

- The low temperature treatment to anther or flower bud enhance the haploid formation.
- The low temperature effects the number of factors such as dissolution of microtubules lowering of absicisic acid maintenance of higher ratio of viable pollen capable of embryogenesis.

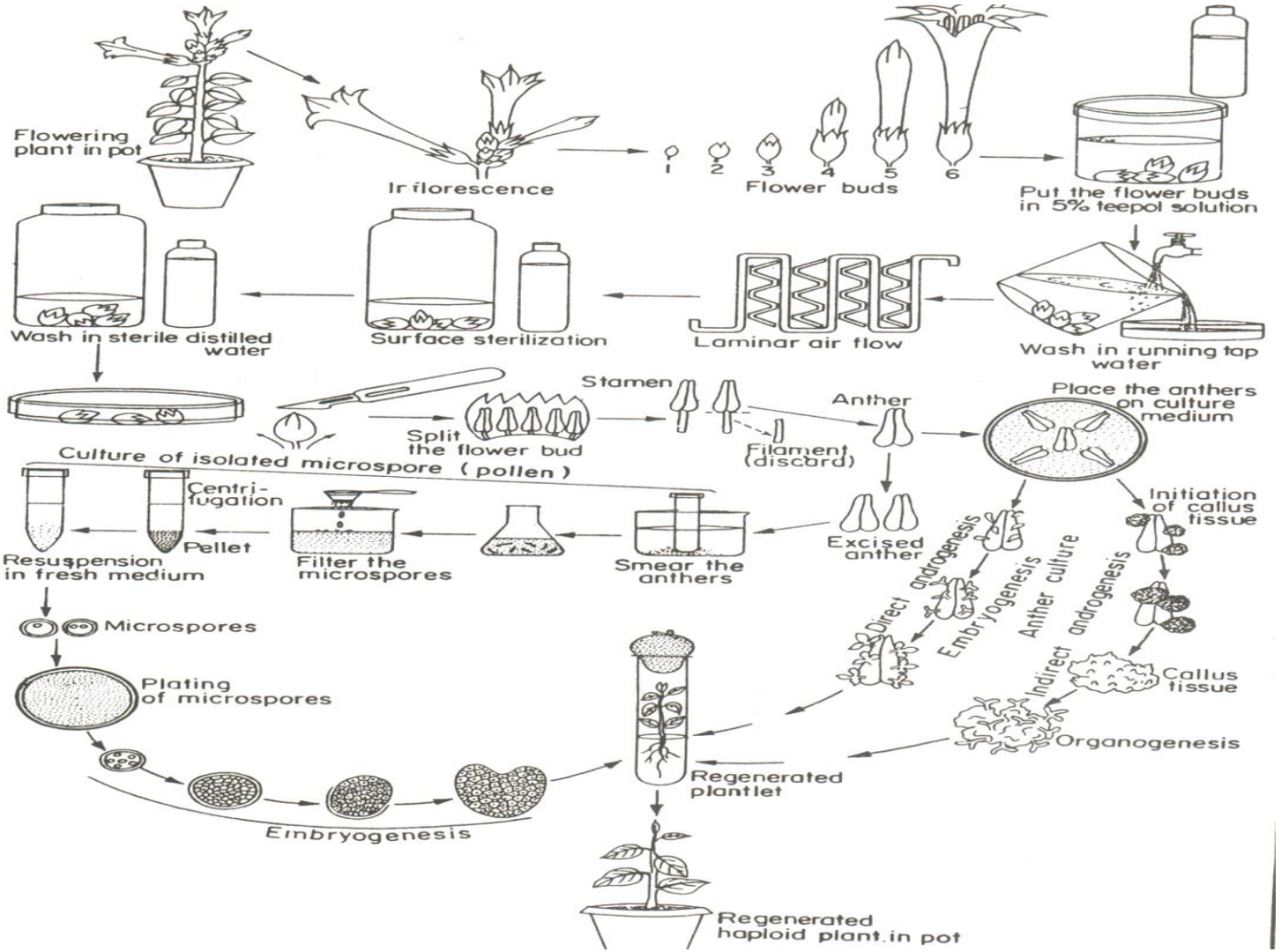
FACTOR INFLUENCING ANTHER CULTURE

6) PHYSIOLOGICAL STATUS OF DONAR PLANT:-

- Physiological status of donor plant such as water stress nitrogen requirement and age of donor plant highly effect the pollen embryogenesis.

- Plants starved of nitrogen may give more responsive anthers compared to those that are well fed with nitrogenous fertilizers.

METHOD OF ANTER AND POLLEN CULTURE



ADVANTAGE OF POLLEN CULTURE OVER ANTHER CULTURE

- During anther culture there is always the possibility that somatic cells of the anther that are diploid will also respond to the culture condition and so produce unwanted diploid calli or plantlets.
- Sometimes the development of microspores inside the anther may be interrupted due to growth inhibiting substances leaking out of the anther wall in contact with nutrient medium.

ADVANTAGE OF POLLEN CULTURE OVER ANTHER CULTURE

- Of interest because formation of embryo is known to be from one cell only and thus no chimeras are formed
- Much more difficult than anther culture
- Cultured either isolated microspores or pollen
- –*Brassica oleracea*

Ovule Culture

- •Haploids can be induced from ovules
- •The number of ovules is less and thus is used
- less than anther culture
  •May be by organogenesis or embryogenesis
  •Used in plant families that do not respond to

- androgenesis
- –*Liliaceae*
- –*Compositae*
-

IMPORTANCE OF POLLEN AND ANTHER CULTURE

- (1)Utility of anther and pollen culture for basic research:-
- (a) cytogenetic studies.
- (b) Study of genetic recombination in higher plants.
- (c) Study of mode of differentiation from single cell to hole organism.
- (d) Study of factor controlling pollen embryogenesis of higher plants.
- (e) Formation of double haploid that are homozygous and fertile.

2) Anther and pollen culture are use for mutation study. Example :- Nitrate reductae mutants are reported in Nicotiana tabacum.

3) Anther and pollen culture use for plant breeding and crop improvement.

4) Anther culture are use to obtain the alkaloid Example :- Homozygous recombination Hyoscyamus niger having higher alkaloid content is obtain by anther culture.

5) Haploid are use in molecular biology and genetic engineering. Example:- Haploid tissue of Arbidopsis and lycopersicon have been used for the transfer and expression of three genes from Escherchia coli....

Embryo Culture and Associated Techniques

- Embryo culture
  - most important apps
    - rescuing interspecific and intergeneric hybrids
      - wide hybrids often suffer from early spontaneous abortion
      - cause is embryo-endosperm failure

Embryo Culture and Associated Techniques

- Embryo culture
  - most important apps
    - rescuing interspecific and intergeneric hybrids
      - e.g., Gossypium, Brassica, Linum, Lilium
    - production of monoploids
      - useful for obtaining "haploids" of barley, wheat, other cereals
      - the barley system uses Hordeum bulbosum as a pollen parent

Embryo Culture and Associated Techniques

- Embryo culture
  - most important apps
    - production of monoploids
      - H. vulgare is the seed parent
      - zygote develops into an embryo with elimination of HB chromosomes
      - eventually, only HV chromosomes are left
      - embryo is "rescued" by culturing 10 PP to avoid abortion

Embryo Culture and Associated Techniques

- Embryo culture
  - reqs for embryo culture
    - excision of the immature embryo
      - hand pollination of freshly opened flowers
      - surface sterilization – EtOH on enclosing structures
      - dissection – dissecting scope necessary
      - plating on solid medium – slanted media are often used to avoid condensation

Embryo Culture and Associated Techniques

- Embryo culture
  - reqs for embryo culture
    - culture-medium factors
      - mineral salts – K, Ca, N most important
      - carbohydrate and osmotic pressure
        - 2% sucrose works well for mature embryos
        - 8-12% for immature embryos
        - transfer to progressively lower levels as embryo grows
        - altern. to high sucrose – auxin & cyt PGRs

Embryo Culture and Associated Techniques

- Embryo culture
  - reqs for embryo culture
    - culture-medium factors
      - amino acids
        - reduced N is often helpful
        - up to 10 amino acids can be added to replace N salts, incl. glutamine, alanine, arginine, aspartic acid, etc.
        - requires filter-sterilizing a portion of the medium

Embryo Culture and Associated Techniques

- Embryo culture
  - reqs for embryo culture
    - culture-medium factors
      - natural plant extracts
        - coconut milk (liquid endosperm of coconut)
        - enhanced growth attributed to undefined hormonal factors and/or organic compounds
        - others – extracts of dates, bananas, milk, tomato juice

Embryo Culture and Associated Techniques

- Embryo culture
  - reqs for embryo culture
    - culture-medium factors
      - PGRs
        - globular embryos – require low conc. of auxin and cytokinin
        - heart-stage and later – none required, usu.
        - GA and ABA regulate "precocious germination"

Embryo Culture and Associated Techniques

- Embryo culture
  - reqs for embryo culture
    - culture-medium factors
      - PGRs
        - GA and ABA regulate "precocious germination"
        - GA promotes, ABA suppresses

Embryo Culture and Associated Techniques

- In vitro pollination and fertilization
  - methods used to overcome prezygotic barriers – e.g., pollen – stigma incompatibility
  - various methods have been used
  - e.g., in vitro ovular pollination
    - a flower bud is cultured on nutrient medium
    - aseptically-collected pollen is applied directly to exposed ovules in vitro
    - intergeneric hybrids of Caryophyllaceae
    - interspecific hybrids of Solanaceae and Brassicas

Embryo Culture and Associated Techniques

- In vitro pollination and fertilization
  - prereqs for culturing ovules or ovaries
    - emasculate and cover flower buds to control pollination, and collection of pollen grains
    - remove sepals and petals, surface-disinfest excised pistil w/70% EtOH, rinse with sterile distilled water
    - place pistil into culture
  - several alternate treatments can be used

Embryo Culture and Associated Techniques

- In vitro pollination and fertilization
  - several alternate pollination treatments can be used
    - pollination thru a slit or pore
    - pollinate on the stigma
    - cut up the pistil into small pieces of placental tissue with attached ovules
    - culture individual ovules
  - Collecting pollen

Embryo Culture and Associated Techniques

- In vitro pollination and fertilization
  - Collecting pollen
    - surface-sterilize buds (with anthers)
    - keep in sterile petri dishes till anthesis
    - anthers are then taken from open flowers and pollen is collected and applied to cultured ovules, placenta or stigma, depending on the method
  - Factors affecting seed set after pollination

Embryo Culture and Associated Techniques

- In vitro pollination and fertilization
  - Factors affecting seed set after pollination
    - the less parental tissue removed, the better seed set is later
    - some species (maize) are more tolerant than others (Trifolium, Brassica)
    - not wetting the surface of ovules or stigma
    - time of excising the explant

Embryo Culture and Associated Techniques

- In vitro pollination and fertilization
  - Factors affecting seed set after pollination
    - a pollinated pistil provides better (unfertilized) ovules that later have better seed set
    - medium reqs – simple mineral salts, a few vitamins, and sucrose
    - sucrose at 4-5% is typical, but some workers use higher levels

Embryo Culture and Associated Techniques

- In vitro pollination and fertilization
  - Factors affecting seed set after pollination
    - a pollinated pistil provides better (unfertilized) ovules that later have better seed set
    - medium reqs – simple mineral salts, a few vitamins, and sucrose
    - sucrose at 4-5% is typical, but some workers use higher levels

Embryo Culture and Associated Techniques

- In vitro pollination and fertilization
  - some have used a simpler technique than any presented here: culture of ovules after pollination in vivo
  - E.g., Gossypium arboreum x hirsutum, Trifolium repens x hybridum, Helianthus annuus x maximiliani, H. annuus x tuberosum
- True in vitro fertilization

Embryo Culture and Associated Techniques

- True in vitro fertilization
  - only Zea mays, using single egg and sperm cells and fusing them electrically
  - fusion products were cultured individually in 'Millicell' inserts in a layer of feeder cells
  - the resulting embryo was cultured to produce a fertile plant
  - one suggested app: fusion of genetically modified gametes

THANKS!