ANTHER CULTURE

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introduction

- 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 gives rise to haploid plantlets either through organogenesis or embryogenesis.

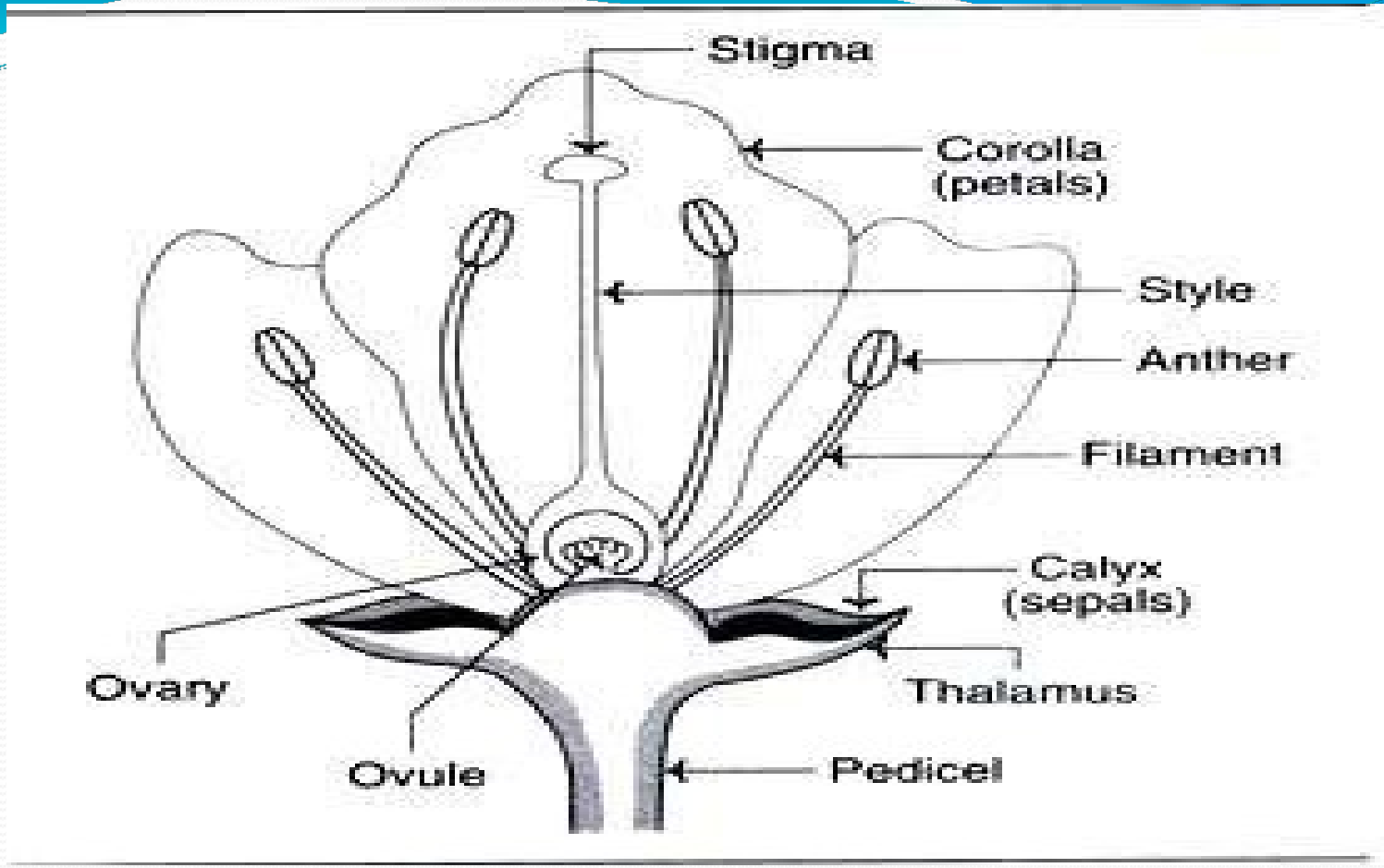


Fig : parts of flower

What is androgenesis ?

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

history

- The development of numerous pollen plantlets in anther cultures of *Datura innoxia* was reported by two Indian scientists GUHA and MAHESHWARI in 1966.

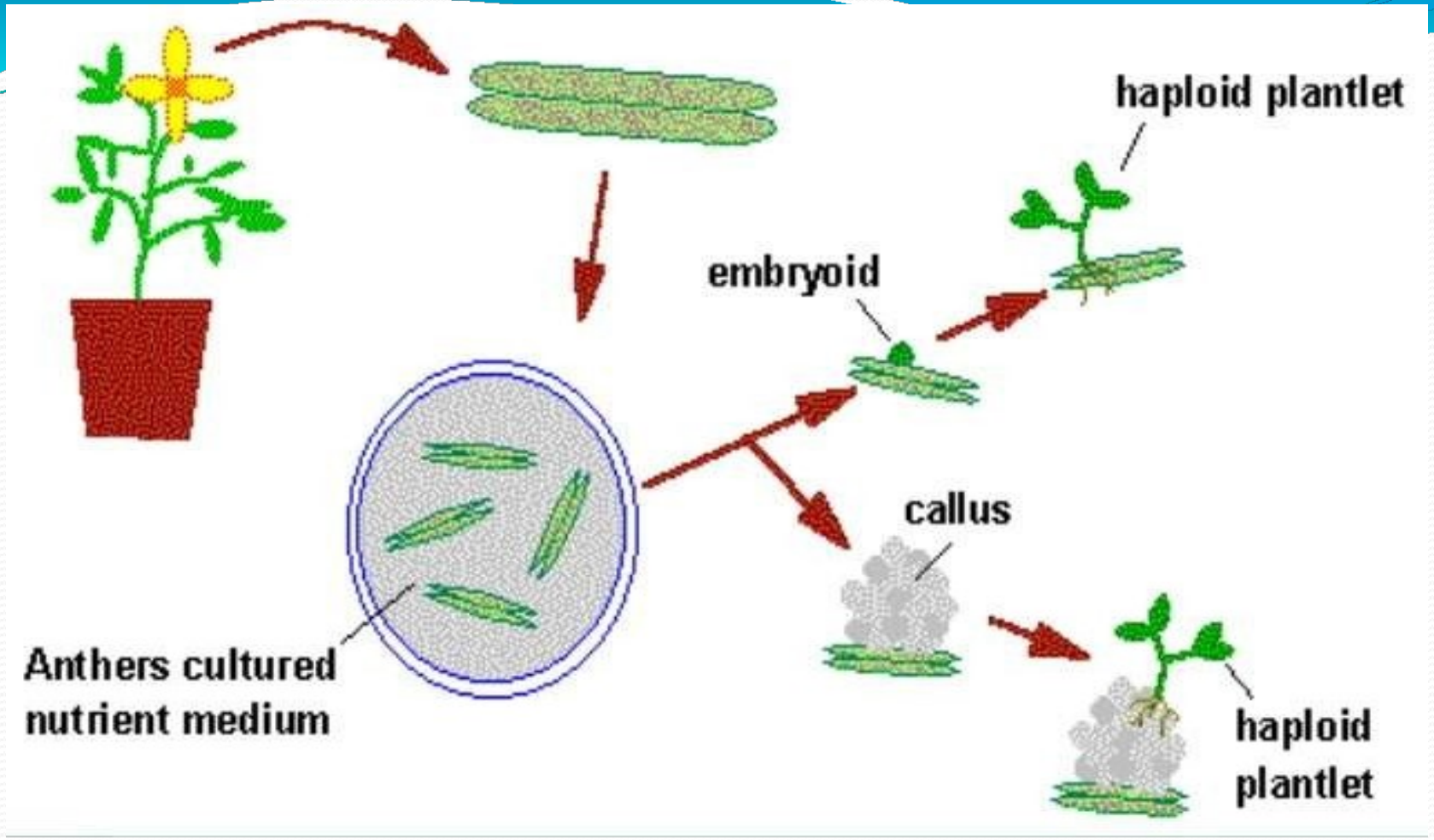


Fig: anther culture

The haploid plants can be isolated from three different techniques

- Anther culture
- Isolated pollen culture
- Gynogenesis

Anther culture

- The experimental plants for anther culture should be grown undercontrolled conditions of temperature, light and humidity.
- Anthers should be taken fromyoung plants.
- The selected buds are surfacesterilized with a suitabledisinfectant.
- Anthers along with their filaments are excised under aseptic conditions and placed on a sterilized petri plate.

- One of the anther is crushed in acetocarmine to test the stage of pollen development and if it is found to be of the correct stage the anthers of the remaining stamens are gently detached from their filaments, without injuring the anthersand placed horizontally on the medium.
- Sometimes complete inflorescences have been cultured to obtain androgenic haploids.
- The anther cultures are generally maintained in alternating periods of light (12-18 hrs) at 28°C and darkness (12-6 hrs) at 22°C.

- After 3-8 weeks they burst open due to the pressure exerted by the growing pollen callus or pollen embryo.
- The embryos germinate on the medium.
- After they have attained a height of about 3-5 cm, the individual plantlets or shoots are excised and transferred to sterilized potting mix in small pots orseed trays.

Isolated pollen culture

- Anthers are collected from sterilized flower buds in small beaker containing basal media.
- Microspores are squeezed out by pressing anthers against the side of the beaker with glass rod.
- Anther debris areremoved by nylon sieve.
- The filtrate is centrifuged, supernatantwith debris is discarded and pellet of pollen is resuspended in fresh media and several washes are given.

- Final suspension is pipetted into small petri dish with solid or liquid media.

ADVANTAGES OF MICROSPORE CULTURE OVER ANTHER CULTURE

- It is a haploid single cellsystem.
- Isolated microspores can be genetically modified.
- A homologous population of pollen grains at the developmental stage most suitable for androgenesis can be obtained by gradient centrifugation.

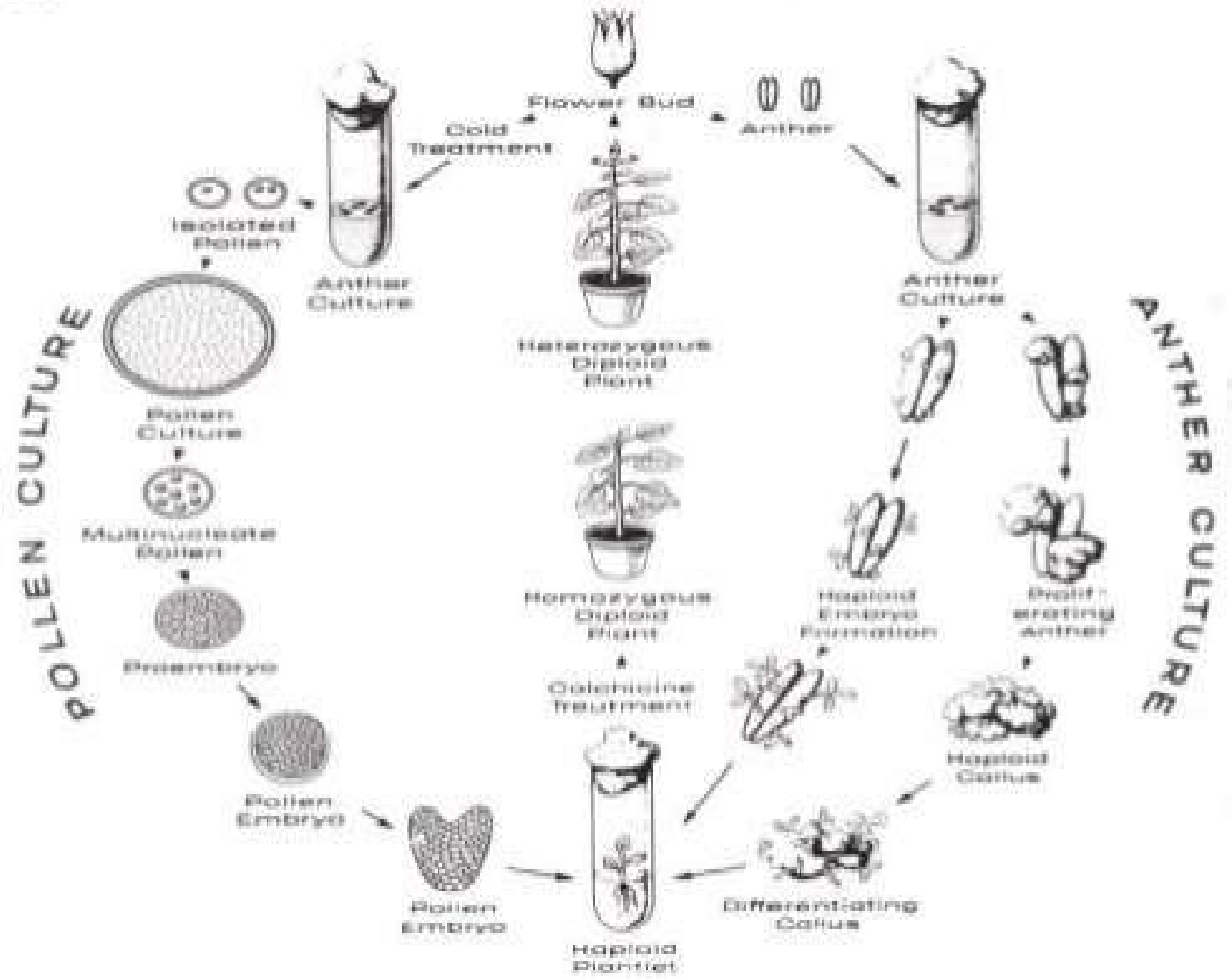


Fig : anther and pollen culture

Ontogeny of androgenic haploids

- INDUCTION –

- For most species a suitable stage for the induction of androgenesis lies between just before to just after pollen mitosis.
- A variety of stresses applied during the labile developmental period of the pollen grain can mask the gametophytic programs.

- And induce the expression of sporophyte specific genes and thus, induce the grains to switchover from gametophytic mode to sporophytic mode of development.
- Treatments such as temperatureshocks (high or low).
- High osmolarity and starving the grains of sugar or other nutrients are required to induce or promote the induction of androgenesis.

- The cultured microspores mainly follow fourdistinct pathways during the initial stages of in vitro androgenesis.
- PATHWAY 1-
- The microspores divide by anequal division, and the two identical daughter cells contribute to the sporophytic development.
- In this pathway distinct vegetative and generative cells are not formed.

- PATHWAY 2 –
- The uninucleate microspores divide by a normal unequal division and thesporophytes arises through further divisions in the vegetative cell.
- PATHWAY 3 –
- The pollen embryos are predominantly formed from the generative cellalone.
- In such cases the vegetative cell either does not divide at all or does so only to a limited extent.

- PATHWAY 4 –
- As in pathway 2, vegetative and generative cells are formed but in this case both the cells divide further and participate in the development of the sporophyte.

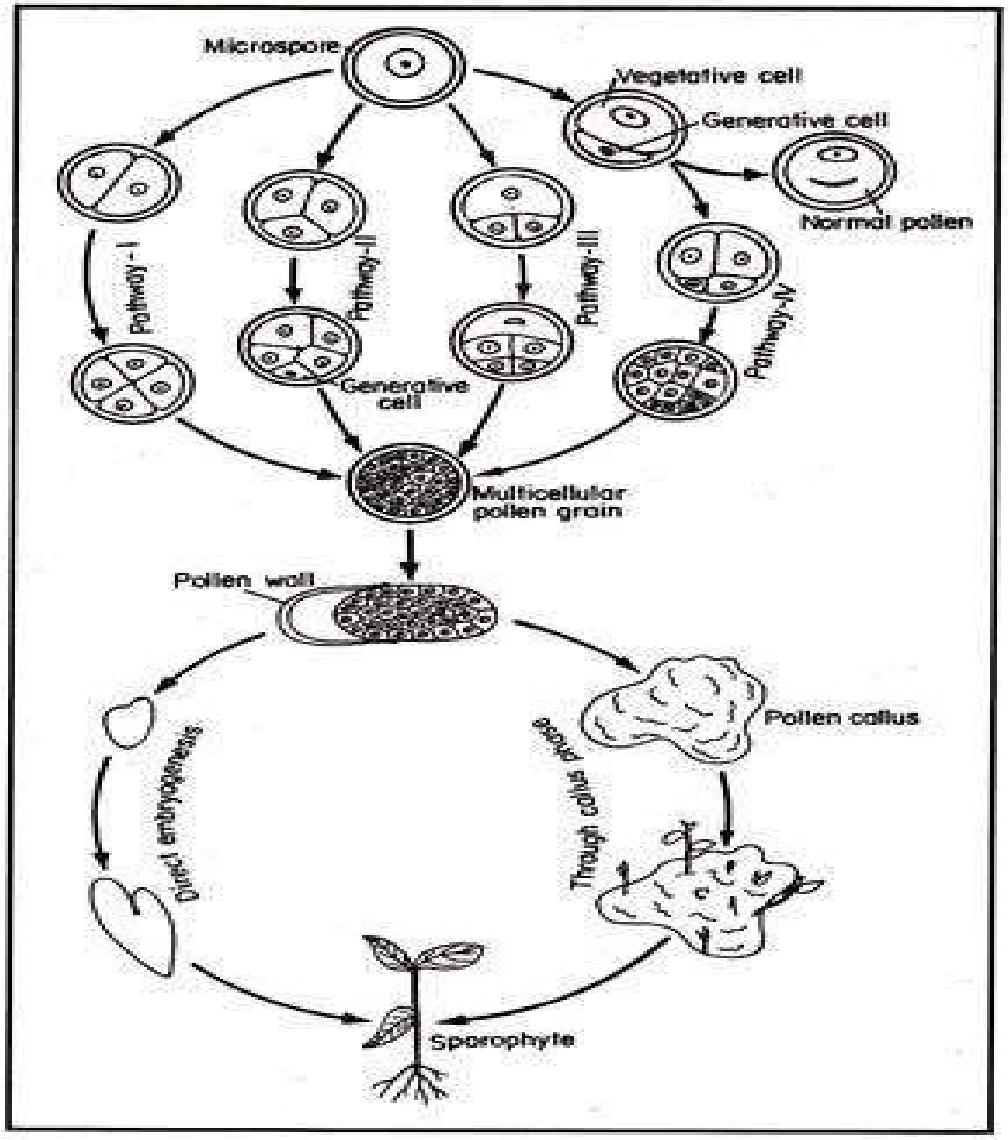


fig : four distinct pathways of in vitro androgenesis

- LATER DEVELOPMENT-
- Irrespective of the early pattern of divisions, the responsive pollen grains finally become multicellular and burst open to release the tissue.
- This cellular mass gradually assumes the form of a globular embryo and undergoes the normal stages of post-globular embryogeny.

Gynogenesis

- haploid plants can bedeveloped from ovary or ovule cultures. It is possible to trigger female gametophytes (megaspores) of angiosperms to develop into asporophyte.
- The plants so produced are referred to as gynogenic haploids.
- Gynogenic haploids mostly arise from unfertilized egg cell (parthenogenesis), as observed in barley.

- Gynogenic haploids were first developed by SAN NOEM in 1976.
- EXPLANT –
- Young flowers, ovaries or ovules have been used as the explant to produce gynogenic haploids.
- Generally, ovules attached to the placenta respond better than isolated ovules.
- The explant cultured at nearly mature embryo sac stage gives the best result.

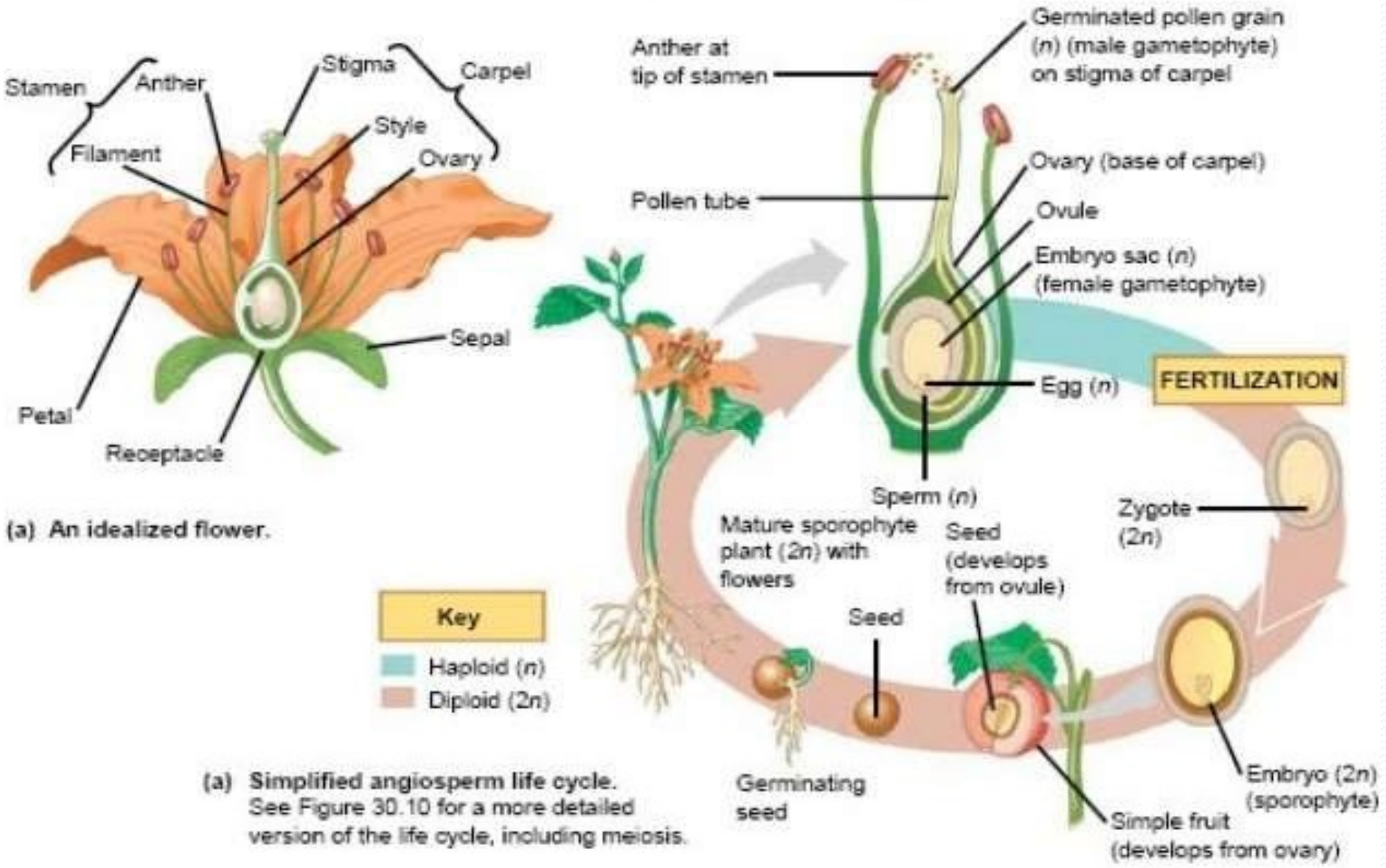
- PRETREATMENT –
- A beneficial role of cold treatment on gynogenesis has been reported. Temperature shock (both low 4-5 °c to high 30-35 °c) for 24-48 hrs.
- CULTURE MEDIUM –
- The most widely used basal medium in these studies happens to be N6 and MS.
- Generally, sucrose is used at higher levels 2-12 %.

PROCESS OF DEVELOPMENT –

- After removal of calyx, corolla and stamens the ovaries are subjected to surface sterilization.
- The ovary, with a cut end at the distal part of pedicel, is inserted in the solid culture medium.
- The ovaries are placed on a filter paper or allowed to float over the medium with pedicel inserted through filter paper.
- Production of gynogenic haploid is particularly useful in plants with male sterile genotype.

- The production of haploid via gynogenesis is more tedious and less efficient.

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.

Factors affecting androgenesis

1. Physiological status of the donor plants:

The age of the donor plants and the environment conditions under which it has been grown significantly affects the androgenic process.

- Generally, the buds from the first flush of flowers show better response than those born separately.

- 2. Stage of pollen development :
- The pollen grains around the first mitosis is most responsive.
- 3. Genotype :
- Hybrids are more androgenic than their parents.
- 4. Pretreatment of cultured anthers/pollen grains :
- Application of certain physical(temp. shock, centrifugation) and chemical (auxin) treatments to cultured anthers or pollen grains prior to standard culture room conditions has proved essential or promotory for in vitro androgenesis.

- 5. Culture medium :
- Addition of etherel (2-chloroethyl phosphonic acid), sucrose, agar and other nutrients specific to certain genotype found to increase thesuccess rate of androgenesis.
- 6. Affect of light :
- Isolated pollen cultures are more sensitive to light than another culture.

Applications of androgenesis

- Shortening of breeding cycle.
- Gameto clonal variations : besides yielding haploids, in vitro androgenesis provides aunique opportunity to screen the gametophytic variations, caused by recombination and segregation during meiosis, at the sporophytic level.
- Induction of mutation.
- Genetic transformation : production of disease resistance plants.

limitations

- low yield only 5-8% of total pollengrains.
- Conversion of pollen embryos intoplants.
- Albinism in cereals.
- Instability of genetic material during androgenesis.

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