PRE - FERTILISATION: STRUCTURES AND EVENTS

Even before you see a flower on a plant, the plant has already decided it's time to flower. Lots of changes happen in the plant's hormones and structure before the actual flowering begins. The shoot's tip turns into a reproductive center, which then grows into a structure called inflorescence axis. On this axis, tiny buds called floral primordia appear. These primordia turn into buds and eventually become flowers. In the flowers, two important parts called androecium and gynoecium start to form and develop.

Stamen, Microsporangium, Pollen Grain

A typical stamen consists of two parts:

1. **Anther**
   It is terminal bilobed structure.
2. **Filament**
   It's a long and thin stem. One end stays connected to the base of the flower or the petal.

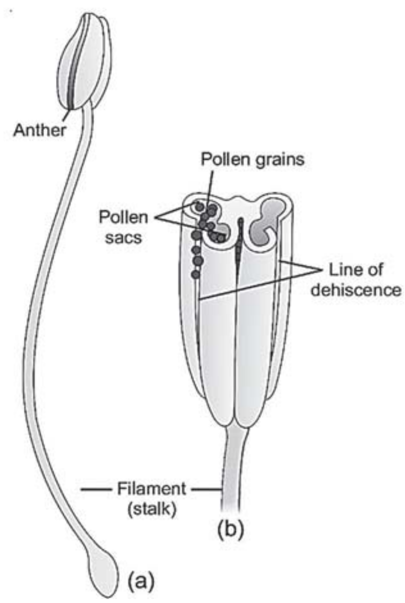


Fig.: (a) A typical stamen, (b) Three-dimensional cut section of an anther

Structure of Typical Anther :

- A usual anther has two parts. There's a deep groove in the middle that separates the two parts, and they stick together with a band of sterile tissue called connective.
- Each anther is like a four-sided shape with four small pollen-making parts, two in each part. So, a fully grown anther has four of these pollen-making parts, making it tetrasporangiate.
- The pollen-making parts, called microsporangia, create sacs that fill up with pollen grains as they mature.

Structure of Microsporangium

In a cross-section view, a regular pollen-making part looks almost round. It's made up of a group of similar cells called primary sporogenous cells, and they're surrounded by the wall of the pollen-making part. These primary cells turn into mother cells, which then produce tiny spores.

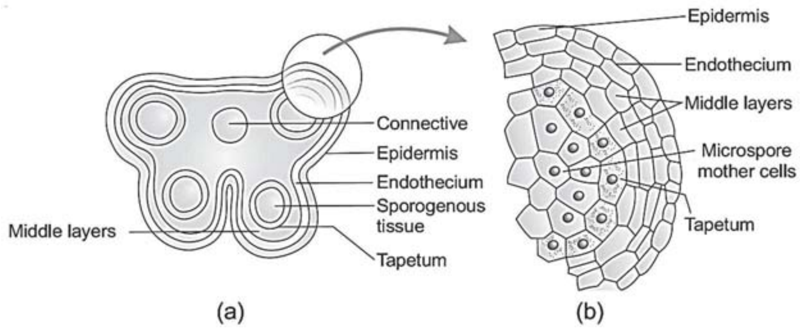


Fig. : (a) T.S. of a young anther, **(b)** Enlarged view of one microsporangium

Anther Wall Layers:

Anther wall consists of following layers:

(i) Epidermis

The outermost layer is like a single protective sheet. In Arceuthobium, the outer layer, called the exothecium, gets some fibrous thickenings to add extra protection.

(ii) Endothecium

The cells in this layer have fibrous bands made of cellulose. These bands come from the inner wall and play a role in the anther opening up because they can absorb moisture. However, in water plants like Hydrocharitaceae, these fibrous bands are not present.

(iii) Middle layer

The cells in this layer don't last long, and there are usually 1 to 3 layers of them. As they mature, these cells break down and disappear.

(iv) Tapetum

This layer is found at the very center of the wall around the pollen-producing tissue in a flower. The tapetal cells in this layer provide nutrients to the growing pollen grains. These cells have a thick liquid inside and typically contain more than one center. They have multiple sets of chromosomes, and the amount of genetic material in their cells increases.

Sporogenous Tissue

In the early stages of the flower's development, there's a group of closely packed and similar cells known as sporogenous tissue right at the center of each small pollen-producing unit called microsporangium.

Microsporogenesis

The process where single-cell microspores with half the usual genetic material are formed from a special cell called microspore mother cell inside the pollen sac is called microsporogenesis. These tiny microspores, originating from a single cell, arrange themselves in groups of four called microspore tetrads.

As the flower's anthers mature and lose water, these microspores separate from each other and grow into pollen grains. Each microsporangium produces thousands of these microspores or pollen grains, and they are released when the anther opens up.

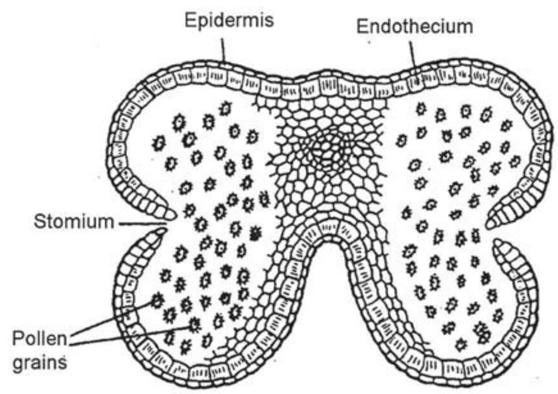


Fig. : A mature dehisced anther

Pollen Grain

Pollen grains serve as the male reproductive cells.

- They are generally round structures, measuring around 25-50 micrometers in diameter.
- The outer wall of a pollen grain is known as sporoderm, consisting of two layers: exine and intine.

a. **Exine**

This is the tough outer layer composed of sporopollenin, one of the strongest organic materials known. It can withstand high temperatures, strong acids, and alkalis. No known enzyme can break down sporopollenin.

- This feature also contributes to the preservation of pollen grains as fossils because sporopollenin makes them durable.
- Its hardness provides protection to pollen grains in challenging environments when they are carried by living or non-living agents.
- The exine displays interesting patterns and designs, which are important for classifying different species.
- It has distinct openings called germ pores where sporopollenin is not present.

b. **Intine**

This is the inner layer, thin and smooth, and is composed of cellulose and pectin.

c. **The cytoplasm** inside a pollen grain is enclosed by the plasma membrane.

Pollen Allergy

Pollen from various plants, especially those that rely on wind for pollination (anemophilous plants), can cause strong allergic reactions and respiratory issues in some individuals, often leading to persistent conditions like asthma and bronchitis.

Examples of plants causing pollen allergy:

(i) Parthenium/carrot grass (introduced to India accidentally through imported wheat).
(ii) Amaranthus
(iii) Chenopodium

Pollen Products

Pollen grains are packed with nutrients. Some claim that consuming pollen can enhance the performance of athletes and racehorses. In recent times, using pollen tablets as dietary supplements has become a trend. In Western countries, a variety of pollen products in the form of tablets and syrups are readily available in the market.

Fig. : Pollen products

Pollen Viability

- The time during which pollen grains can still grow after reaching the stigma is known as pollen viability.
- This duration can vary a lot and is influenced by the current temperature and humidity.

Pollen viability periods in plants:

1. 1.30 minutes ($\frac{1}{2}$ hour) Rice and Wheat (cereals)
2. Several months - Leguminosae, Rosaceae and Solanaceae

Pollen Banks

Keeping pollen grains stored in liquid nitrogen (-196°C) for many years, with the intention of using them in future plant breeding efforts, is referred to as cryopreservation. These facilities where pollen is stored are known as pollen banks.

The pollen grain, also known as a microspore, undergoes a process where it divides into two cells:

Vegetative cell

This is the larger cell with plenty of stored food and a large, irregularly shaped nucleus.

Generative cell

A smaller cell that hovers in the cytoplasm of the vegetative cell. It has a spindle shape, dense cytoplasm, and its own nucleus.

In 60 percent of flowering plants (angiosperms), pollination or the release of pollen grains occurs when they are at the two-celled stage. In the remaining plants, the generative cell undergoes division to create two male gametes, and pollen is released at the three-celled stage. These male gametes are not capable of moving and have an amoeboid shape. They are slightly different in size, and when the pollen is at the three-celled stage, it is referred to as three-celled pollen or mature male gametophyte.

The Pistil, Megasporangium (Ovule) And Embryo Sac

1. **Gynoecium**

This is the female part of the flower responsible for reproduction. It can either have a single pistil (monocarpellary) or multiple pistils (multicarpellary).

A pistil with multiple carpels can be of two types:

a) **Apocarpous -** Carpels are separate from each other, for example, in Michelia.

b) **Syncarpous -** Carpels are fused together, for example, in Papaver.

The pistil has three main parts:

i. **Stigma:** Acts as a landing spot for pollen grains after pollination.

ii. **Style:** The slim, elongated part beneath the stigma.

iii. **Ovary:** The swollen base of the pistil.

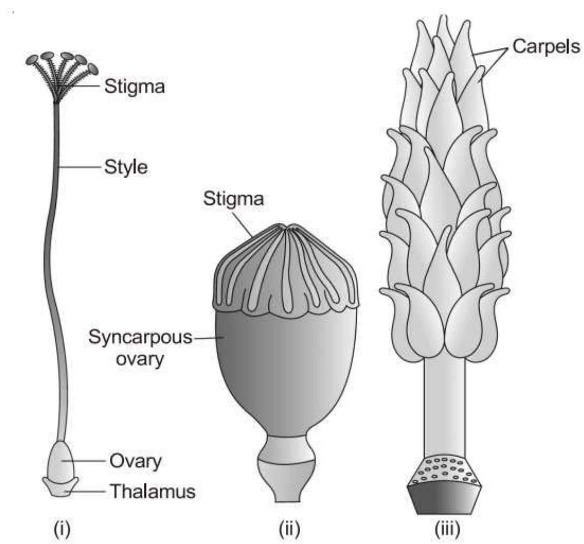


Fig.: (i) A dissected flower of Hibiscus showing pistil, (ii) Multicarpellary, syncarpous pistil of Papaver, (iii) A multicarpellary, apocarpous gynoecium of Michelia

The ovary contains a space called the ovarian cavity, which can have one or more chambers known as locules. Inside the ovarian cavity, you'll find the placenta. From the placenta, there are structures called megasporangia, often referred to as ovules. Depending on the plant, an ovary can have a single ovule, as seen in wheat, rice, mango, or multiple ovules, as in papaya, watermelon, and orchids.

2. **Ovule (Integumented indehiscent megasporangium)**

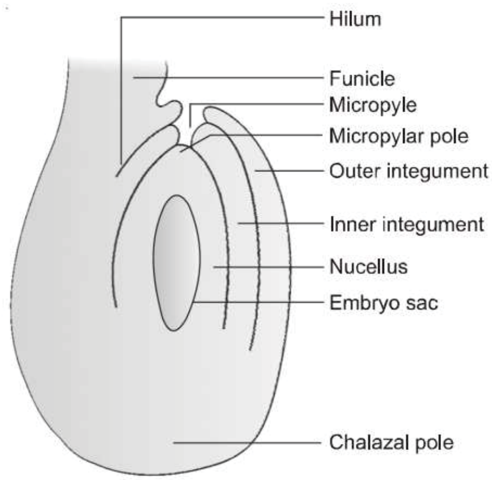


Fig. : A typical anatropous ovule

The ovule's structure can be examined in the following categories:

(a) **Funicle**
This is the stem-like part of the ovule that keeps it attached to the placenta.

(b) **Hilum**
The point where the ovule and funicle connect, or where the funicle is attached to the body of the ovule.

(c) **Integument**
One or more protective layers that surround the ovule.

(d) **Micropyle**
A pore or passage at the tip of the ovule where there is no integument.

(e) **Chalaza**
Found opposite to the micropylar end, representing the base of the ovule.

(f) **Nucellus**
The parenchymatous (soft tissue) mass enclosed by the integuments, forming the main part of the ovule. Depending on its development, ovules are of two types:

(i) **Crassinucellate ovule**
Well-developed nucellus, seen in polypetalae.

(ii) **Tenuinucellate ovule**
Poorly developed nucellus, found in gamopetalae.

(g) **Embryo sac**
Also known as the female gametophyte, it is situated in the nucellus. Typically, an ovule has a single embryo sac formed from a megaspore.

There are six types of ovules based on the arrangement of micropyle, ovule body, and funicle, indicating the degree of curvature:

i. **Orthotropous**
The micropyle, chalaza, and funicle form a straight line. This is the most basic type of ovule, seen in plants like Piper, Polygonum, and Cycas.

ii. **Anatropous**
The ovule turns at a 180° angle, making it an inverted ovule. The micropyle is close to the hilum or at the side of the hilum. This is the most common type, found in 82% of angiosperm families.

iii. **Hemianatropous**

The ovule turns at a 90° angle, or the body of the ovule is at a right angle to the funicle. This type is seen in plants like Ranunculus.

iv. **Campylotropous**

The ovule is curved more or less at a right angle to the funicle, with the micropylar end bending down slightly. Examples include plants in the Leguminosae and Cruciferae families.

v. **Amphitropous**

Both the ovule and the embryo sac are curved like a horseshoe, observed in plants like Lemna, Poppy, and Alisma.

vi. **Circinotropous**

The ovule turns at an angle greater than 360°, causing the funicle to coil around the ovule. This type is found in plants like Opuntia (Cactaceae) and Plumbaginaceae.

Fig. : Types of Ovule: **a.** Orthotropous **b.** Anatropous **c.** Hemianatropous **d.** Campylotropous **e.** Amphitropous, **f.** Circinotropous

3. **Megasporogenesis**

Definition

Megasporogenesis is the process where megaspores are formed from a special cell called the megaspore mother cell (MMC).

- Typically, in ovules, there is one megaspore mother cell (MMC) located in the micropylar region of the nucellus.
- The MMC is a large cell with a lot of material inside and a noticeable nucleus.
- The MMC undergoes a type of cell division called meiosis, resulting in a linear group of four haploid megaspores. Among these, one stays active at the chalazal end, while the other three break down at the micropylar end.

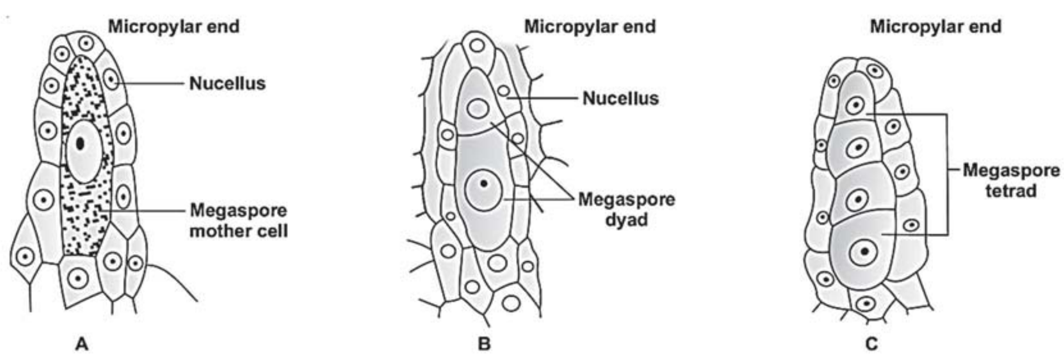


Fig. : (A) Large megaspore mother cell, **(B)** A dyad of megaspore, **(C)** A tetrad of megaspore

Significance of Meiosis in Megaspore Mother Cell

Meiosis is essential to make sure that a single-cell female reproductive unit becomes haploid before fertilization occurs.

Female Gametophyte or Embryo sac

P. Maheshwari categorized the embryo sac based on the number of megaspore nuclei involved in its into the following types:

a. **Monosporic embryo sac:** The creation of an embryo sac from a single megaspore is known as monosporic development, observed in plants like Polygonum and Oenothera.
b. **Bisporic embryo sac:** Two megaspore nuclei participate in the development of the embryo sac, seen in plants like Allium and Endymion.
c. **Tetrasporic embryo sac:** All four megaspore nuclei contribute to the formation of the embryo sac, found in plants like Adoxa, Plumbago, Drusa, Fritillaria, Paenaea, Plumbagella, and Peperomia.

Development of Monosporic Embryo sac (Polygonum type)

a. In most angiosperms, one of the megaspores becomes functional while the other three degenerate. Only the functional megaspore (n) develops into the female gametophyte. This process is called monosporic development.
b. The Polygonum type of embryo sac is present in 80% of flowering plants. Strasburger studied this development in Polygonum. The nucleus of the chalazal functional megaspore (4th from the micropyle) undergoes mitotic division to form two nuclei that move to opposite poles, creating the 2-nucleate embryo sac. Two more consecutive mitotic nuclear divisions lead to the formation of the 4-nucleate and later the 8-nucleate stages of the embryo sac. One nucleus from each pole moves to the middle, forming polar nuclei. These mitotic divisions are strictly free nuclear, meaning cell wall formation doesn't immediately follow nuclear divisions. At this stage, the following changes occur:
   (i) Three of the nuclei (n) organize into cells at the micropylar end, forming the egg apparatus. One is the egg cell (n), and two are synergids (n).
   (ii) Three nuclei organize into antipodal cells (n) at the chalazal end.
   (iii) Two nuclei in the center are called polar nuclei (n).

   This results in a 7-celled and 8-nucleated embryo sac.

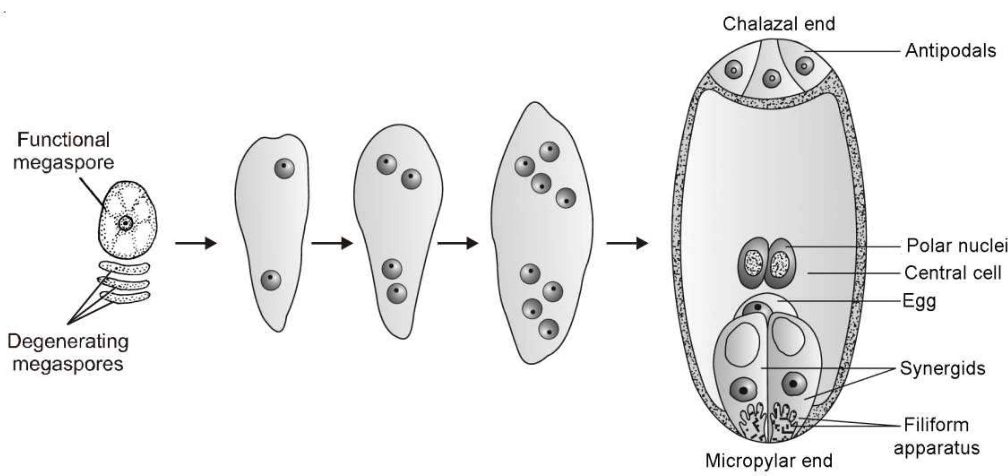


Fig. : Development of female gametophyte (*Polygonum* type)

Organization of Embryo Sac

a. **Synergids or helper cells or co-operative cells:** These cells typically have a nucleus near the micropyle and a vacuole at the chalazal end. Detailed studies using electron microscopy show that, as they mature, synergids lack a cell wall on their chalazal side. They are identified by the presence of a filiform apparatus at the micropylar tip, shaped like finger-like projections. Each projection consists of a core of microfibrils enclosed in a sheath. Often, one synergid starts to break down right after pollination. Synergids likely release some chemical substance that guides the growth of the pollen tube inside the embryo sac.

b. **Egg:** The egg has cytoplasmic polarity opposite to the synergid, and its wall is thicker at the micropylar end. Typically, the egg contains a vacuole near the micropyle and a nucleus at the chalazal end. Plasmodesmata connections exist between the egg and synergids.

c. **Antipodals or vegetative cells:** These are the vegetative cells of the embryo sac. In most plants, there are three antipodal cells.

d. **Central Cell:** This is the largest cell in the embryo sac. Initially, it contains two polar nuclei that fuse just before fertilization, forming a secondary nucleus or definitive nucleus (2n).

Pollination

The transfer of pollen grains shed from the anther to the stigma of pistil is called pollination.
Pollination is of two types.

Kinds of Pollination :

Depending on the source of pollen, pollination can be divided into three types.

(A) **Autogamy**
In this type, pollination is achieved within the same flower. Transfer of pollen grains from the anther to the stigma of the same flower.

Contrivances for Autogamy:
Bisexuality or hermaphrodite
When male and female both reproductive part present within flower.
Homogamy
Male and female reproductive parts in bisexual flowers mature at the same time
Flowers are open (chasmogamous).
Eg: Convolvulus, Gardenia, Catharanthus, Mirabilis, sunflower (Fail-safe device).

(i) **Cleistogamy:** Sometimes bisexual flowers remain closed and never open, such flowers are known as cleistogamous
Eg: Commelina benghalensis, Groundnut, Viola and Oxalis.

- In cleistogamous flower, the anthers and stigma lie close to each other. When anthers dehisce in the flower buds, pollen grains come in contact with the stigma to effect pollination.
- Thus, cleistogamous flowers are invariably autogamous as there is no chance of cross-pollen landing on the stigma.
- Cleistogamous flowers produce assured seed-set even in the absence of pollinators.

Note: Commelina benghalensis bears two types of flowers.

(a) **Chasmogamous -** These are open aerial flowers.
(b) **Cleistogamous -** These are subterranean closed flowers.

Such flowers are called chasmocleistogamous flowers. This phenomenon is called Amphicarpy.

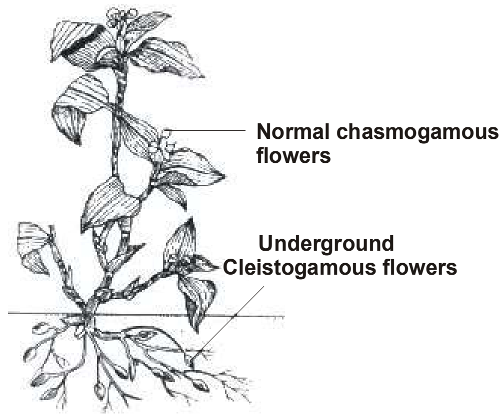


Fig. Chasmocleistogamy in Commelina

Merits:

(i) Flowers do not possess showy petals, presence of scent and nectar to attract pollinators.
(ii) The purity of the generation is maintained.
(iii) Pollen grains are not wasted.

Demerits:

(i) New and healthier varieties are not formed.
(ii) It results in weaker progeny producing weaker seeds and plants.

(B) Geitonogamy

Transfer of pollen grains from the anther to the stigma of another flower of the same plant. Although geitonogamy is functionally cross-pollination involving a pollinating agent, genetically it is similar to autogamy since the pollen grains come from the same plant.

(C) Xenogamy or Cross pollination

Transfer of pollen grains from anther to the stigma of a different plant. This is the only type of pollination which during pollination brings genetically different types of pollen grains to the stigma.

Agents Of Pollination

There are different types of pollinating agents which are as follows-:

1. Zoophily

The zoophilous type of cross-pollination occurs when pollination is carried out with the assistance of animals. Flowers with hairy pollen grains stick to the body of animals, so zoophilous pollination plants
have flowers with hairy pollen grains. When these creatures come into contact with another plant's blossoms, they transfer pollen to the stigma.

2. Entomophily

Cross-pollination is accomplished through the use of a variety of agents. This type of cross-pollination is known as entomophilous cross-pollination because it is pollinated by insects.
Insects pollinate the flowers when they are huge, vividly coloured, emanate a perfume, and generate nectar.

3. **Anemophily**
Wind pollination, also known as anemophily, is pollination carried out with the assistance of the wind. Anemophilous pollination is the term for this type of pollination. Wind pollinating flowers are those that carry out this form of pollination. They are little and light-coloured, with no scent or nectar. The stamens of these flowers are lengthy and protrude from the flower to aid pollination by the wind. The pollens are very light and dry, and the anthers are loosely linked to the filament. The stigmas of the flower are feathery and protrude from the blossom. Take maize, for example.

4. **Hydrophily**
Pollination is known as hydrophilous cross-pollination since it is done with the help of water. It's most common in aquatic plants, where pollen is generated in vast quantities and with a certain weight, causing them to float below the water's surface.
The male flower in Vallisneria floats on the water's surface until it comes into contact with the female blooms. Pollinating agents are substances that help plants reproduce.

Examples Of Pollinating Agents

- Many grass plants are pollinated by the wind.
- Pollination is also aided by bats. They're only found in a few bananas (Musa spp.).
- Evening primrose and tobacco plants are both pollinated by moths.

Outbreeding Devices

Self-pollination is common and more likely to happen in the case of hermaphrodite flowers but a successive series of self-pollination affects negatively and causes inbreeding depression. This also results in homozygous genes. Thus plants are adapted to promote cross-pollination. This is known as outbreeding. Factors which encourage cross-pollination are as follows:

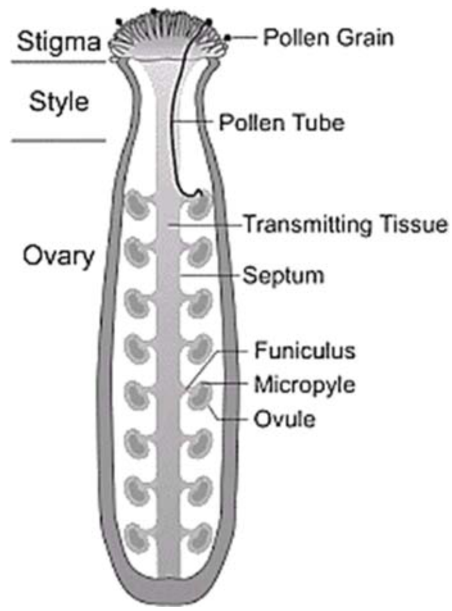
- **Unisexual flower:** If a flower is unisexual i.e., containing only one sex either female or male, cross-pollination is the only choice.
- **Non-Synchronization:** Timing is important for successful self-pollination. Pollen release and receptivity of stigma should happen for successful self-pollination. Sometimes, pollen matures and releases before the stigma is open which leads to loss of pollen vitality or vice-versa. This prevents self-pollination; even though the flower is hermaphrodite.
- **Self-incompatibility:** Incompatibility within a flower (or plant) includes self-sterility; structural barriers. Self-sterility means even though pollination takes place it can't proceed to fertilization due to further pollen growth failure. Structural barriers include height difference between gynoecium and androecium and some structures which hinder the stigma from receiving pollen. These are the more or less genetic mechanism.

Pollen-Pistil Interation

The process of transfer of pollen grains from the anther of a male flower to the stigma of the female flower is known as Pollination. Pollination can be of two types which are cross-pollination and self-pollination. Most of the plants are bisexual or hermaphrodite which promotes self-pollination. Is this self-pollination desirable? What are the factors promoting cross-pollination?

What is pollen-pistil interaction?

The sequence of events, which is carried out from the time of pollen deposition over the stigma and till the entry of the pollen tube inside the ovule is called pollen pistil interaction. The process of pollination during which the transferring of pollen grains takes place from the anther to the pistil is the first step of the pollen-pistil interaction.



Artificial Hybridization

Pollen-stigma compatibility is essential for successful pollination and fertilization. Once compatible pollen is accepted by pistil, events of fertilization proceed, whereas incompatible pollen will be rejected. This interaction where a pistil is capable of recognizing its pollen is the result of long-term pollen-pistil interaction and chemicals released by pollen.

It is very important to understand pollen-pistil interaction in hybridization. It is one of the innovative methods of the crop production improvement program. During artificial hybridization, only the desired pollen grains are introduced to the stigma through pollination. This helps to avoid unwanted pollen rejection and saves time. Also, the plants with the desired characteristics can be grown.

Steps in Artificial Hybridization

Hybridization proceeds in two steps:

- Emasculation
- Bagging

Emasculation

We know hybridization is the method of selective breeding. Thus, anthers have to be removed from a bisexual flower before they release pollen grains. This step of removal of anther using forceps is termed as emasculation. In the case of unisexual flowers, this step is not necessary.

Bagging

Bagging is the protection of emasculated flower from contamination by undesirable pollen grains. Here the flower is masked by a bag, still, the flower attains receptivity. In unisexual flowers, bagging is done before the flowers are open.

Emasculation and bagging ensure that the female flower is completely protected from contamination. Once the flower attains stigma receptivity, the desired pollens are dusted on the stigma. This is resealed for further developments.

Hence, artificial hybridization ensures that the right type of pollen has been transferred to the stigma of the flower. In addition, the chance of fertilization is high. Through this approach, a variety of strains of crops can be developed and it improves the quality of crop with desirable characters.