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MB-06

PLANT DEVELOPMENT AND REPRODUCTION

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PLANT DEVELOPMENT AND REPRODUCTION

MB-06

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Unique Features of Plant Development

Plant development has the following unique features:

1. In plants, there is no germ line at all. Because the germ line is not set aside in plants, germ cells can arise from vegetative cells that have undergone extensive divisions.

2. The haploid phase dominates the lifecycle in some lower plants, but it plays a lesser role in the life cycles of higher plants. The gametophytes, the embryo sac and pollen grains are haploid structures in higher plants. Gametophytes are small and multicellular.

3. In higher plants, embryos do not contain any of the organs found in the adult plant. Plant embryos are composed of organs that make up the embryo and seedling, but not the mature plant.

4. Plant cells and tissues do not migrate in embryonic development because plant cells have cell walls that cement them in place. As a result, the division dictates the development of plant form and expansion of otherwise immobilized cells.

5. Plants have the remarkable capacity to regenerate from vegetative parts. Thus, they are totiopotent in nature.

6. Plants have fewer cell types and produce only certain organs, not a variety of organs.

7. Cytoplasmic bridges called plasmodesmata interconnect plant cells.

Differences Between Plant and Animal Development

There are many differences between plant and animal development. Some of them have been discussed below:

Germ Line Development

In animals, germ and somatic cell lines diverge very early in embryonic development. In mammals, the germ cell line in the female is set aside and germ cells undergo very few divisions. In males also, the germ line is set aside early; however, germ-line cells undergo many more divisions in the male, and like somatic cells, they are more subject to mutations.

In plants, it is argued that there is no germ line at all. At least, the germ line is not set-aside during embryogenesis. In plants, the cell layer from which the germ line is derived (L2) is not exclusively reserved for germ cell production; rather, it gives rise to too much of the vegetative part of the plant as well as germ cells. Thus in plants, germ cells can arise from somatic cells that have undergone extensive divisions. The genetic makeup of germ cells derived from various pats of a plant might differ due to the differential accumulation of mutations.

Role of the Gametophyte

The haploid phase dominates the life cycle in some lower plants, but it plays a lesser role in the life cycles of higher plants. In higher plants, the gametophytes, the embryo sac and pollen grains, are haploid structures. Although gametophytes are small, they are multicellular, and their development must therefore be accounted for in the life cycle of plants.

In higher animals, all that remains of the haploid phase are gametes.

Post Embryonic Development

In higher animals, most adult organs are formed during embryogenesis. For example, the human embryo appears almost as a miniature version of the adult.

In higher plants, embryos do not contain any of the organs found in that adult plant. Plant embryos are composed of organs that make up embryo and seedling, but not the mature plant. Plant organs are formed from shoot and root meristems during post embryonic development.

The body parts of animals are predetermined by embryonic development, while those of plants have greater plasticity in adapting to different environmental pressures.

Cell Movements and the Planes of Cell Division

Plant cells have cell walls that cement them in place. Plant cells and tissues do not migrate in embryonic development as they do in animals.

Regeneration and Totipotency

Plants have the remarkable capacity to regenerate from vegetative parts. Many terminally differentiated plant organs, tissues, and cells retain their capacity to regenerate. For example, a stem segment broken off from a prickly pear cactus will regenerate a new plant. In tissue culture, plants can be regenerated from undifferentiated cells. It occurs by two routes: by organogenesis or by somatic embryogenesis.

In higher animals, embryogenesis requires genetic input from both parents because alleles are imprinted by parental source. Imprinting serves to promote sexual reproduction in higher animals. In plants, imprinting also acts as sexual reproduction checkpoint, but it has a greater impact on endosperm than embryo development.

Variety of Plant Organs and Cell Types

Higher animals have a greater variety of organs and cell types than do higher plants. Plants, however, produce certain organs, like leaves, in great numbers and variation. Embryos of dicots have only four organs: epicotyls, cotyledons, hypocotyls and the radicle. Mature plants consist of only three vegetative organs- sepals, petals, stamens and pistils. Because of having fewer, different organs, higher plants have fewer cell types than higher animals. Lyndon has listed only forty cell types in plants, while hundreds of cell types are found in higher animals. In animals, organs often stamp special identity on their cells. In plants, the organs do not always confer special cell-type characters.

Because there are fewer cell types and organs in plants, the intercellular signaling network between different cells and organs is less complicated in plants than it is in animals. Animal cells bristle with receptors for the hundreds of extra cellular signaling molecules and hormones. Far fewer hormones, growth factors, and signaling molecules have been described in plants.

Intercellular Communication

Plant cells are separated by cell walls. Cytoplasmic bridges called plasmodesmata interconnect these. Many small molecules, such as sucrose, flow in the "symplasm" from cell to cell. The vast interconnections between plant cells do not accommodate movement between cells by macromolecules, such as protein and nucleic acids.

Seedling

Seedling stage is more vulnerable than any stage. For survival, the seed must sense suitable conditions to germinate, and the seedling must respond to changing environmental conditions. The seedling is equipped with an array of sensors and mechanisms that allow it to respond to light, gravity, water conditions, and so on. The seedling is a transition form between embryonic and postembryonic development. In seedlings, such as *Arabidopsis* seedling, postembryonic shoot development is initiated by activation of the shoot apical Meristem (SAM) and the appearance of the first true leaves.

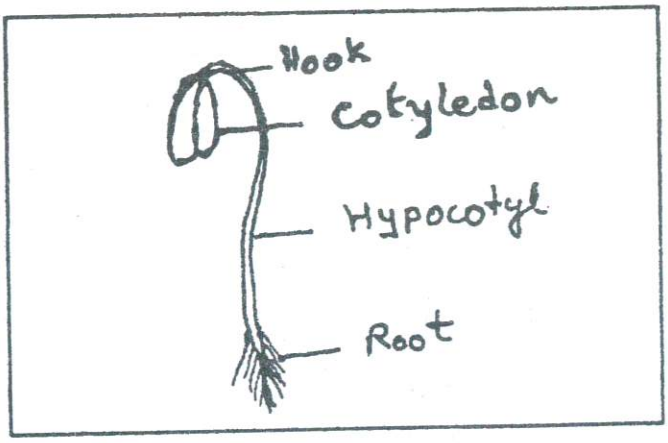


Figure 1.1: Soybean Seedlings Emerging from the Soil

Metabolism of Nucleic Acids, Proteins and Mobilization of Food Reserves

Seed is defined as a ripened ovule consisting of an embryo and its coats. Botanically, a seed consists of an embryo, variable amounts of endosperm and the seed coat, or testa. The normal seed contains materials that it utilizes during the process of its germination. These substances are frequently found in the endosperm. Thus, endosperm may contain variety of stored materials such as- starch, oils, proteins etc. In some plants, however, the reserve food material is present in cotyledons.

Seed Germination

Germination represents a dynamic period in the life cycle of plants as a seed makes the transition from a metabolically quiescent to an active and growing entity. Seed germination represents the termination of a developmental phase and the commencement of a growth phase. Germination is controlled by dormancy and various environmental factors. The environmental conditions necessary are access to water and air; a suitable range of temperatures; freedom from high concentrations of inorganic salts, poisons and inhibitors, and for some seeds, exposure to light.

Thus, germination is a separate developmental phase in the life cycle of the higher plants. This phase is preceded by embryogenesis and seed development, which is followed by seedling growth, and development. The term 'germination' includes those processes beginning with the imbibitions of water by a dry seed and ending when a portion of the

embryo penetrates the seed coat. When dry seeds are placed in water or exposed to humid atmospheres, the first measurable process associated with germination is an increase in water content of the seed and its associated components.

It is the root or radicle that first penetrates the seed coat, but in some plants, the plumule or shoot emerges first.

Morphological and Biochemical Changes Accompanying Seed Germination

There are three successive main phases, which make up the process of germination. The sequence of germination in its simplest form is: water imbibitions, enzyme activation, hydrolysis and catabolism of storage material, initiation of growth, anabolism and formation of new cell cultures, rupture of seed coat, and emergence of the seedling.

Phase. I Water Imbibitions/Catabolic Phase of Germination

It takes place for 4-24 hours. Imbibitions of seed is the first phase. As the seed imbibes water, all the cells in the embryo, cotyledons and endosperm become hydrated, resulting in cell expansion and size increases. Due to adsorption of water, protoplasm changes its form from gel to sol, which is an active form. Cells get expanded. Enzymes get activated. Seed coat becomes soft. Respiratory activities are initiated. Food reserves in the endosperm or cotyledons are mobilized to provide substrates for continuous growth of the embryonic axis. Rapid rise in activation of phytochrome occurs.

On hydration of seed, various enzyme systems are activated, and cellular activity increases. The activation of enzymes following hydration may be from enzymes formed during maturation of the seed or newly synthesized enzymes. Enzymes formed during maturation are of two types:

1. Drying reverses those requiring only hydration to become active, and this activity. Cytochrome reductase and adenylate cyclase are a few examples of these enzymes.
2. Those enzymes requiring the action of a hormone, or other enzymes, to gain activity within minutes to several hours. Newly synthesized enzymes are also of two types:
    a. Those enzymes produced by pre-existing mRNA utilizing a pool of pre-existing amino acids. Activity of these enzymes may occur in 2 to 4 hours.

b. Those enzymes produced from newly synthesized mRNA and utilize amino acids derived from degradation of storage proteins, which begins as early as 6-12 hours after hydration.

Phase II. Enzymatic Degradation of Storage Materials

It occurs within 24-120 hours. The major food reserves in the seeds are starch, fat protein, nucleic acid and phytin. These are utilized during initial phases of germination. This is also a catabolic phase. Here, seed is having heterotrophic nature, because it cannot synthesize its own food.

i) Metabolism of Nucleic Acids:

Mainly, nucleic acids are of two types-DNA and RNA. Their further degradation is shown below-

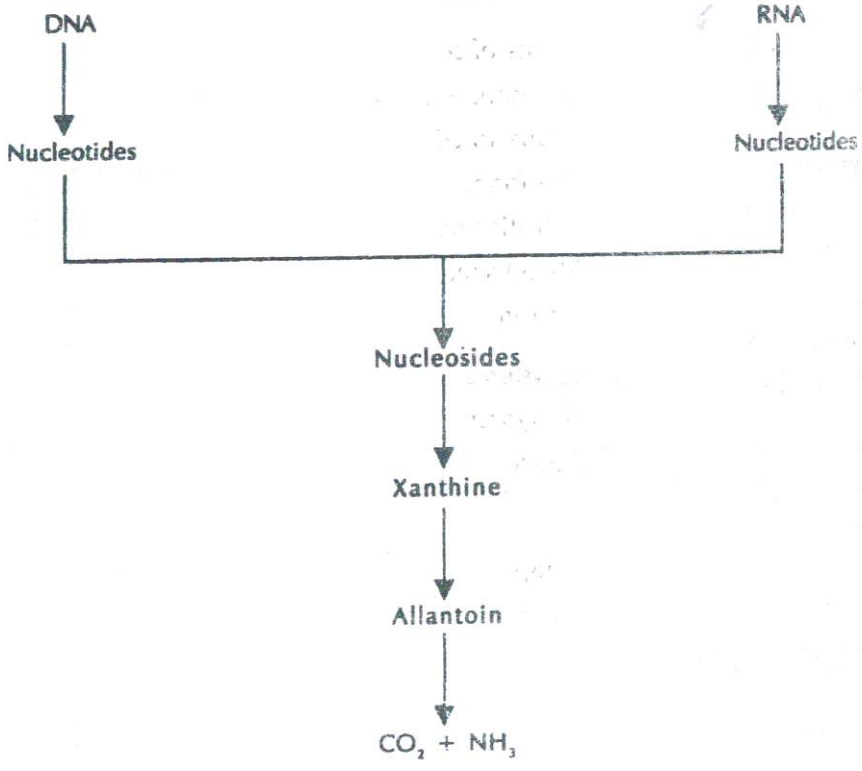


Figure 1.2

NH_3 (Ammonia) then takes part in amino acid synthesis. Nucleotides formed here take part in DNA and RNA synthesis in embryonic axis.

ii) **Metabolism of Proteins:**

Protein catabolism involves protease degradation of insoluble storage proteins. The soluble proteins are further hydrolyzed by peptidases to the component amino acids. The released amino acids are transported to the embryo where they are resynthesized into new cell proteins.

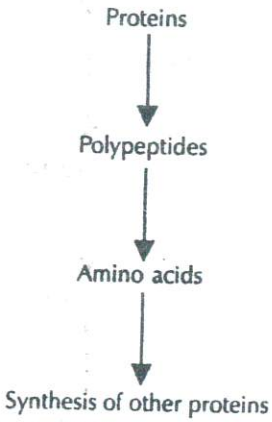


Figure 1.3

Protein synthesis plays an important role in germination, in the growth of the embryonic axis and in the synthesis of the hydrolytic enzymes and the other cellular machinery used for the mobilization of the reserves. Dry seeds contain two classes of mRNA for protein synthesis- residual mRNA, which codes for proteins. They are synthesized during embryogenesis, and stored mRNA, which was synthesized during embryogenesis, but is translated during germination to yield proteins that are required for the germination process.

Dure and Colleagues found in cottonseeds that two enzymes involved in germination are synthesized de novo following water imbibitions. The first enzyme Carboxypeptidase, catalyses the breakdown of proteins to amino acids, whereas the other isocitric acid lyase, is a key enzyme in the glyoxylic acid cycle.

Mobilization of Food Reserves

In the embryo, reserves are stored in virtually all the living cells, and it seems that the enzymes, which are synthesized in the same cells, mobilize them. Mobilization of stored material is the first mass process in the sequence of metabolic events during germination. This consists in such a transformation of reserves that they can be used for energy generation and for building of the new compounds. Sphaerosomes and protein bodies are enclosed in a single membrane envelope. The lipid bodies disappear as a result of hydrolysis of

stored substances, whereas the protein bodies transform into vacuoles. Starch seems to be hydrolyzed by cytoplasmic enzymes and the granules disappear when its hydrolysis is completed.

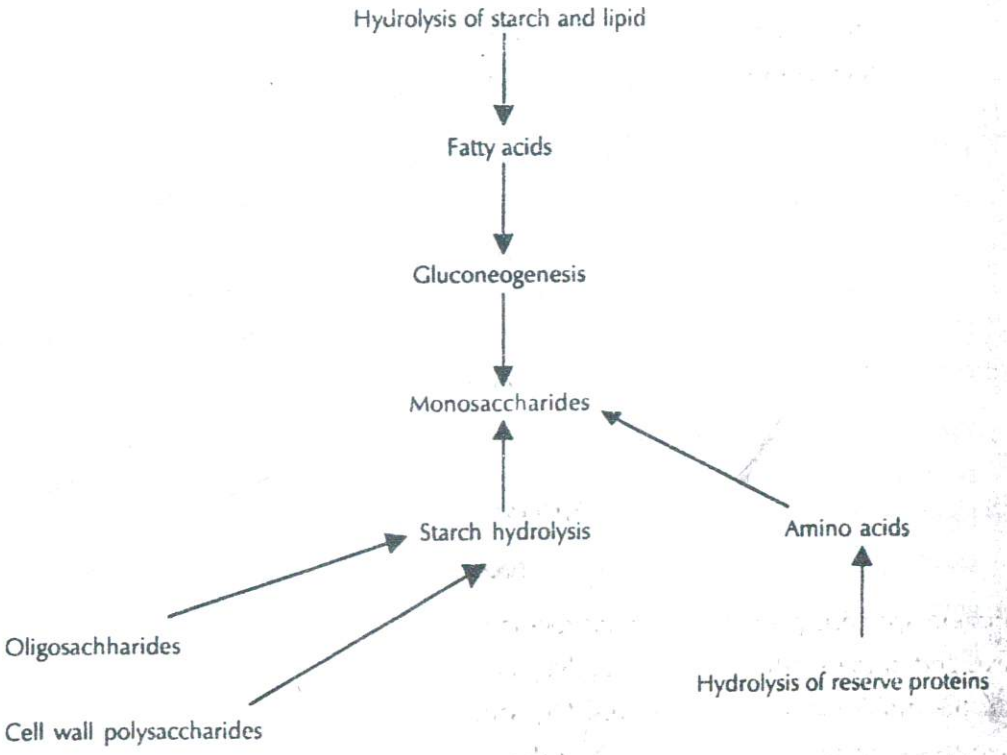


Figure 1.4: Mobilization of Reserve food Materials During the Catabolic Phase of Germination

It can be observed in the endosperm of castor bean (*Ricinus communis*). In this, the main reserve food material is lipids, which is mainly converted into sucrose, the sucrose being absorbed by the cotyledons, after which most was translocated to the embryonic axis. The whole complex mechanism for the conversion of triglycerides to sucrose develops in the endosperm itself, and involves enzyme synthesis and thus lipids get metabolized in this way.

Similarly, the cells of endosperm of cereals and grasses, which contain starch, are dead. The starch is hydrolyzed by á-amylase secreted by aleurone cells.

Regulation of Mobilization of Food Reserves

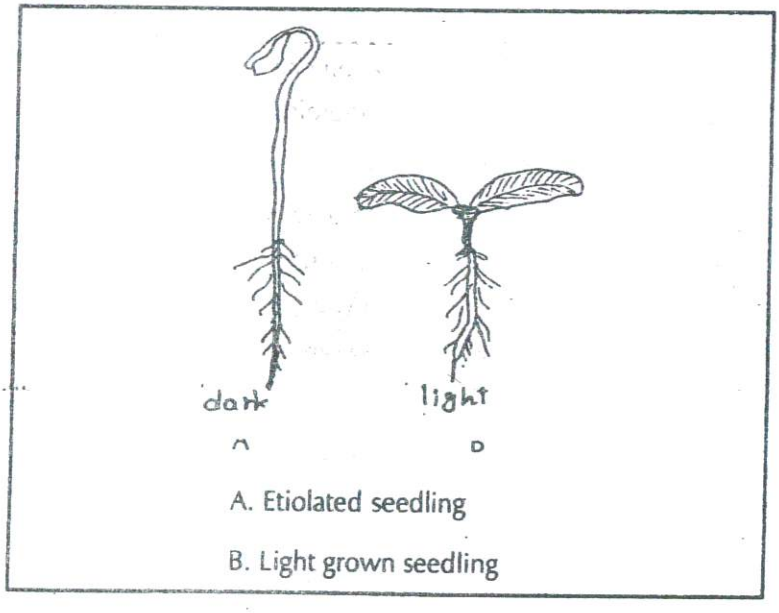
It is clear in many cases that mobilization of food reserves is controlled by the embryonic axis, i.e., the radicle and plumule or where the endosperm is the storage tissue, by the

embryo as a whole. For example, in *Cucumber* seeds, it has been seen that after the germination of an intact seed, the triglycerols stored in the cotyledons rapidly become depleted, but if the axis is removed from a newly imbibed seed, the amount of these stored reserves hardly falls.

Tropisms (Photomorphogenesis)

Light is a major environmental factor influencing seedling development. It is also an important germination signal in some species. Seeds that germinate beneath the surface of the soil undergo a form of development in the dark called skotomorphogenesis (dark development). Seedlings grown in the dark are bleached, lanky and said to be etiolated. In an etiolated seedling of *Arabidopsis*, long hypocotyl (apical hook), and unexpanded, unopened cotyledons are found.

When a seedling emerges to the surface of the soil and is exposed to light, development switches from a program of skotomorphogenesis to photomorphogenesis, and seedlings begin to de-etiolate (i.e., they become green and begin to grow as normal light-grown seedlings). Light-grown seedlings have short hypocotyls, lack an apical hook, and have green cotyledons that are opened and expanded, the SAM is activated, and the first true leaves emerge.



A. Etiolated seedling

B. Light grown seedling

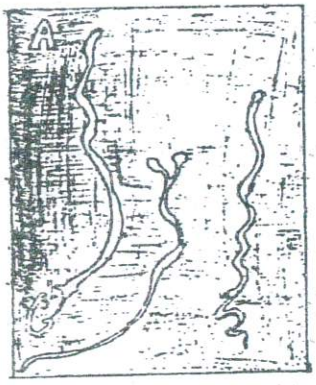
Figure 1.5

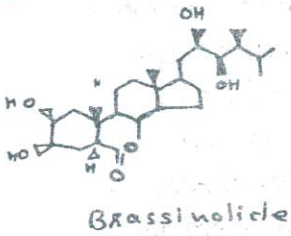
Hormonal Control of Seedling Growth

Mainly, 2 hormones control seedling growth-

1. **Brassinosteriods :**

   Brassinosteroids are required to suppress photomorphogenic development in the dark.





Brassinolide

A. Etiolated (Wild type)
B. De-etiolated
C. Etiolated

In dark, brassinosteroids level is high. So, etiolation occurs. While in light, brassinosteriods level is low. So, de-etiolation occurs. Brassinosteroids play a major role in Arabidopsis development.

The first constitutive photomorphogenic mutant that was reported to involve brassinosteroids was det2. It is a mutant of Det2. It is identified by its photomorphogenic development in the dark (i.e., the inhibition of hypocotyls elongation, the opening of cotyledons, and the precocious development of true leaves in the dark.

If det2 is mutant, then its many characters are similar to det1. In this condition, mutants become dwarf and dark green. Its apical dominance and fertility reduces which is similar to photomorphic effects. Although if externally brassinosteroid hormone is provided, then the plant shows the same character as indicated in the dark. It means Det2 gene is related with synthesis of brassinotseroids. When this gene was cloned and its activity was observed, then its activity was similar to the enzyme related with steroid synthesis. It shows that in the absence of brassinosteroids, photomorphogenesis is not suppressed in the dark. Similarly,

constitutive photomorphogenesis and dwarfism movements are also observed, which code a cytochrome P-450. Structurally, it is similar to steroid hydroxylase enzyme. So it can be concluded that brassinosteroid is related with cell elongation of hypocotyls.

2. **Ethylene :**

Ethylene is the triple response hormone. It shows three activities, which are-

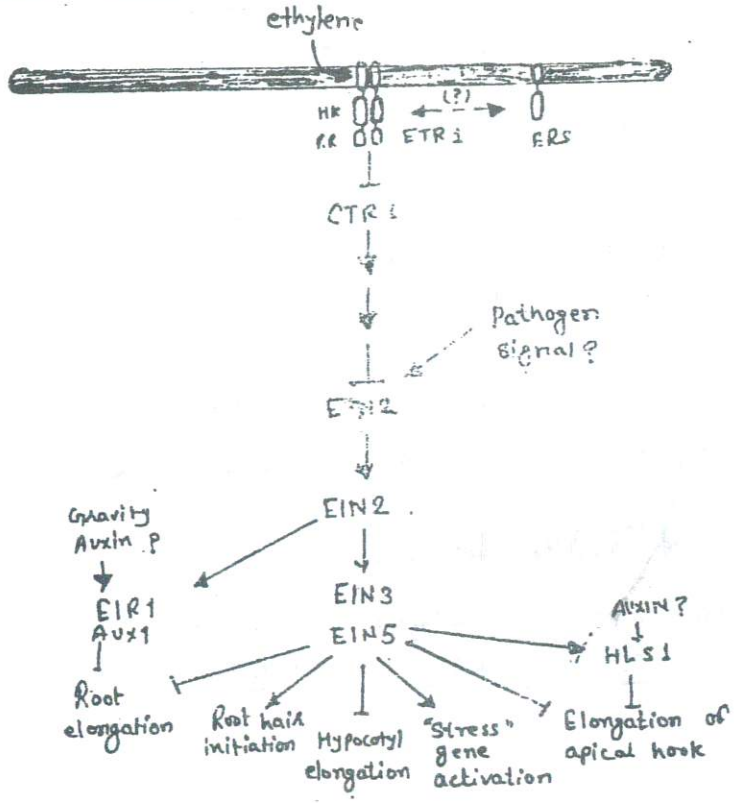
a. Inhibition of stem elongation

b. Increasing radius of stem

c. Absence of normal geotropic responses, which is negatively geotropic.

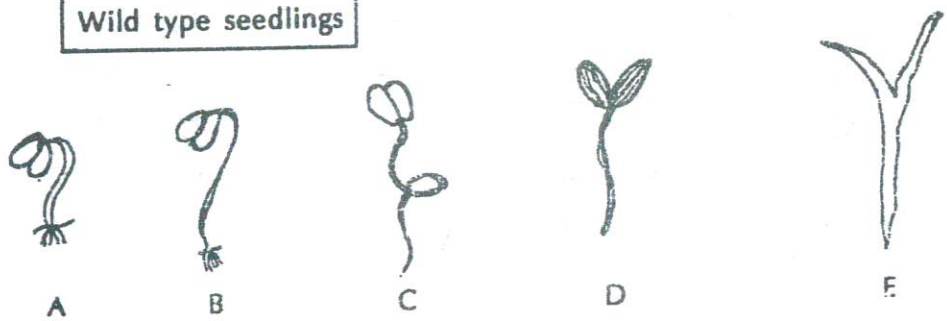
It is also related with coiling of apical hook, which protects SAM. It shows cotyledons in the downward direction, but if ethylene is absent, then this hook is moved towards downward side. Actually, ethylene imbalances the auxin concentration, so unequal growth takes place. When ethylenes over producer mutants were observed, then it shows triple response normally. Similarly, hls1 is another mutant, which shows growth without apical hook. It is grown in the absence of ethylene. If ethylene is supplied, then hook is formed. The same results are observed with ethylene insensitive genes (ein-2). If it is mutant, then it does not show hook formation even in the presence of ethylene. It means this gene is responsible for ethylene sensation.

In *Arabidopsis*, the two-component system is observed for ethylene response. This component is made up of histidine kinase and response regulator proteins. When ethylene binds with this component, then it inhibits a constitutive gene CTR1 (Constitutive Ethylene Response1). It shows downstream activities and it negatively regulates the EIN2. This gene activates other EIN genes, like-EIN3 and EIN5, which activate the expression of many events associated with ethylene action. These genes inhibit root elongation, initiate root hair formation, activate stress genes and inhibit hypocotyls elongation and apical hook elongation.



Model for ethylene action

Wild type seedlings



A. In the presence of ethylene

B. In the absence of ethylene

C. eto1 (Ethylene overproducer1) mutant grown in absence of ethylene

D. hls1 (Hookless1) in the absence of ethylene

E. ein z1(ethylene insensitive gene) mutant grown in the absence of ethylene

Figure 1.7: Effects of Ethylene on Growth of Arabidopsis Wild Type and Triple Response Mutant Seedlings.

Use of Mutants in Understanding Seedling Development

Gene Expression

Photomorphogenesis or de-etiolation has been intensively studied as a developmental process. Photomorphogenic mutants have been isolated and shown to have defects in light perception or light signal transduction. Two very different types of mutants have been found:-

a. Light-insensitive photomorphogenic mutants

b. Mutants that undergo photomorphogenesis in the dark

Light Insensitive Photomorphogenic Mutants

Koorneef and co-workers (1980) isolated a group of light- insensitive, photomorphogenic mutants in *Arabidopsis* called long hypocotyls or hy mutants.

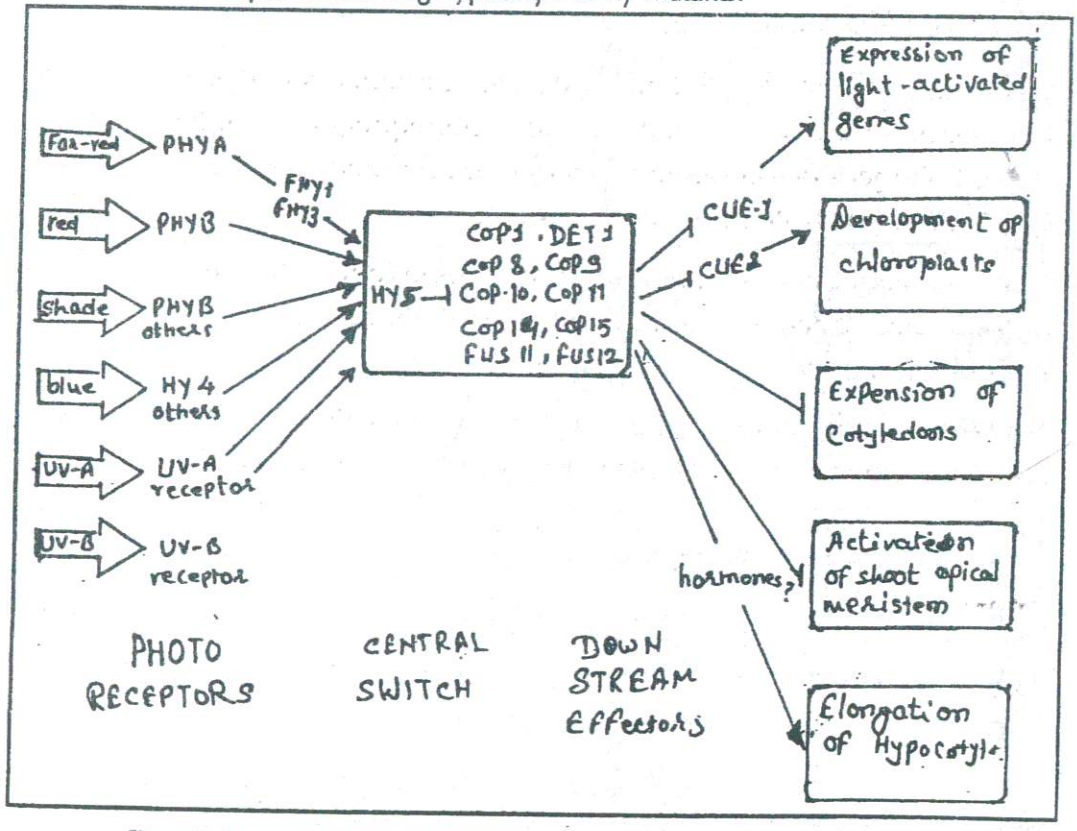


Figure 1.8: Regulatory Pathway for the Control of Photomorphogenesis in Arabidopsis Seedling

In wild type seedlings, hypocotyls elongate rapidly in the dark but slowly in the light. Hy seedlings fail to perceive the light, and their hypocotyls elongate rapidly as though they are growing in the dark. On the other hand, most of the hy mutants have defects in photoreceptors, but not in other steps in the light-signaling pathway. The hy mutants have therefore been key in identifying light receptors in photomorphogenesis.

Different wavelengths of light are perceived by different photoreceptors. Most loss-of-photomorphogenic function mutants (particularly hy or phy mutants) have defects in specific photoreceptor. Light signals from different photoreceptor systems converge on a central processor that involves hy5 , which appears to repress the combined action of cop/det/fus gene products. These products act as negative regulators of photomorphogenesis. Some of the products are known to act as a complex. The pathway involves two negative operator steps. (activation is indicated by an arrow ") while inactivation is represented by ^). Mutants that inactivate COP/DET/FUS genes constitutively activate photomorphogenesis.

The major photoreceptors involved in photomorphogenesis- the phytochromes- are composed of an apoprotein and a light absorbing chromophore. Phytochrome apoproteins are encoded by a family of genes-

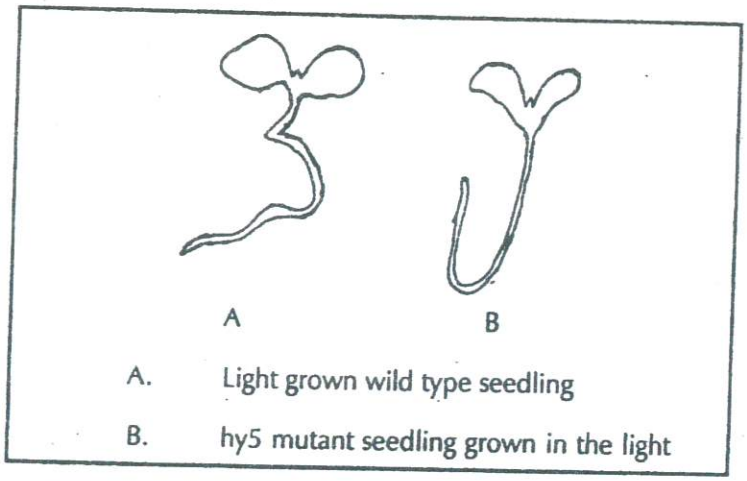
HY1, HY2, HY3, HY4, HY5, HY6 and HY8 genes have been observed. Koornneef isolated a hy3 mutant, who is deficient in PHY B, and photomorphogenesis in hy3 is insensitive to red light. The gene responsible for the mutant has been cloned and found to encode the apoprotein of PHY B. PHY B is a red light acceptor.

Another hy mutant, originally called hy8, is insensitive to far-red light. The gene responsible for this mutant has been cloned and found to encode the apoprotein of PHY A. PHY A is a far-red light acceptor.

Another hy mutant, originally called hy4, is insensitive to blue light, HY4 has also been cloned and found to encode the apoprotein of a putative blue light receptor.

Later on, Chory (1992) proposed that hy1, hy2 and hy6 appear to encode functions involved in the production of the phytochrome chromophore.

Only hy5 does not encode a photoreceptor component, but it does appear to be involved in a step downstream in the photomorphogenic response pathway. HY5 gene, it means, a regulator gene. The hy5 mutants make the plants insensitive to light.



A. Light grown wild type seedling

B. hy5 mutant seedling grown in the light

Figure 1.8: Comparision in Growth of Wild Type and Long Hypocotyl 5 (hy 5)

Constitutive Light Response Mutants- DET and COP Mutants

DET= De-etiolated gene

COP= Constitutive Photomorphogenic gene

Generally it is thought that light drives the photomorphogenic pro- but Chory *et al.* (1989) discovered a set of mutants that undergo photomorpho- nesis in the dark. These are called de-etiolated or det mutants. These have short hypocotyls and open cotyledons in the dark, which are characteristics of seedlings grown in the light. (Det mutants are not green because chlorophyll biosynthesis requires light). DET gene is active in dark, while det mutants are inactive in dark.

Deng and Quail isolated similar mutants, and these were called Constitutive Photomorphogenic (COP) mutants. COP mutants are similar to the DET mutants. COP 1 mutant has short hypocotyls, open and expanded cotyledons and chloroplast like differentiation in cotyledon plastids in the dark. COP and DET mutants are highly pleiotropic.

It is clear that DET and COP genes normally suppress photomorphogenesis in the dark. A pathway of gene action with two negative operators can represent the action of the DET and COP genes. Pathway is shown as in figure below-

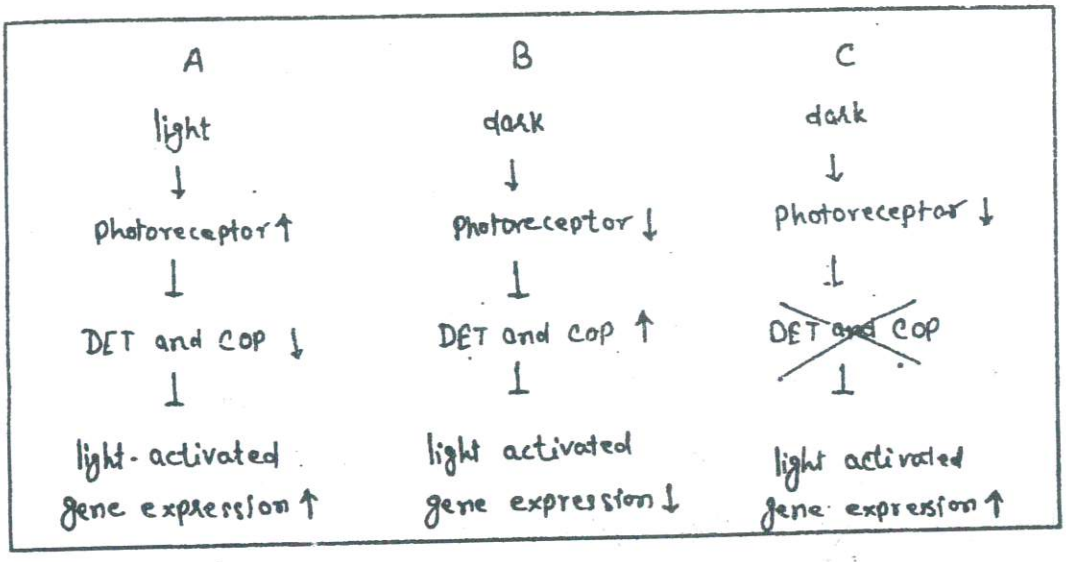


Figure 1.10: Operational Logic of the Photomorphogenesis Pathway

Several of the COP genes have been cloned.

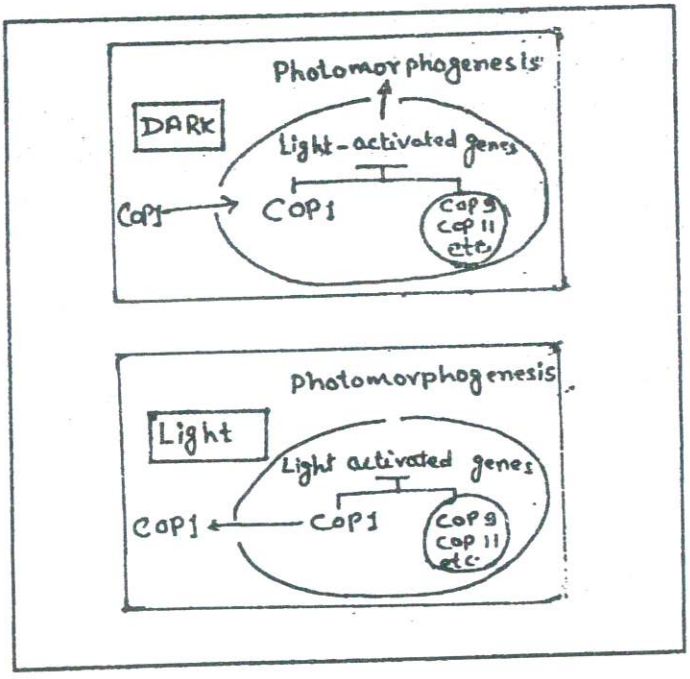


Figure 1. 11: Change in Location in the Dark and Light of COP1 Protein in Arabidopsis Hypocotyl Cells

FUSCA Mutants

Fus mutants are a class of seedling-lethal mutants that accumulate anthocyanins during embryo development. It was found that nearly all of the pleiotropic mutants were allelic to fusca (fus) mutants. For example, fus1 is allelic to cop1, fus2 to det1, and so forth. The allelism between the det, cop and fus mutants in *Arabidopsis* represents an example of the complexity and the overlapping action of genes in seedling development.

The fus mutants accumulate anthocyanin pigments in the cotyledons of developing seedlings (the term fusca means dark purple color). Anthocyanin biosynthesis in plants is tightly regulated by light and other environmental and hormonal influences. This can be shown by the fact that a double fus6 and anthocyanin biosynthesis mutant fails to accumulate anthocyanin, but still suffers from the developmental defects. The pleiotropic character of the fus mutants indicates a defect in some basic mechanism that affects a number of regulated processes.

Downstream Events in Photomorphogenesis

A number of key effector genes are typically used to monitor photomorphogenic development, such as the CAB genes. CAB genes encode the chlorophyll A/B binding proteins, which are the major chlorophyll binding proteins found in chloroplast membranes. Light activates the transcription of CAB genes in etiolated seedlings as part of program of chloroplast development in greening tissue. CAB gene expression has been used to identify mutations in genes encoding trans activating factors that positively regulate effector genes in photomorphogenic development.

CHAPTER 2

SHOOT DEVELOPMENT

LEARNING OBJECTIVES

- Introduction
- Organization of the Shoot Apical Meristem (SAM)
- Cytological and Molecular Analysis of SAM
- Molecular Biology of SAM and Control of Cell Division
- Control of tissue Differentiation, Especially Xylem and Phloem
- Secretory Ducts and Laticifers
- Wood Development in Relation to Environmental Factors

Introduction

Shoot is the aerial portion of the plant consisting of stem, branches, leaves etc. Shoot actually develops from Shoot Apical Meristem (SAM) and Axillary Bud Meristem. Meristems are self-renewing structures. They house stem cells that divide and give rise to the primary shoot, and they produce a variety of lateral organs, such as leaves, branches, tendrils and thorns.

During vegetative growth, SAMs give rise to repeating units of the shoot called phytomers. Successive phytomers are often composed of the same organs, but phytomers differ in internode length, leaf size and shape, axillary bud potential. A typical phytomer consists of

a node to which a leaf is attached, a subtending internode, and an axillary bud at the base of the leaf.

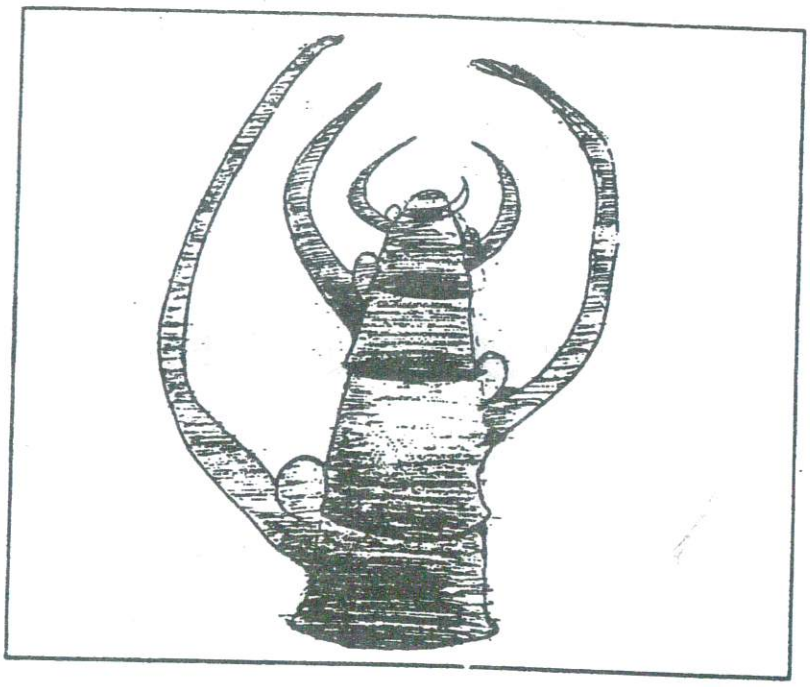


Figure 2.1: Generalized Diagram of Shoot Apex

Plant shoots can be single and unbranching or multiple and highly branched. The SAM of the main shoot is formed embryonically, whereas the others are formed postembryonically.

Organization of the Shoot Apical Meristem (SAM)

The anatomy of the SAM is defined in terms of layers and radial zones.

A. SAM is organized in 3 fundamental cell layers. The outer layer in dicots constitutes the tunica and is composed of L1 and L2, whereas the inner layer or corpus is made up of L3. Tunica cells (L1-L2) undergoes anticlinal cell division, and have a sheet-like appearance, whereas the corpus cells (L3) undergoes periclinal division.

L1 layer forms epidermis.

L2 layer gives rise to subepidermal tissue, the procambium, and part of the ground meristem (the cortex and sometimes part of pith).

L3 gives rise to the rest of the ground meristem and the pith.

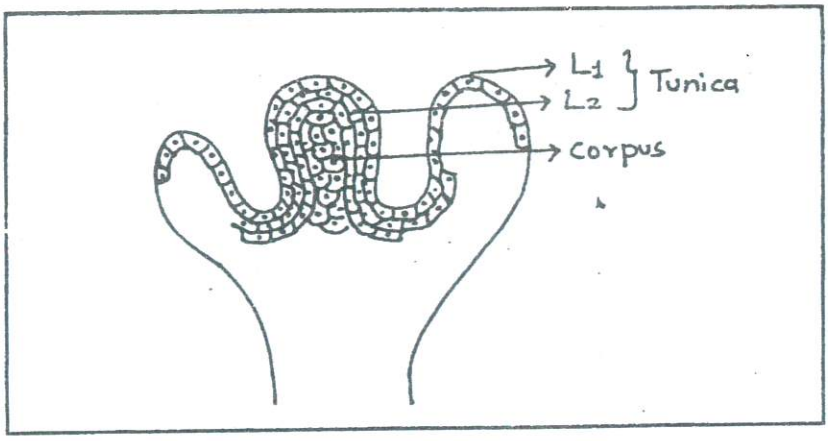


Figure 2.2: Organization of Shoot Apical Meristem (SAM) into Layers

These three fundamental layers have separate cell lineages. It is evident from the analysis of periclinal chimeras. In periclinal chimeras, one of the cell layers in the Meristem differs

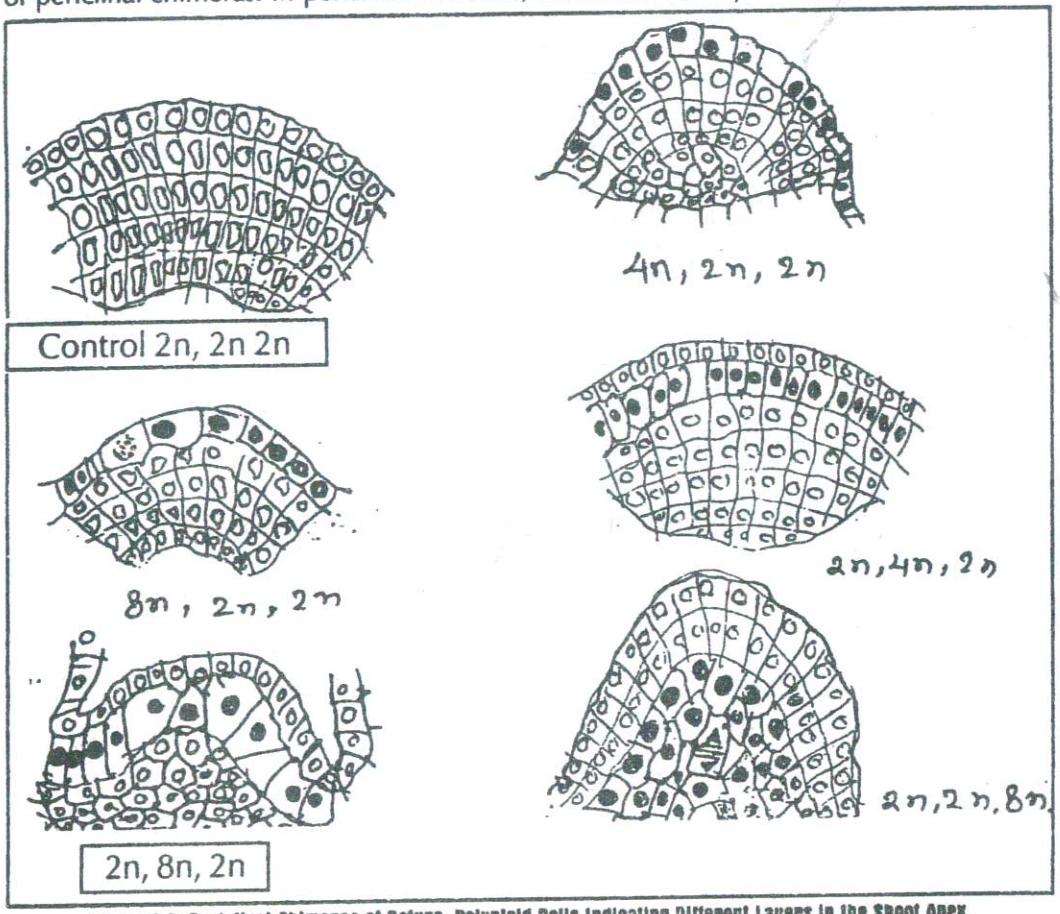


Figure 2.3: Periclinal Chimeras of Datura. Polyploid Cells Indicating Different Layers in the Shoot Apex.

genetically from the other layers. Satina et al. (1940) demonstrated the existence of layers by generating chimeras in datura using colchicines to induce the formation of polyploid cells. In this experiment, shoots were treated with colchicines to induce polyploidy in various layers. These polyploid cells were recognized cytologically as cells with enlarged multiple nucleoli. Polyploid cells occupied one of the three fundamental cell layers-L1, L2, or L3. The ploidy number of layers in the various examples is indicated for the various shoots. This experiment indicates that all cells in the shoot are organized into three fundamental layers derived from the SAM.

B. SAM is also organized radially into zones: a central zone composed of central mother cells and a peripheral zone composed of rapidly dividing apical initial. Lateral organs (leaf primordial) form on flanks of the SAM (morphogenic form). The initial cells below the apical dome constitute rib meristem. It helps in stem elongation.

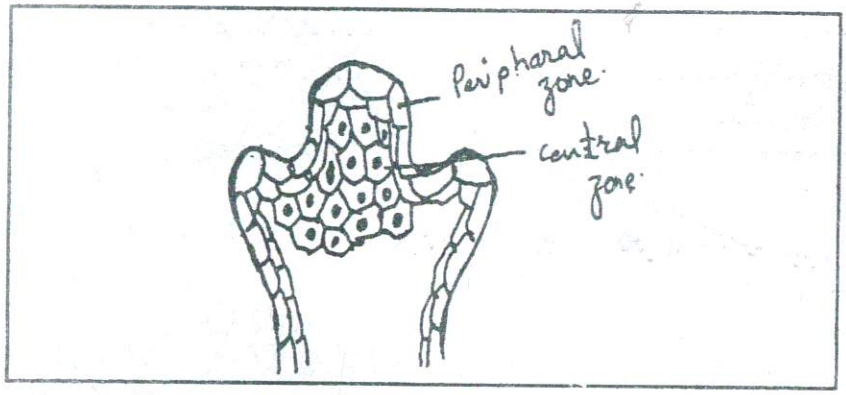


Figure 2.4: Diagram indicating peripheral and central zones

Cytological and Molecular Analysis of SAM

Proliferation

The operation of the SAM is so important to the understanding of shoot development that there has been considerable interest in mutants that affect the formation of the SAM. One of the mutant in Arabidopsis is Shoot Apical Meristemless (STM). In wild type seedling, first true leaves, covered with trichomes, emerge from the functional SAM. The stm-1 fails to organize a recognizable SAM during embryogenesis, and true leaves do not emerge at the seedling stage. These mutants, however, produce normal seedlings with cotyledons and hypocotyls. It means STM gene does not control seedling formation. It controls only SAM

activation. Another allele, stm-2, also failed to organize an embryonic SAM, although the mutant developed a SAM at the seedling stage. Shoots were produced in stm-2, but they were determinate structures that terminated after producing just a few leaves.

STM was cloned by Long et al. (1996) and used as a hybridization probe to detect STM expression during embryonic development in normal (non-mutant) plants. STM was expressed only in those cells predicted to form the embryonic SAM. STM was first expressed in only 1-2 cells in early to midglobular stage embryo. In heart-shaped embryos, STM was expressed in the notch between the cotyledons where the presumptive SAM forms. In older plants, STM was expressed in the SAM and axillary bud meristems.

Long et al. found that STM encodes a transcription factor belonging to the same class of homeodomain-containing transcription factors as encoded by the knotted1 (Kn1) gene in maize. This gene affects meristematic activity. Kn1 mutants in maize produce knots in maize leaf. In normal maize plants, the expression pattern of kn1 is similar to STM in Arabidopsis in that both genes are active in meristematic tissue. The kn1 is expressed in undifferentiated cells in the Meristem. Overexpression of kn1 results in ectopic formation of shoots meristems.

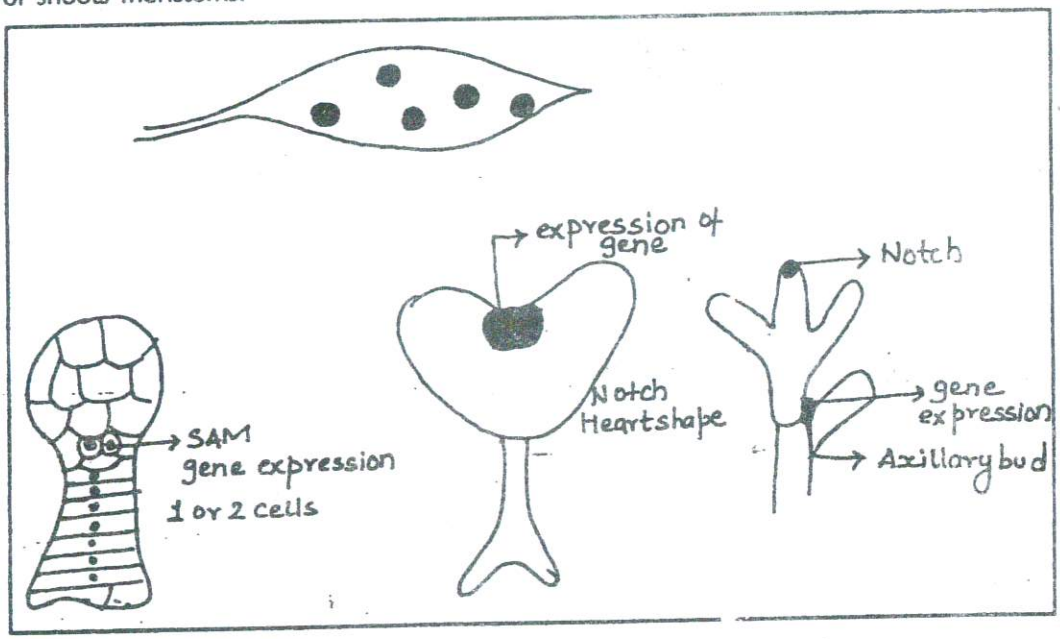


Figure 2.5: Diagram Indicating Gene Expression (Sam and Kn1)

Another Arabidopsis mutant that fails to maintain a functional SAM is wuschel (wus). Wus mutants form defective SAMs that prematurely terminate growth after having

produced only a few leaves. The wus mutant inhibits proliferation and promotes cell differentiation, whereas wild-type (WUS) initiates proliferation and inhibits the cell differentiation.

Differentiation

In this process, undifferentiated cells differentiate to form different tissues (as- hypodermis, endodermis, pericycle, V.B.).

Arabidopsis mutants have been found with defects in maintaining the balance of cell populations in peripheral zone. One mutant clv1 (clavata1), produces a much larger SAM during vegetative and reproductive growth. Another clavata mutant (clv3) is recessive in nature, has been identified and it is similar to clv1. CLV genes might promote cell differentiation at the boundary of the peripheral zone.

The clv1/clv3 shows proliferation. It has been proposed that CLV1 negatively regulates WUS. CLV1 promotes cell division, while WUS promotes proliferation. STM also promotes proliferation, so STM and CLV have opposing effects. Actually, the proper balance of STM and CLV1 and/or CLV3 activity is required for normal Meristem growth and development. STM and CLV have counteracting effects because clv1 can suppress the effects of stm1. The development and function of the SAM is very sensitive to the dose of STM and CLV genes.

CLV1 has been cloned and found to encode a putative receptor protein kinase. These kinases are similar to some receptor kinases involved in plant defense responses.

Double stm1/stm1 clv1/clv1 mutants produce SAMs that are often larger or smaller than wild type SAMs. Actually, we can conclude that in the stm clv double mutants, STM is not really needed for meristem formation in the absence of CLV. This means that STM and CLV are truly regulators involved in balancing the proliferative and differentiation functions of the Meristem, but are not really required for the development of the meristem.

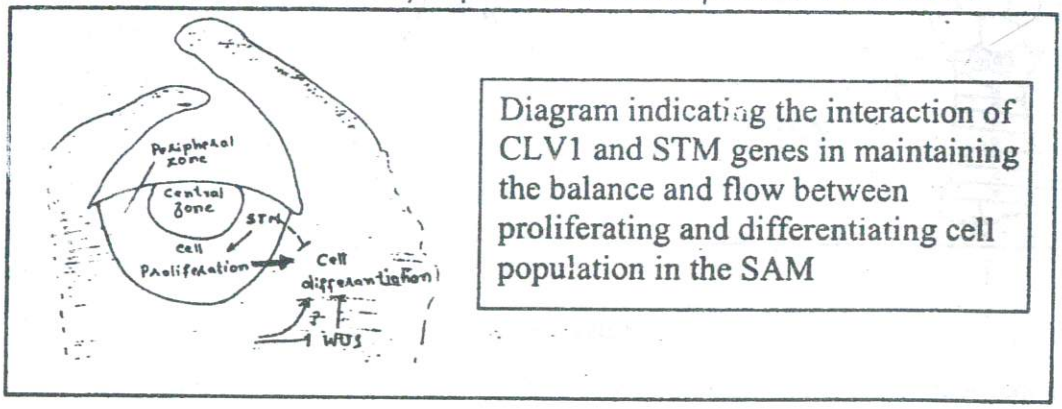


Diagram indicating the interaction of CLV1 and STM genes in maintaining the balance and flow between proliferating and differentiating cell population in the SAM

Figure 2.8

Molecular Biology of SAM and Control of Cell Division

Medford et al. (1992) recognized that cauliflower heads were an ample source of SAMs and used them to isolate meristem mRNAs (cauliflower heads are formed because of a defect in genes that promote the floral transition. The heads are composed of inflorescences that repeatedly branch, but do not flower, forming a head literally covered with meristems.

The SAM is a site of active cell proliferation, and genes involved in the regulation of the cell cycle are expressed in the SAM. In general, the genes involved in regulating the cell cycle in other eukaryotes have also been found in plants. Cell cycle progress is controlled by Cell Division Kinases (CDPKs) that are activated by proteins called cyclins and held in check by negative regulators. In yeast, cyclins transiently appear at specific cell cycle stages, and their synthesis and proteolysis drives the cell cycle. Cyclins have been cloned from a number of plants, including soyabean. The pattern of expression in the SAM of a B-type cyclin is represented by cyc5Gm; the expression of an S-phase marker, histone H4, was also visualized by in situ hybridization.

The cell cycle genes were actively expressed in a punctate pattern in the peripheral zone and rib meristem and rarely in the central mother cells. The punctate pattern indicates that the cells in the meristem are at different cell cycle stages, and the gene is expressed only during a short interval of the cell cycle (G2-M). Thus, cyclin gene is expressed during G2-M and histone H4 is expressed during S-phase.

Gene trap technology can also be used to identify genes in SAM. A gene trap is a mobile DNA element that contains a promoterless reporter gene that can insert itself at random locations in the genome. If the gene trap inserts within a gene that is expressed in a tissue-specific manner, then the reporter gene adopts the pattern of expression of the disrupted gene.

An exon trap is a form of a gene trap that expresses a reporter gene when it inserts into an intron of a plant gene. Gene trap technology has been used to find genes in an Arabidopsis collection that are expressed in various organs or tissues. One insertion occurred in a gene called PROLIFERA (PRL), which is expressed in various dividing cells in Arabidopsis including the SAM. PRL encodes a gene product that is similar to the MCM2. 3-5 family of genes are required for DNA replication. Unlike cyclins, PRL is not expressed in a punctate or cell cycle stage-specific manner, but it is expressed throughout the SAM.

Control of Tissue Differentiation, Especially Xylem and Phloem

In higher plants, the primary stem vasculature is usually organized in separate vascular bundles or in a cylinder that forms a ring around the central pith. The vascular system in vegetative shoots is leaf-oriented.

The vasculature of the stem is either primary or secondary, depending on its developmental origin. Primary vasculature arises from the procambium in the embryo or the Shoot Apical Meristem (SAM). The primary vasculature of the root arises from stele initials those that are located in the promeristem. Secondary vasculature, which is found extensively in woody dicots, is derived from the vascular cambium, a lateral meristem generally sandwiched between the xylem and phloem.

The vascular system has a remarkable capacity for regeneration, and revascularization can occur when a system is wounded or when a graft is formed. The stem vasculature differentiates basipetally (from tip to base). Vascular bundles in the stem that continue their course to the next internode are called sympodial bundles.

The vasculature is an arterial system that transports water, minerals, photoassimilates, hormones, woundifng signals, and so on. The primary vasculature links shoots to roots, and serves as a major channel for the upward and downward movement of substances in a plant.

The vascular system is largely made up of vascular bundles or strands that contain two kinds of conducting tissue: phloem that moves photoassimilates from sources to sinks, and xylem that conducts water and minerals from roots to other parts of the plant. The conducting cells of xylem are non-living, tracheary elements, whereas the conducting elements of phloem are living, enucleate cells called sieve elements. Conducting or tracheary elements of the xylem are tracheids and vessel members. They differ from each other in that vessel members are perforated and cells fused into long continuous tubes. Vessel members have single or multiple perforations in their end walls that form a perforation plate. Tracheids, on the other end, usually do not have perforations in their end walls; rather they have pits that occur in pit-pairs on the common lateral walls with other xylem elements. Other cells in xylem include fibers and parenchyma cells.

At maturity, the tracheary elements of the xylem are large, nonliving cells. The differentiation of tracheary elements involves the formation of secondary cell walls with helical, annular, reticulate or pitted wall thickenings. During development, tracheary elements undergo

autolysis, losing their nuclei and cytoplasm, and leaving only cell walls. The dead cells serve as conduits for the unimpeded movement of water and solutes. They also provide mechanical support for stems and other structures.

Phloem consists of many different cell types. Conducting cells of phloem are living. The conducting cells, which are sieve elements, are interconnected at their end walls by sieve areas or plates. The sieve areas or plates are specialized cell walls formed by the deposition of callose around pores that permit the flow of materials from cell to cell. the cells are interconnected through the sieve plate by cytoplasmic bridges or strands. Sieve areas or plates develop as an elaboration of plasmodesmata in the primary pit fields that interconnect sieve elements. The pores in the sieve plates are much larger than plasmodesmata. Because sieve elements are living cells, the phloem is actually a continuous stream of cytoplasm that courses through the plant. Although sieve elements retain a cytoplasm at maturity, their nuclei and other cellular components, including ribosomes, degenerate during development. Sieve elements are associated with nucleated companion cells, but are cordoned off during sieve element development.

In the development of phloem, sieve elements and companion cells are closely related in their ontogeny. The cell that gives rise to the sieve element divides one or more times longitudinally, and the larger cell that results from the divisions usually become a sieve element whereas the others become companion cells. The number and size of companion cells can vary among species.

Phloem and xylem differentiate at a late stage in vascular bundle development. In general, phloem differentiation precedes xylem. Furthermore, phloem is often found in the absence of xylem, but xylem is usually not found in the absence of phloem. Xylem and phloem differentiate in response to similar hormonal signals, but the difference seems to be quantitative. The development of the vasculature and differentiation of xylem and phloem involve issues in programmed cell death. The conducting or tracheary elements in xylem are dead cells, and the death of these cells is a normal part of their development. There are aspects of death in these cells that are similar to apoptosis in animal cell systems. Unlike cell death in animal cells, the process in tracheary cell maturation appears at a morphological level to be initiated by the rupture of the vacuole membrane. Following vacuole rupture other organelles quickly follow suit, in which organelles with single membranes such as ER and Golgi compartments lyase followed by organelles with double membranes, including the nucleus. Fragmentation of nuclear DNA in developing vessel elements occurs before the disruption of nuclei. Sieve elements, the conducting cells in phloem, are living cells, but

they lose their nuclei and most organelles during development. As a result, they relay on companion cells for many vital functions.

Vascular development is a successional form of growth. The phloem first formed in the developing vasculature of a newly forming organ, such as a leaf, is called protophloem. Protophloem is a transient form that usually develops before an organ completes its elongation. Phloem formed after elongation is called metaphloem. In herbaceous dicots, the metaphloem may constitute the phloem vasculature of the mature plant. In woody or herbaceous dicots with secondary growth, the metaphloem is usually destroyed by secondary growth. Protophloem is composed of less specialized vascular elements than metaphloem. Sieve elements in protophloem are narrower with thinner walls, and they have less developed sieve plates. Companion and parenchyma cells that are present in dicots metaphloem are less frequently found in protophloem.

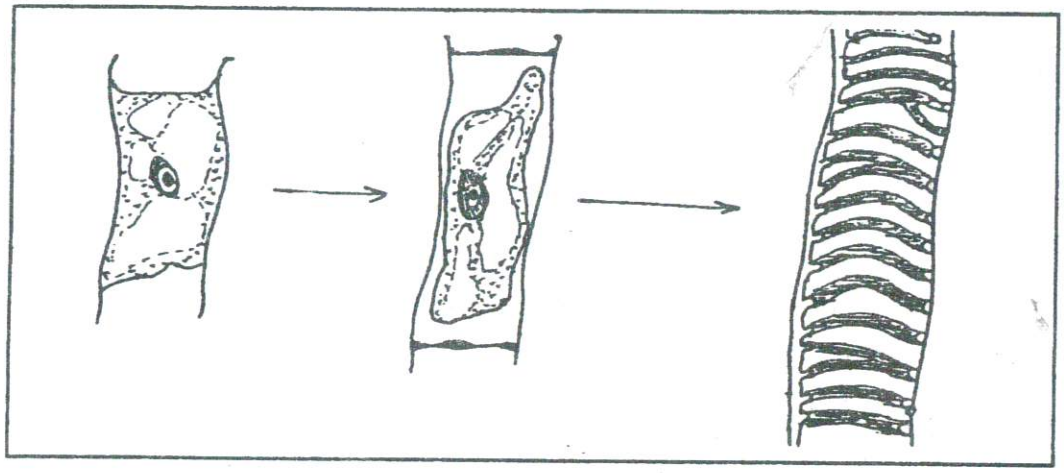


Figure 2.7: Development of Conducting Cells of Xylem

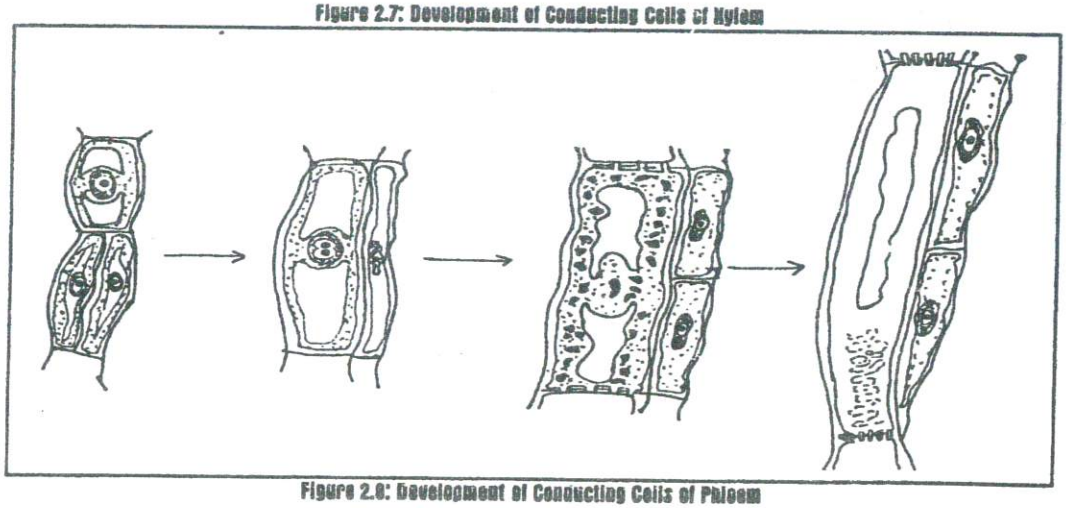


Figure 2.8: Development of Conducting Cells of Phloem

The first xylem laid down in the vasculature of a newly forming organ is called protoxylem. Protoxylem has few tracheary elements and more xylem parenchyma. The cell walls of tracheary elements are thinner with only annular or helical thickenings. During elongation of the organ, protoxylem is stretched, distorted and often distorted. Metaxylem is laid down when the organ is fully expanded. Tracheary elements are more abundant in metaxylem and their cell walls are reinforced with helical, sclariform, and reticulated thickenings.

Secretory Ducts and Laticifers

Secretory Ducts

These are the glandular tissues present in the form of glands in or on various parts of plants, especially in or on stems. These are specialized group of cells that are endowed with the capacity to secrete or excrete products. These are of two types:

1. External glands
2. Internal glands

External Glands

These generally occur on the epidermis of stem and leaves as glandular outgrowth e.g., glandular hair, nectar secreting and enzyme secreting glands.

a) **Glandular hair:** These unicellular or multicellular hairs are present in epidermal layers of leaves and are living. Contents of hair are poisonous and cause irritation and blisters. Ex. *Utrica dioica*.

b) **Nectaries:** Present I leaves or flowers or leaves. Ex. *Euphorbia sp.*

Internal Glands

c) **Oil glands:** Ex. Orange.

d) **Resin glands:** In Pinus, the resin secreting cells form one or two peripheral layers that surrounds a schizogenously developed canal or duct in the leaves and stem.

Laticifers

This tissue is mainly composed of thin walled elongated, branched and multinucleated tube like structures that contain colorless milky or yellow colored juice called latex. These are scattered throughout the ground tissue of the plant and contain stored organic matter in the form of starch, rubber, tannins, alkaloids, mucilage, enzymes, proteins etc. This tissue is of two types:

Latex Cells

They differ from latex vessels in that they are not formed due to cell fusions and with other latex cells to form a network. They are branched or unbranched. They do not anastamose. Ex. *Calotropis*.

Latex Vessels

They are composed of a large number of cells placed end to end with their transverse walls dissolved so as to form a long vessel. They are unbranched in the beginning but get branched later. Two or more latex vessels fuse with each other forming a network. Ex. *Papaver, Argemone*.

Wood Development in Relation to Environmental Factors

The activity of cambium ring is markedly affected by variations in climate, e.g., in temperate regions where changes in climate in different seasons of the year are pronounced, the xylem cells produced in spring season are with wider lumens. The secondary xylem formed during this period of pronounced activity is called **spring wood**. During autumn season, the vessels produced are generally of smaller size and have narrow lumens. The secondary wood formed during this season of the year is called **autumn wood**. In a transverse section of the stem, these two types of wood appear in the form of distinct concentric circles known as annual rings. One spring wood circle and one autumn wood circle constitute an annual ring. Like this, year after year such rings appear and their identity is well marked. The number of annual rings in the oldest part of the tree corresponds to its age. In some woody trees, the vessels in the spring wood are large and arranged in a ring and narrow vessels of the autumn wood are scattered, such a wood is said to be ring porous. In *Eugenia*

jambolana and rose, the vessels are more or less uniformly distributed throughout the springwood and autumn wood. Such a wood is called diffuse porous wood.

Leaf Growth and Differentiation

Leaf is formed from leaf primordia. It develops from the shoot apex. Differentiation in shoot apex occurs in the formation of leaf primordia.

A dicot leaf primordium first emerges as a small ridge or leaf buttress on the shoot apex. As it elongates, the primordium extends laterally and form apical-basal axis, and the leaf blade or lamina expands laterally from the early midrib. The development of dorsiventrality is an early step in leaf development.

Internal structure of leaf has epidermis, mesophyll (palisade and spongy) and vascular tissue.

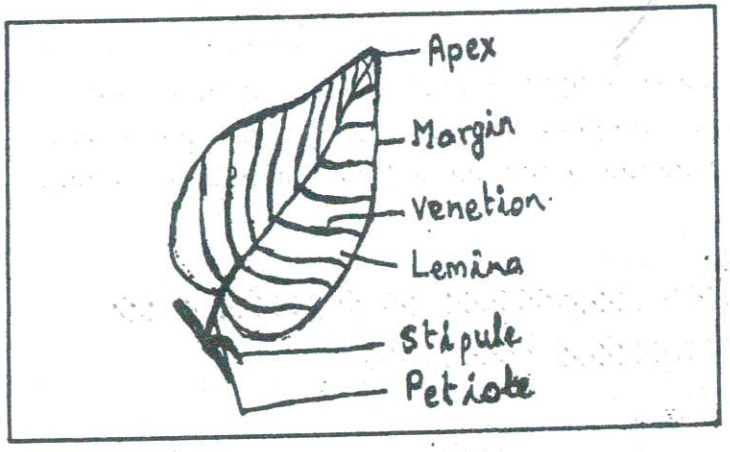


Figure 2.9: Generalized Diagram of Typical Leaf

Leaf Determination

Leaf determination is defined as the stage when explanted leaf primordia will develop into a leaf in tissue culture. We can say, the process through which shoot apex changes into leaf primordia is called as leaf determination. Steeves and Susex (1957) addressed this tissue by explanting leaf primordia at various stages of development. When leaf primordia were explanted at various stages of development in the fern, *Osmunda cinnamomea*, P8-P-10 formed leaves at high frequency, while P1-P5 failed to do so. Thus, leaf determination occurs between P5-P8 in *Osmunda*.

Leaf determination depends upon the influence of more central cells in the apical meristem in *Osmunda*. When P1 was isolated from the apex by making an incision and inserting a thin mica sheet, shoots arose more frequently than old leaves. Thus, conclusion is:

1. Leaf determination occurs after primordia emerge. A brief period is there. When a young primordia is not determined to form a leaf, and other routes of development may be followed depending on environment and developmental cues.
2. Leaf primordia become determined soon after emergence. A leaf primordia less than 1 mm in length, such as leaf primordia from Sunflower, can give rise to a normal leaf in culture.
3. Leaf determination is not a one-step process. It is a multi step process.
4. There are decisions to be made whether growth will be determinate or indeterminate, and also growth will have dorsiventral symmetry or be radial.
5. Removal of margins later in development resulted in loss of leaf parts, tendrils, leaflets or stipules, while these could be regenerated, if margins of leaf primordia are removed in early stage.

Phyllotaxy

Arrangement of leaf on the main stem or branch is called as phyllotaxy. Main purpose of phyllotaxy is to provide sufficient light to the leaves. There may be following types of phyllotaxy possible:

Cyclic

In this type, each node has two or more leaves. It is of two types-

A. **Opposite:** In this type, only two leaves are attached on each node, which are opposite to each other. In other words, both leaves have an angular distance of 180°.

It is also of two types:

i) **Opposite decussate:** Usually both leaves of a node arise at right angle in relation to the leaves of upper or lower nodes. Ex. *Ocimum, Calotropis*.

ii) Opposite superposed: In many plants, leaves arising from a node are in the same direction as of the upper or lower node, e.g., *Quisqualis*.

B. Whorled or Verticillate: In this type, more than two leaves arise from each node, e.g., Three in *Nerium* and five or more in *Alstonia*.

Spiral or Alternate

In this type, one leaf arises from each node. Leaves are arranged on different nodes in such a way that these form a spiral shape. Most of the plants have this type of phyllotaxy, e.g., mango, China rose, mustard, sunflower, tobacco etc.

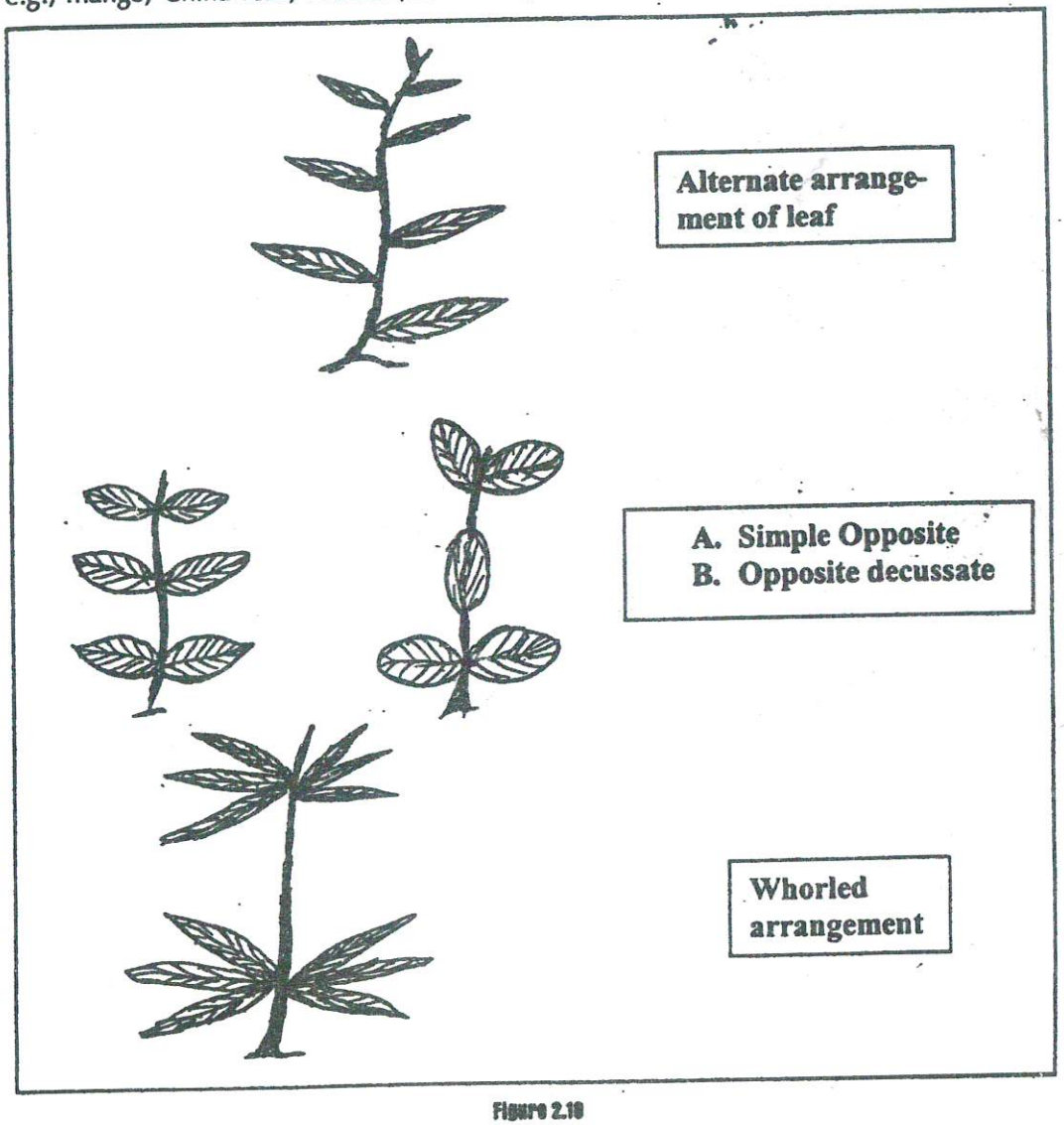


Figure 2.18

If at shoot apex, one primordium is formed at one node, then alternate phyllotaxy gets formed. If two leaf primordia are formed at one node, then opposite phyllotaxy is there; and if many primordia are formed, then whorled (spiral) phyllotaxy gets formed.

Phyllotaxy is established in the SAM by the pattern in which leaf primordia arise in the peripheral zone. There are three basic phyllotactic patterns: whorled with a circle of two or more leaves at a node (including decussate arrangement with two opposite leaves per node, alternating at right angles from node to node); distichous with one leaf per node, but arranged in two longitudinal rows; or spiraled with leaves arranged in a helical pattern (Esau, 1960). Spiral phyllotaxy is shown by Arabidopsis. One property of a spiral phyllotaxy is its direction or handedness. In Arabidopsis, handedness of the spiral is maintained through vegetative development, but it can be clockwise or counterclockwise with equal frequency.

The angle between successive leaf primordia – the divergence angle- is the same at about 137°. The divergence angle is fixed.

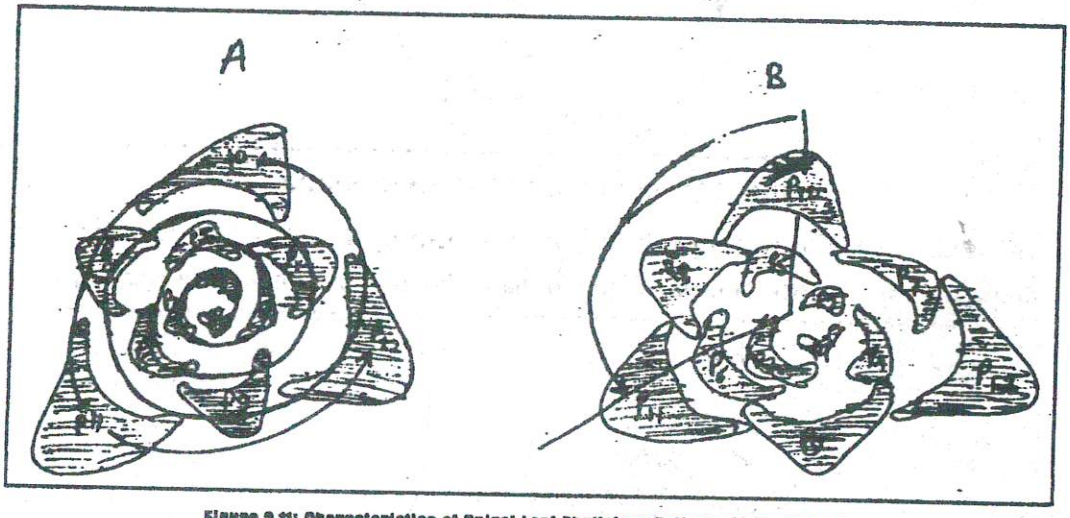


Figure 2.11: Characteristics of Spiral Leaf Phyllotaxy Patterns in Arabidopsis

Phyllotaxy can be reversed experimentally by using expansion. It is indicated with the help of *Arabidopsis*.

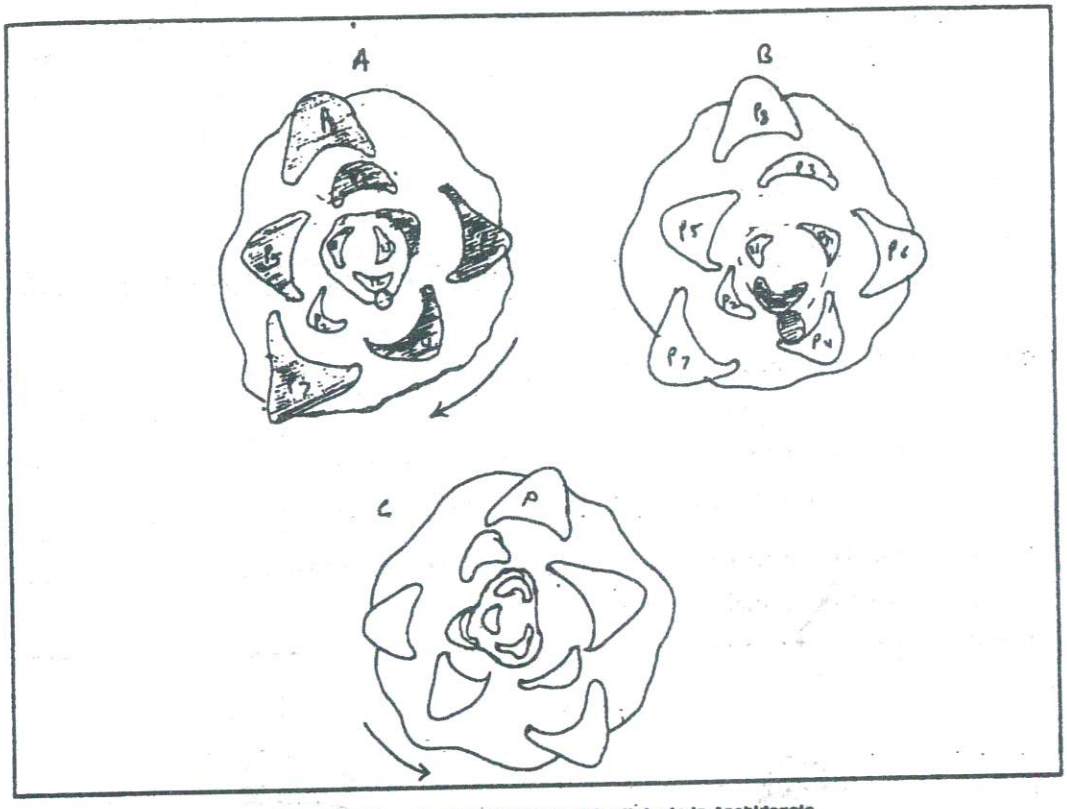


Figure 2.12: Experimental Reversal of Phyllotaxis in Arabidopsis

The positioning of new primordia may be mediated by inhibitory factors or forces that prevent the emergence of new leaf primordia in the local of recently emerged ones.

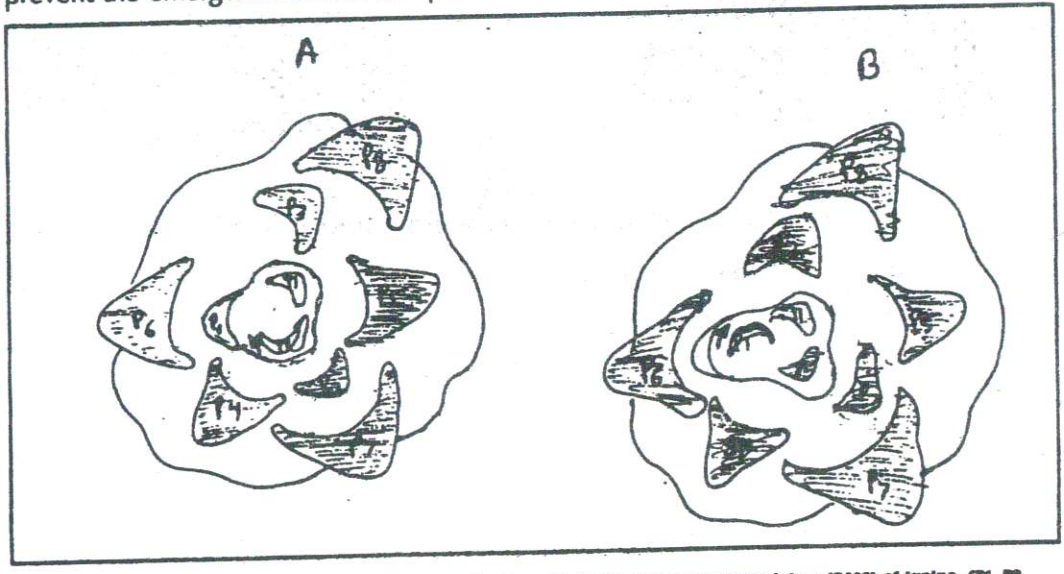


Figure 2.13: Shifting of site of appearance of new leaf primordia in the shoot apical meristem (SAM) of lupine. (P1, P2.... Are newly emerged primordia).

A. Primordia initials are isolated from P1 by incision. Position of 11 does not shift. 12 shifts in position towards p1.

B. Primordia initials are isolated from 11 by incision. Position of 12 does not shift. 13 shifts in position toward 11.

Control of Leaf Form (Differentiation)

Epidermis

Trichome Differentiation

Development of leaf also involves the formation of specialized epidermal cells, such as trichomes and stomata. In Arabidopsis, trichomes are usually located on upper or adaxial side of rosette leaves and stomata on the lower side or abaxial side. In some species, trichomes can be small, unbranched leaf hairs or large, multicellular spikes.

Hulskamp et al. (1994) collected and analyzed a group of mutants with defects in trichome development. The group of 70 trichome mutants represented 21 different genes. They used the mutants to define steps in a pathway of trichome development.

1. Several alleles of trichome mutants such as glabrous 1 (gl 1) or ttg (transport testa glabrous) lack trichome altogether. If GL1 (wild) and TTG1 (wild) are there, then glabrous testa is formed. Its function is to increase width of cell files and to initiate trichome formation.

2. Triptychon gene (wild- TRY) keeps proper spacing in trichome, while try mutant produce trichome cluster.

3. Distorted (dis) group is composed of 8 mutants with similar phenotypes. It causes distorted growth.

4. STA (wild): It controls branching pattern.

5. ZWI (wild): It produces secondary branching. If zwi (mutant) is there, no branching is produced.

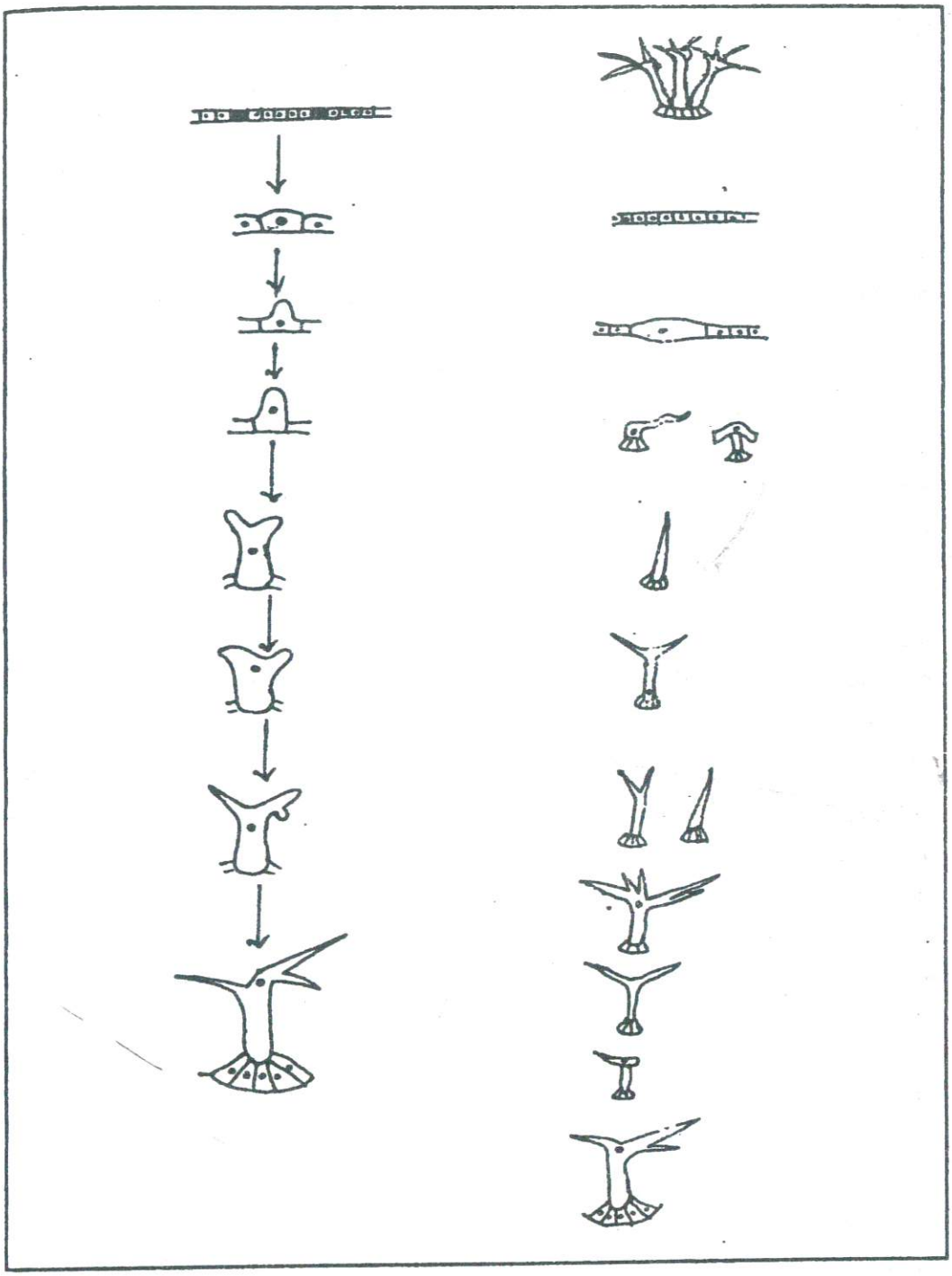


Figure 2.14: Trichome development in (left) *Arabidopsis* and effects of various trichome mutants.

Stomata Differentiation

Stomata are most characteristically found on the underside of leaves and provide passageways for gas exchange. A stoma is composed of guard cells that form a pore, which opens and closes in response to various environmental conditions. Stomata are epidermal derivatives and are associated in some species with other epidermal accessory cells called subsidiary cells. Because stomata are involved in gas exchange, there spacing is of vital importance to the operation of a leaf.

In dicots, stomatal initials appear randomly on the leaf with the constraint that a minimum distance separates any one stoma from another. The spacing pattern suggested that stomata inhibit the formation of other stomata by a mechanism that acts over short distances.

Stomatal spacing in most monocotsdoes, indeed, appears to result from regular patterns of cell division. In monocots, an epidermal cell typically undergoes an asymmetric an polarized division to produce a larger cell towards the base of the leaf and smaller initial toward the leaf tip. The smaller initial becomes a Guard Mother Cell (GMC) and undergoes a symmetric division to form two guard cells. In so doing, an alternating pattern of stomatal initials and neighboring cell is created within cell files in many monocots. In dicots, several asymmetric cell divisions occur in the formation of GMCs. The cell initiating the series of divisions is called a primary meristemoid.

Primary meristemoids appear to be positioned randomly and undergo a few further asymmetric divisions in which larger daughters become epidermal cells. After the last asymmetric division, the smaller meristemoid cell becomes a GMC which gives rise to two equal-size guard cells by a symmetric division. Some dicots, such as Arabidopsis, have another type of meristemoid called a satellite meristemoid. An asymmetric division of a cell, which neighbors a stoma, forms it. The satellite meristemoid can convert to a GMC or undergo additional asymmetric division placing additional neighboring cells between the satellite meristemoid and the stoma. In this way, stomata in Arabidopsis are spaced away from each other.

In an Arabidopsis mutant called *too many mouths* (tmm), the controls on stomatal spacing have been compromised. Another Arabidopsis mutant called *four lips* (flp) is characterized by groups of mostly two adjacent stomata and a few unpaired guard cells.

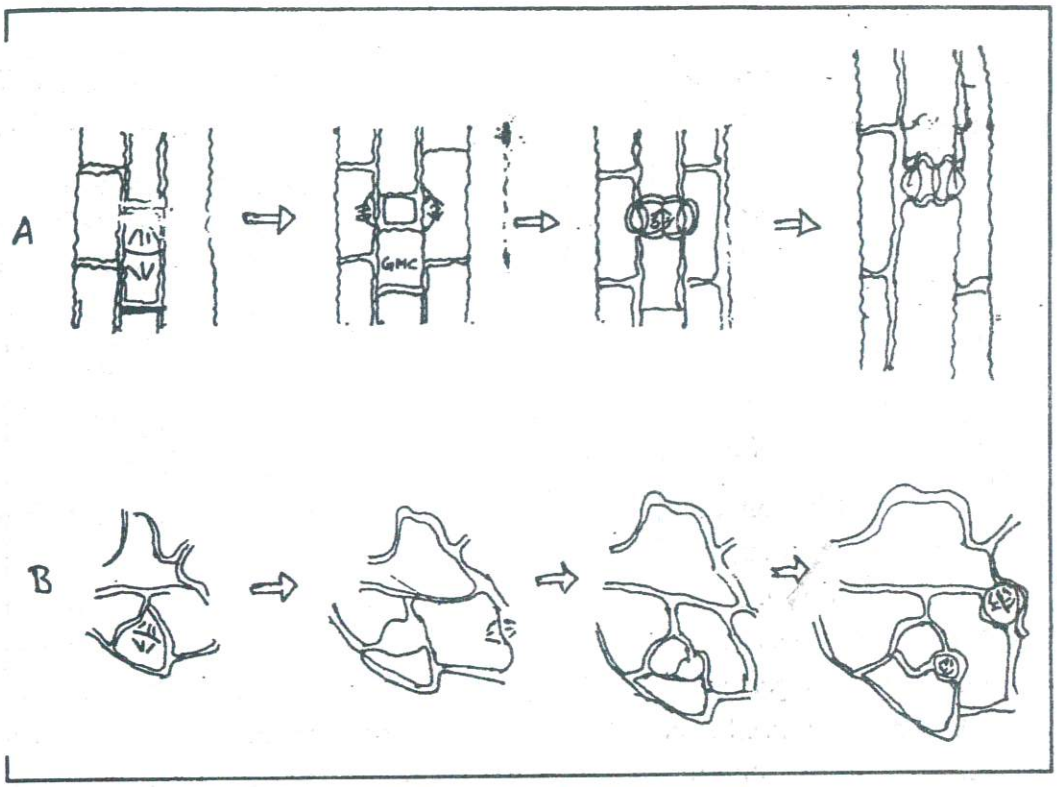


Figure 2.15: Development and Patterning of Stomata

A. In monocot grasses (*Zea mays*) an asymmetric, transverse division of an epidermal cell produces a larger cell towards the leaf base and smaller cells toward the leaf tip, which functions as guard mother cell.

B. In a dicot (*Arabidopsis*), an epidermal cell that serves as primary meristemoid undergoes an unoriented, asymmetric division which gives rise to larger epidermal cell and smaller meristemoid cell.

Mesophyll Differentiation

Mesophyll tissue of dicots is differentiated into spongy and palisade type while in monocots, only spongy type of mesophyll tissue are found. Mesophyll tissue differentiation is totally dependent on genes, which are as follows:

1. **Phan (wild type):** Phantastica gene exhibits pronounced dorsal-ventral asymmetry with respect to the order of cell types from the dorsal to ventral surface of the leaf.

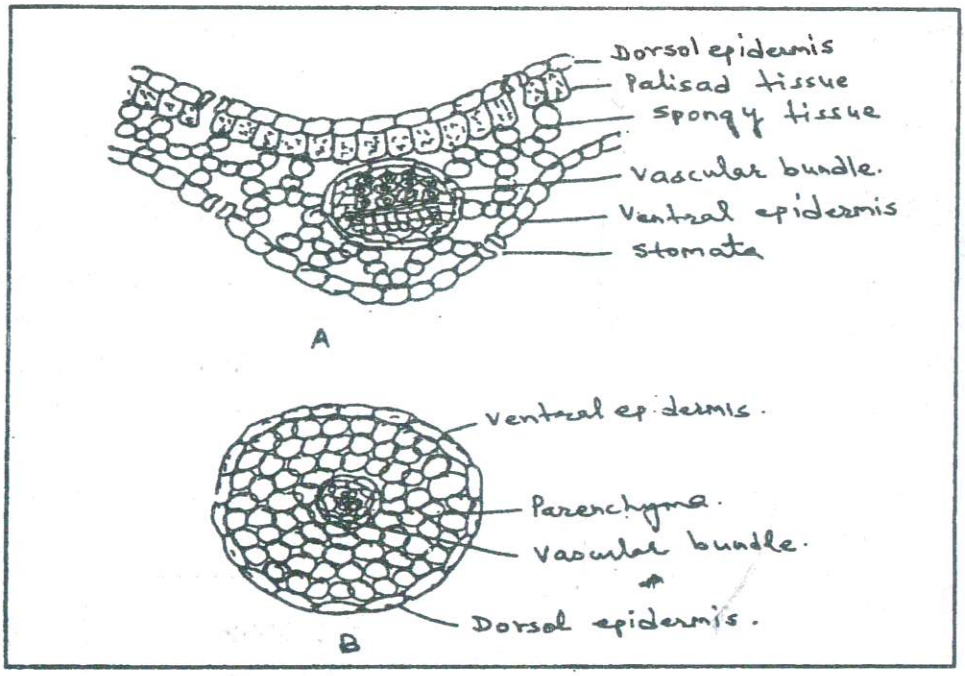


Figure 2.16: Comparision of leaves from Arabidopsis wild type and phantastica (phan) mutants.

A. Wild type leaf exhibits dorsal – ventral asymmetry.

B. Needle like phan leaf mutant shows only ventral like cells arrangement with radial symmetry

Phan mutants show no dorsiventrality in leaves at and above the fifth node. It shows only ventral cell types arranged with radial symmetry around a central vascular bundle.

1. Lam-1 (wild type) causes initiation of the lamina, while lam-1 (mutant) develops leaf dorsiventrality. In lam-1 (mutant), leaves are nearly rod-shaped and lengthen normally.

2. The fat mutant has thickened leaves. Leaves of fat plants have upto four layers of middle mesophyll cells that arise from abnormal periclinal divisions in L2 or L3 cells.

Root Development

Roots are tip-growing structures that grow in length through the action of tip Meristem. Actually embryo develops into radicle and plumule. Radicle develops into primary root Meristem. It develops into RAM (Root Apical Meristem) in seedling, which further develops into root. RAMs do not reduce lateral organs. Primary roots reduce lateral roots but lateral

roots do not arise from the root tip; rather lateral roots are outgrowths from more mature regions of the roots. RAM elaborates and forms root cap, which covers the root tips. Roots are constructed from concentric layers of cells. The root produces no nodes and internodes.

Organization of Root Apical Meristem

The RAM is organized both radially and longitudinally. RAMs are quite simple in the aquatic fern. A single large apical cell at the center of the root tip produces new cells that make up both the root cap and root. RAMs are more complicated in higher plants with larger roots. The distal part of the root apical Meristem may be termed as protomeristem. The young root axis is more or less clearly separated into the future central cylinder (plerome) and cortex (periblem). Apical meristems of roots are analyzed on the basis of three layers, which are dermatogen, periblem and plerome.

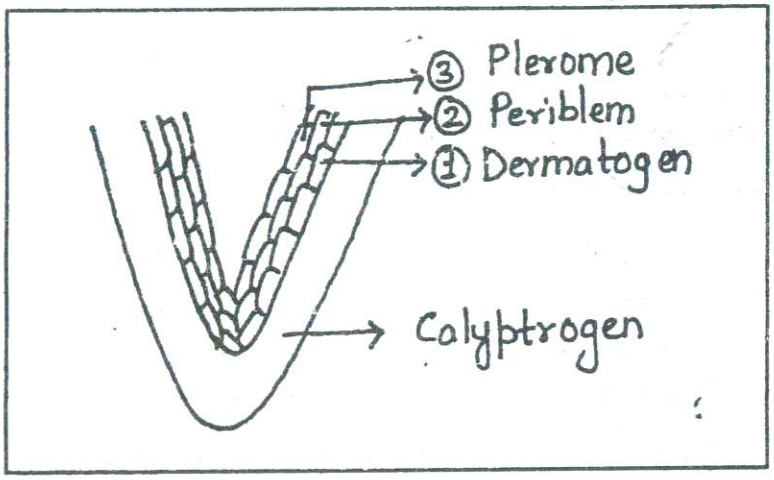


Figure 2.17: Root Apical Meristem Organization

The layers later differentiated and form different type of cells present in different layers. Cells are derived in the embryo from both the proembryo and suspensor.

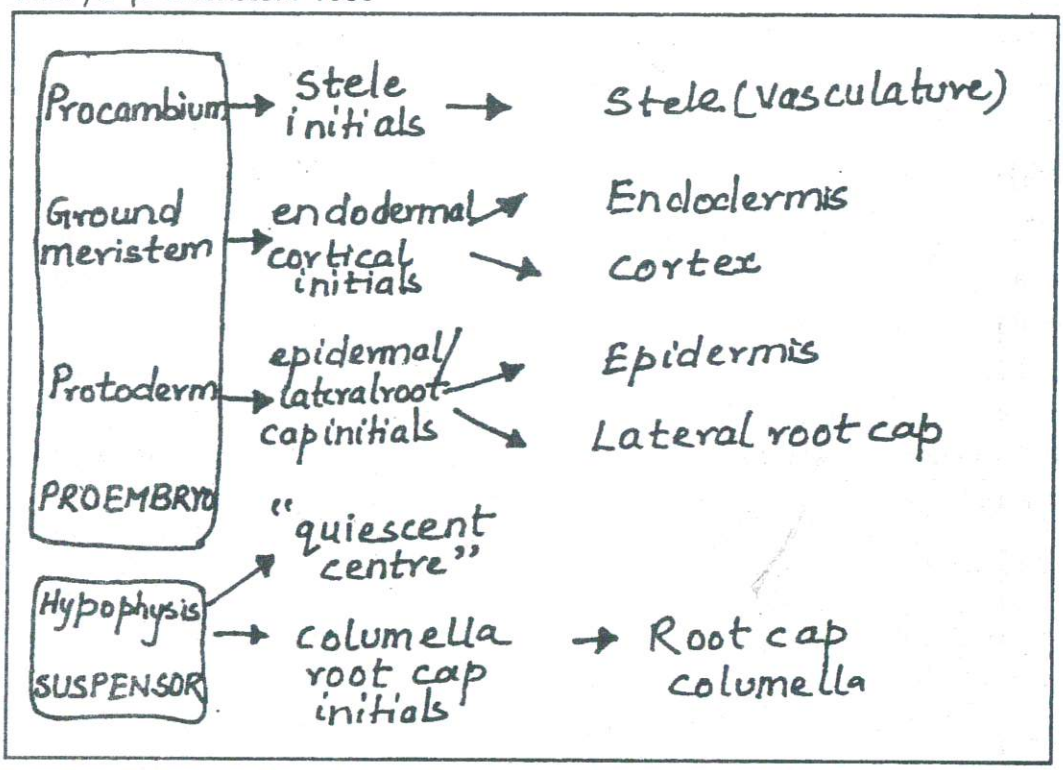


Figure 2.16: Origin of Cells in Various Cell Layers of the Root

Clowes (1954) proposed the promeristem concept. The promeristem is the small group of initial cells at the base of the vascular cylinder that gives rise to the radial pattern of the root and the root cap. The promeristem does not include cells in the zone of cell division. In the Arabidopsis embryonic axis divided periclinally and anticlinally to form promeristem. Periclinal division produce concentric rings of cells and anticlinal divisions subdivide the rings, increasing number of cells. It is shown in following diagram:

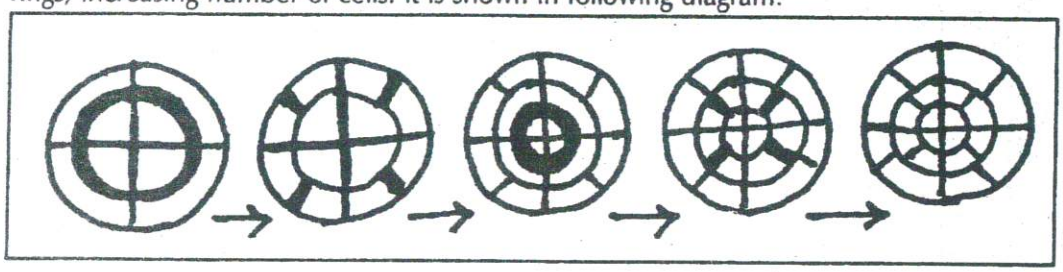


Figure 2.18: Representation of Alternating Anticlinal and Periclinal Divisions in the Embryonic Axis that Gives Rise to the Promeristem in the Root Region of Arabidopsis

At the heart of the promeristem cells, which are thought to be the progenitors of all cells in the root, they constitute a structure called the quiescent center. It is a group of slowly dividing cells flanked by more rapidly dividing initials. The doubling time for cells in the quiescent center in maize is less than 170 hrs, whereas that for root cap initials is only about 12 hrs. In Arabidopsis, Dolan et al. (1993) were unable to find any divisions at all in the quiescent center. But, cells in the quiescent center can be recruited to grow by damaging the root cap, or by nutrient refeeding of growth-arrested roots in culture.

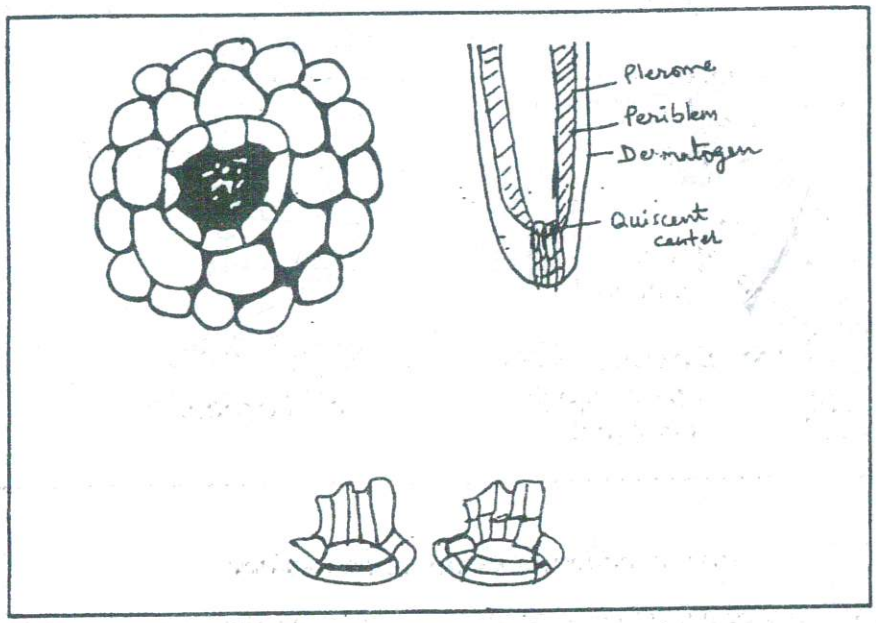


Figure 2.20: Arabidopsis Root Apical Meristem (RAM) and Promeristem Region

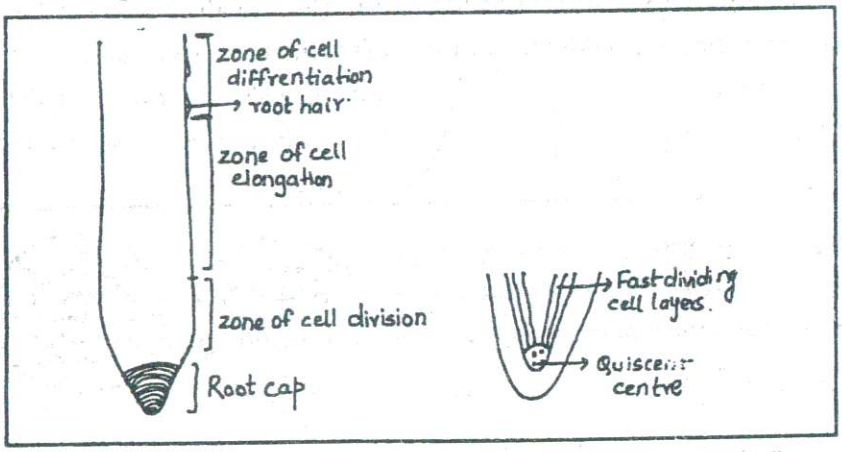


Figure 2.21: Root tip of Arabidopsis Indicating Special Zones Along with the Length

Cell Fates and Cell Lineage Analysis

The fate of RAM cells can be predicted from the structure of the root in Arabidopsis. Arabidopsis roots are composed of concentric layers of cells, which surrounds the central vascular cylinder in constant numbers and invariant pattern. The pericycle, for example, is composed of a ring of 12 cells and is surrounded by an endodermis made up of 8 cells. A cortex of 8 cells, then an epidermal layer of about 16 cells in turn surround the endodermis. The central vascular cylinder or stele has two protophloem elements that are located perpendicular to two protoxylem elements. The structure of the root cap is also regular.

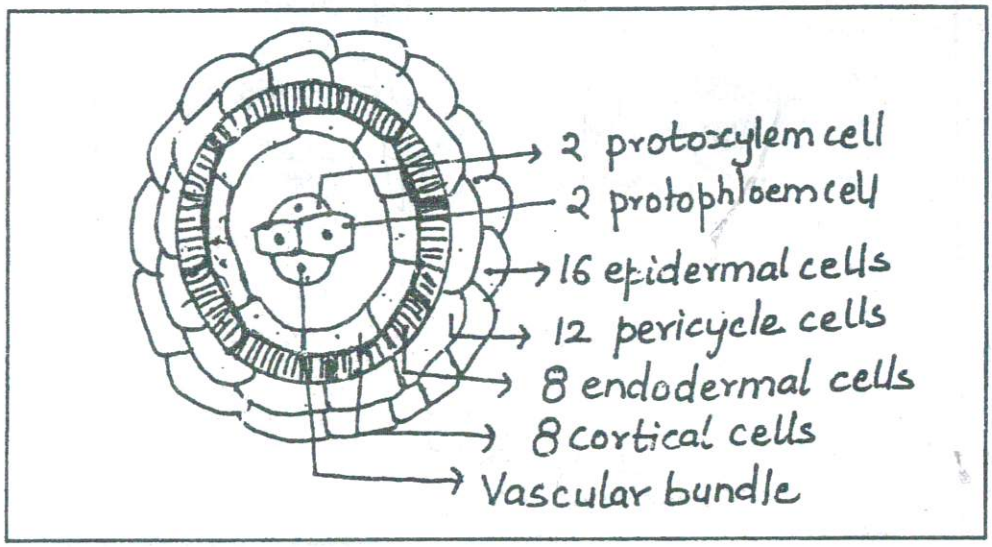


Figure 2.22: Arabidopsis: Cell Lineage System

The Arabidopsis promeristem is composed of three tiers of cells:

Lower Tier

The root cap columella arises from periclinal divisions of the lower (distal) tier in the promeristem. The lateral root cap is derived from a ring of about 16 initial cells that surrounds the columella initials. These initials undergo periclinal divisions to give rise to two different concentric layers of cells: the epidermal and lateral root cap cells.

Middle Tier

Another ring of initials that surrounds the middle tier of cells in the promeristem also gives rise to 2 concentric layers: the cortical and endodermal cells. The cells in the center of the middle tier of promeristem cells are quiescent cells.

Upper (proximal) Tier

The plate of cells in the upper (proximal) tier about the central vascular cylinder and are initials for the stele (pericycle and vascular bundles). Lower cell forms quiescent center.

Dolan et al. (1994) carried out a cell lineage analysis in Arabidopsis roots to confirm the apparent cell lineage pattern. They introduced a receptor gene marker into transgenic Arabidopsis plants. The CaMV 35S promoter is normally expressed in roots.

Vascular Tissue Differentiation

The vasculature is an arterial system that transports water, minerals, photoassimilates, hormones, wounding signals, and so on. The primary vasculature links shoots to roots, and serves as a major channel for the upward and downward movement of substances in a plant.

The vascular system is largely made up of vascular bundles or strands that contain two kinds of conducting tissue: phloem that moves photoassimilates from sources to sinks, and xylem that conducts water and minerals from roots to other parts of the plant. The conducting cells of xylem are non-living, tracheary elements, whereas the conducting elements of phloem are living, enucleate cells called sieve elements. Conducting or tracheary elements of the xylem are tracheids and vessel members. They differ from each other in that vessel members are perforated cells fused into long continuous tubes. Vessel members have single or multiple perforations in their end walls that form a perforation plate. Tracheids, on the other end, usually do not have perforations in their end walls; rather they have pits that occur in pit-pairs on the common lateral walls with other xylem elements. Other cells in xylem include fibers and parenchyma cells.

At maturity, the tracheary elements of the xylem are large, non living cells. The differentiation of tracheary elements involves the formation of secondary cell walls with helical, annular, reticulate or pitted wall thickenings. During development, tracheary elements undergo autolysis, losing their nuclei and cytoplasm, and leaving only cell walls. The dead cells serve as conduits for the unimpeded movement of water and solutes. They also provide mechanical support for stems and other structures.

Phloem consists of many different cell types. Conducting cells of phloem are living. The conducting cells, which are sieve elements, are interconnected at their end walls by sieve areas or plates. The sieve areas or plates are specialized cell walls formed by the deposition of callose around pores that permit the flow of materials from cell to cell. The cells are interconnected through the sieve plate by cytoplasmic bridges or strands. Sieve areas or

plates develop as an elaboration of plasmodesmata in the primary pit fields that interconnect sieve elements. The pores in the sieve plates are much larger than plasmodesmata. Because sieve elements are living cells, the phloem is actually a continuous stream of cytoplasm that courses through the plant. Although sieve elements retain a cytoplasm at maturity, their nuclei and other cellular components, including ribosomes, degenerate during development. Sieve elements are associated with nucleated companion cells, but are cordoned off during sieve element development.

In the development of phloem, sieve elements and companion cells are closely related in their ontogeny. The cell that gives rise to the sieve element divides one or more times longitudinally, and the larger cell that results from the divisions usually become a sieve element whereas the others become companion cells. The number and size of companion cells can vary among species.

Phloem and xylem differentiate at a late stage in vascular bundle development. In general, phloem differentiation precedes xylem. Furthermore, phloem is often found in the absence of xylem, but xylem is usually not found in the absence of phloem. Xylem and phloem differentiate in response to similar hormonal signals, but the difference seems to be quantitative. The development of the vasculature and differentiation of xylem and phloem involve issues in programmed cell death. The conducting or tracheary elements in xylem are dead cells, and the death of these cells is a normal part of their development. There are aspects of death in these cells that are similar to apoptosis in animal cell systems. Unlike cell death in animal cells, the process in tracheary cell maturation appears at a morphological level to be initiated by the rupture of the vacuole membrane. Following vacuole rupture other organelles quickly follow suit, in which organelles with single membranes such as ER and Golgi compartments lyase followed by organelles with double membranes, including the nucleus. Fragmentation of nuclear DNA in developing vessel elements occurs before the disruption of nuclei. Sieve elements, the conducting cells in phloem, are living cells, but they lose their nuclei and most organelles during development. As a result, they relay on companion cells for many vital functions.

Vascular development is a successional form of growth. The phloem first formed in developing vasculature of a newly forming organ, such as a leaf, is called protophloem. Protophloem is a transient form that usually develops before an organ completes its elongation. Phloem formed after elongation is called metaphloem. In herbaceous dicots, the metaphloem may constitute the phloem vasculature of the mature plant. In woody or herbaceous dicots with secondary growth, the metaphloem is usually destroyed by secondary growth. Protophloem is composed of less specialized vascular elements than metaphloem. Sieve elements in protophloem are narrower with thinner walls, and they have less developed

sieve plates. Companion and parenchyma cells that are present in dicots metaphloem are less frequently found in protophloem.

The first xylem laid down in the vasculature of a newly forming organ is called protoxylem. Protoxylem has few tracheary elements and more xylem parenchyma. The cell walls of tracheary elements are thinner with only annular or helical thickenings. During elongation of the organ, protoxylem is stretched, and often distorted. Metaxylem is laid down when the organ is fully expanded. Tracheary elements are more abundant in metaxylem and their cell walls are reinforced with helical, sclariform, and reticulated thickenings.

Lateral Root

Plants produce different classes of roots that are distinguished by their site of origin in the plant and/or by the time when they are produced in development. Primary roots are formed in the embryo at the base of the hypocotyls. The RAM forms from the primary root but not at the root tip of lateral roots. Lateral roots emerge instead from the pericycle at some distance from the root tip. The lateral root primordial, bulge out at predicted circumferential positions, which are usually adjacent to xylem poles. The emerging primordial, burrows their way through the endodermis and cortex and burst through the epidermis.

During this outgrowth, the emerging lateral root primordium organizes a RAM. Lateral roots have meristems that are indistinguishable morphologically and functionally from primary RAMs. A lateral root is derived from approximately 10 or 11 pericycle cells

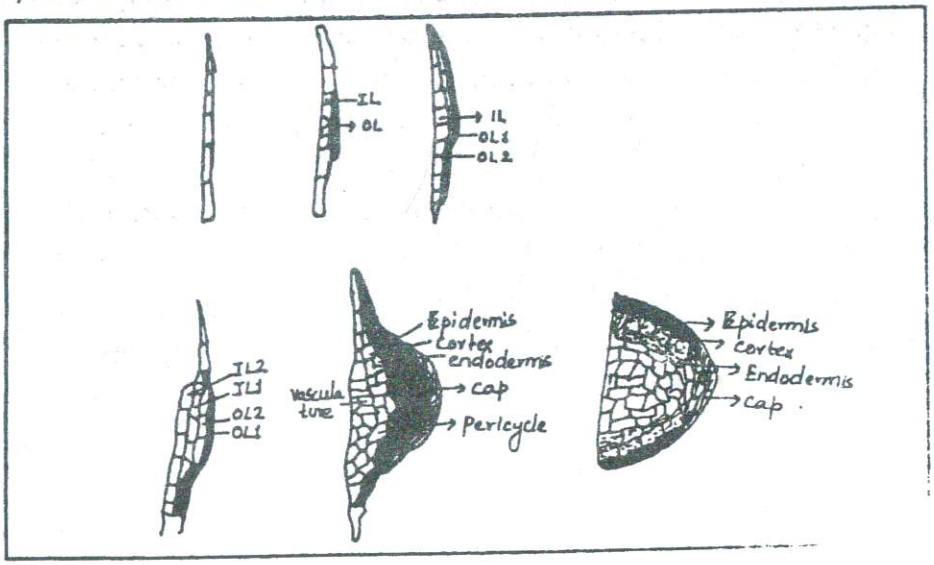


Figure 2.23: Lateral Root Primordia Development in Arabidopsis

The process of lateral root formation is highly ordered. Its various stages (as in Arabidopsis) are:

Stage-I In it, anticlinal divisions occur in pericycle wall.

Stage-II In it, pericycle division generates two-cell layers;

-Outer layer (OL)

-Inner layer (IL)

In the next two stages, cells in OL and IL divide periclinally, which again gives rise to 4 cell layers.

OL1, OL2, IL1 and IL2.

OL1 gives rise to epidermis.

OL2 gives rise to cortex and endodermis.

IL1 and IL2 give rise to inner tissues.

The development of lateral roots in culture can be induced by the hormone auxin. Cyclin promotes cell division in pericycle.

Root Hairs

Root hairs are produced on epidermal cells in the zone of cell differentiation. The root hairs grow in length when the epidermal cell stops elongating. These are unicellular, long and unbranched. Root hair- forming cells are called trichoblasts, which are separated from non-root hair forming atrichoblast cells.

Trichoblasts are more cytoplasmically dense and do not elongate as much as atrichoblast.

Root Hairless (RHL1-RHL3) genes promote root hair initiation in all epidermal cell files of the root. Their action is suppressed in atrichoblast cells by the action of TTG gene and GL2 gene. TTG appears to suppress all aspects of atrichoblast differentiation, and GL2 affects only root hair initiation. Ethylene stimulates root hair initiation as do mutation in CTR1 shows activation and inactivation)

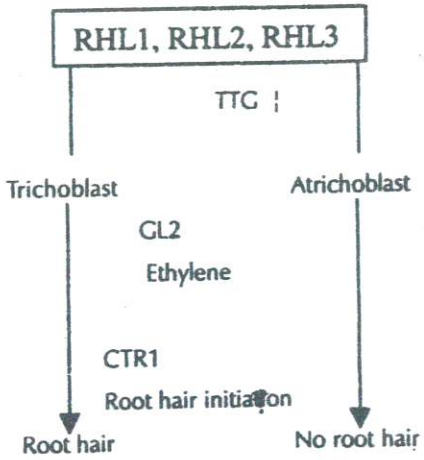


Figure 2.24: Scheme for the Control of Root Hair Initiation in Arabidopsis

Root - Microbe Interactions

It mainly involves mycorrhizal association and root nodule formation in leguminous plants.

Mycorrhiza

Mycorrhiza is an association of root of higher plant and fungus. It is an example of mutualism among vascular plant and fungus. It is (+,+) type of relation. By this association, fungus and plants both are benefited (Albert, Burnard, Frank 1885).

These microbes play an important role in natural environment, agricultural and land development. In about 80% vascular plants, mycrorhizae shows symbiotic association. Fossil evidences show that during Devonian period of Paleozoic era, (387-408 million years ago) mycorrhizal association with vascular plant was found. In this era, vascular plants were originated. Therefore, according to scientists, this association was helpful in the development of terrestrial plant. Since up to that time, soil was not developed completely and minerals present in soil were less in number, they were complex. In this condition, because of mycorrhizal association phosphorus and other minerals become available to the vascular plant. This is the only reason that even today when plants grow in less developed soils, then mycrorhizal association play a vital role. Mycorrhiza also helps in plantation in wasteland.

Types of Mycorrhizae

till now three types of mycrorrhizal association have been seen in vascular plants:

1. Ectomycorrhiza: Ectomycorrhizae are mainly seen in temperate forest, where it shows general association in trees and shrubs. Like, pine trees, which grow in the form of timberline. In its roots, dense mycelia of fungus are present at the outer side. In it fungal component basidiomycetes, ascomycetes or zygomycetes may be present. Ectomycorrhizae are present in the form of sheath outside the root and it shows limited intercellular structure. They are limited upto upper surface of root cortex. They are found in beech, oak, birch and other conifers trees. More than 5000 fungal species, which is often, of basidiomycetes shows ectomycorrhizal association. Most of the part of these mycelia present in soil, which take nutrient from soil and transfer in plant, such as *Pisolithus tinctorius* that grows continuously and work as physical support.

2. Ect-endomycorrhizae: These mycrorhizae shows more intracellular infection in cortical cells. Mainly these mycorrhizae have been seen in orchids. This type of mycelia is present in root cortex and outside of it.

3. Endomycorrhizae: These mycorrhizae are completely found in cortical cells of root. They are present as intra cellular structure in which two main structures are found.

   a. Vesicles

   b. Arbuckle's.

Because of these structures it is called vesicle-arbuscular mycorrhizae or VAM. This type of fungi, mainly are the members of zygomycetes which shows obligate symbiosis. These fungi die out of the plants. In this association, fungal hyphae rupture the outer cortical cell, and enter in it, where it forms a coil by growing intracellular. These type of mycorrhizae are found in tropical plants such as wheat, corn, beans, tomatoes, apples, oranges and many other industrial (commercial) crops and grasses. Plant- mycorrhizae association gives strength to plants and improves the competition capacity. However, this capacity depends on environment. In wet environment they increase the availability of phosphorus and other nutrient. In arid atmosphere, it increases the water absorption capacity of plant. Therefore, these plants show more transpiration as compared to non-mycorrhizal plant. In this way rate of photosynthesis, availability of water and mineral is increased. Out of it some

products are given to fungi. Likewise plant gets water and nutrient and gives food to fungi and shows trade of photosynthetic substances.

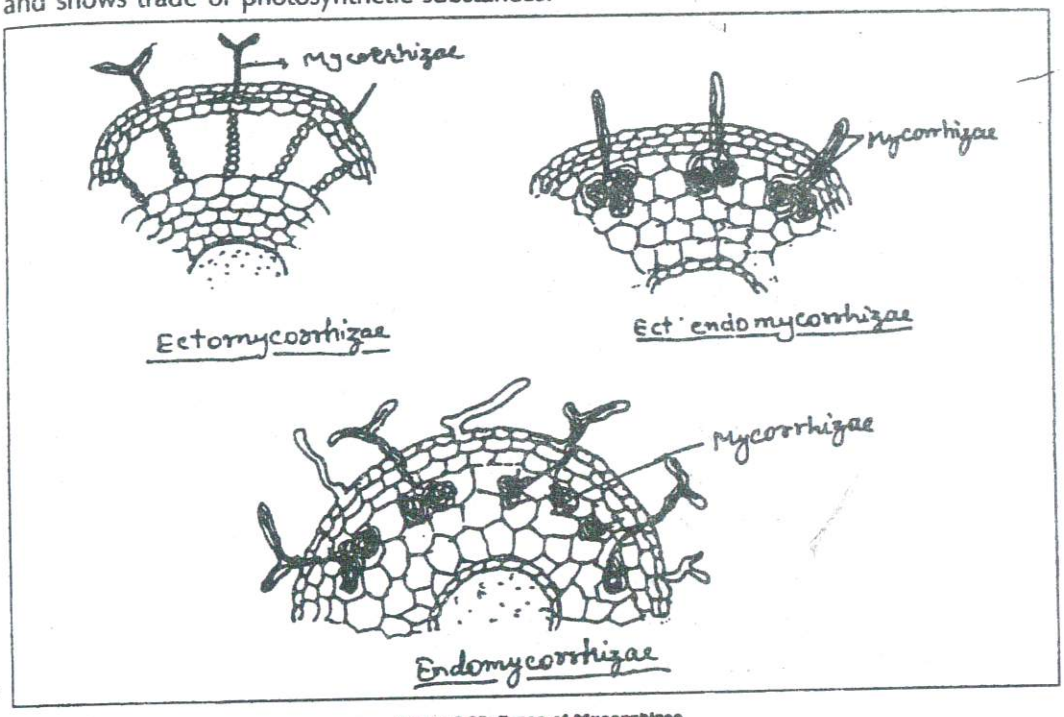


Figure 2.25: Types of Mycorrhizae

Root Nodules in Leguminous Plants

A relatively large group of plants- the legumes, are capable of fixing atmospheric nitrogen through a symbiotic association with soil bacteria called *Rhizobium*. These bacteria or nodule bacteria usually dwell in the roots of various beans, peas, lupines, cloves and other leguminous plants. Roots of most of the leguminous plants possess circular outgrowth or swelling called nodules, which are formed after infection of certain types of nitrogen fixing bacteria particularly the different species of *Rhizobium*. These bacteria have the capacity to fix free atmospheric nitrogen of soil in nodule. They infect or get their entrance in root through the soft hair or other epidermal cells by damaging them.

Those plants, which do not bear root-nodules, will never be able to fix atmospheric nitrogen in the plants. The rate of nitrogen fixation of nodule is directly proportional to the volume of the effective nodule. The nodules are usually larger and pink in color. This is due to the presence of red colored leghaemoglobin. It acts as an electron carrier. The nitrogenase enzyme of bacteria fixes nitrogen by converting nitrogen gas into ammonia using hydrogen ions and energy by host plant.

CHAPTER 3

REPRODUCTION

LEARNING OBJECTIVES

- Vegetative Options and Sexual Reproduction
- Floral Development
- Sex Determination
- Male Gametophyte
- Role of Tapetum
- Pollen Development and Gene Expression
- Sperm Dimorphism and Hybrid Seed Production
- Female Gametophyte
- Megasporogenesis

Vegetative Options and Sexual Reproduction

Plants can be reproduced by vegetative means or sexual reproduction. In many plants vegetative reproduction is common although majority of flowering plants produces sex organs as stamens and pistils in the flower and take part frequently in the reproduction.

Vegetative Options

In the plants different modes of the sexual reproduction can be seen. Some important means are following:

1. Propagation by apomitic seedlings
2. Propagation by special vegetative structures
    - A. Bulb: like onion, garlic
    - B. Tubers and tuberous roots: Tulip, Iris.
    - C. Rhizomes: Banana, Ginger.
    - D. Corms: Gladiolus, Crocus
    - E. Suckers: Pineapple, Banana, Guava
    - F. Runners: Strawberry
    - G. Offsets: Datepalm, Pineapple
3. Propagation by cutting
    - A. Root cutting: Apple, Guava, Pear
    - B. Leaf cutting: Bryophyllum
    - C. Leaf bud cutting: Begonia
    - D. Stem cutting
        - a. Herbaceous cutting: Dahlia, Geranium.
        - b. Soft wood cutting: Evergreen plants
        - c. Semihard wood cutting: Lemon, Pomegranate
        - d. Hard wood cutting: Perennial woody plants.
4. Propagation by layering
    - A. Simple layering
    - B. Tip layering
    - C. Trench layering
    - D. Compound or serpentine layering
    - E. Mound or stool layering
    - F. Air layering or gootee

5. Propagation by grafting
    A. Splice or whip grafting
    B. Tongue grafting
    C. Saddle grafting
    D. Cleft grafting
    E. Slide grafting
    F. Veneer grafting
    G. Bark grafting
    H. Approach grafting
    I. Root grafting
6. Propagation by budding
    A. T-budding
    B. Flute or tube budding
    C. Patch budding
    D. Chip budding
    E. Ring budding
    F. I budding
    G. Forkert budding

Sexual Option

During sexual reproduction many important events occur. Which are following:

1. Microsporogenesis
2. Megasporogenesis
3. Formation of embryosac or female gametophyte development
4. Fertilization
5. Endosperm development
6. Embryogenesis

Although many times embryo is formed without fertilization, which is known as parthenogenesis. But it is included into vegetative reproduction.

Floral Development

For the genetic and molecular dissection in the research laboratories mainly *Aradibopsis*, Maize, *Petunia*, *Snapdragon* and Tobacco plant are used but most important information about *Arabidopsis* is known.

When flowering is induced in *Arabidopsis* then apical meristem produces flower.

Primary Inflorescence: Main flower bearing stem, which prouuces flowers, independently.

Secondary Inflorescence: Bears flowers along their length, arise from the auxillary buds of cauline leaves on the primary shoot.

Arabidopsis flowers contain four sets of organ arrangement in four whorls:

Flower is a sexual part of plant. In the flower four different types of organs are present which are organized in the four concentric rings, called Whorls.

In the flower, parts are two types:

1) Vegetative parts
2) Sexual part.
   - A) Sepals: This is an outer most whorl that is arranged in cruciform & is green coloured & resemble leaves in their overall shape and cellular composition.
   - B) Petals: Petals have cruciform arrangement present inside the sepals at 45°.
   - C) Stamens: Six stamens are arranged in the inner whorl of petals. Stamens are the male reproductive organs. Each contains a long filament and anther.
   - D) Gynoecium : In the central whorl only one organ, Gynoecium is present which is female reproductive organ. Gynoecium is having three main regions. Basic unit of gynoecium is carpel that is divided into stigma, style & ovary. Stigma promotes pollen germination while style promote directional growth of pollen tube. Ovary is the major structural part. Ovary contains ovule that has attached placenta.

Flower is spirally arranged around the meristem. New bud is formed clockwise or counter clockwise but after choosing the direction, all flowers arise in that direction only.

Flower development is acropetal type where oldest flower is at lower while youngest flower is present at the tip. The development of floral organs is basipetal type where oldest part is at tip & youngest is present at lower portion.

Flower development : Flower organs are developed from primordial in which the cells of different organ are of different types. Outer epidermal cells identify these. Like-

Sepals: Epidermal cells are large, irregular & elongated.

Petals: ,, conical in shape.

Stamens: Elongated cells along the filament & interlocking cells on the surface of the anther.

Gynoecium : This is most complex organ.

Stigma: Papillar cells at the top of the stigma.

Style : Less regular, elongated cells on the surface of style.

Ovary : Very symmetrical elongated cells surface of the ovary.

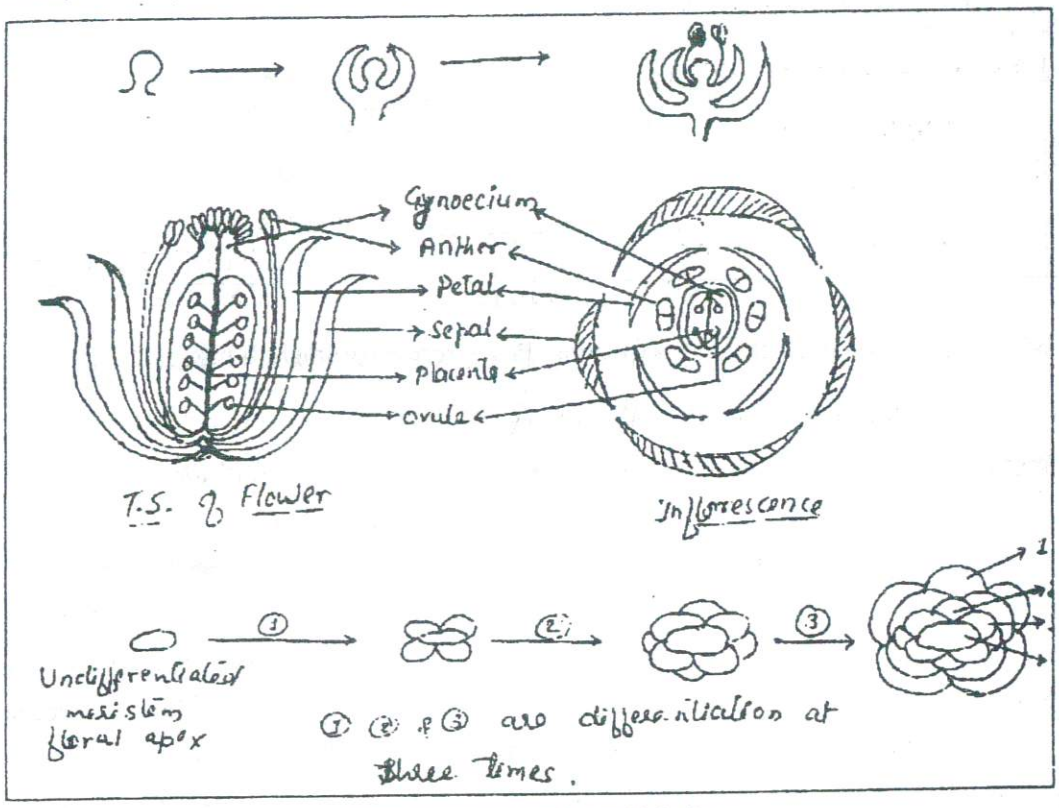


Figure 3.1: Role of Genes in Floral Induction

Phytochrome is actually a protein, so genes are responsible for their synthesis. *Arabidopsis* has 5 phytochrome genes (Phy A, Phy B, Phy C, Phy D & Phy E) each of which encodes a distinct phytochrome protein (multigene family).

Phy A & Phy B are involved in regulating the time to flowering, although neither is essential for the repression or induction of flowering.

The phenotype of the Phy B mutant, which flowers early indicates that Phy B delays flowering time. In contrast Phy A accelerates the time to flowering in response to flower promoting light signals. For example although wild type plants flower early when exposed to one hour light periods in the middle of the dark period, phy A mutants do not. However if the phy A & phy B both are mutant then phy A phy B double mutant will flower even earlier than the phy B mutant.

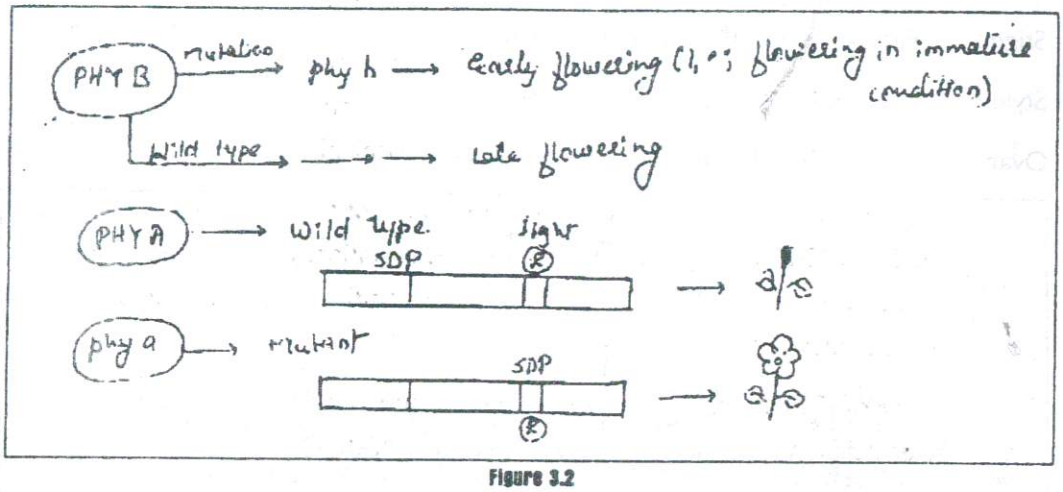


Figure 3.2

Night Break does not affect SDP flowering. Phy A recognizes night break.

phy a + phy b (mutant) ® Earliest flowering

Flowering is under the control of a multifactorial system. These factors are –

Light, Temperature, Phytochromes, GA —(Produced by Genes)→ Phy A-E

The multifactorial control model also suggests that different factors will become limiting for flowering in different plants or under different growth conditions, a result consistent with observations on flowering in many species including *Arabidopsis*. For example – When the GA deficient *Arabidopsis* mutant gat-3 is grown in long days, flowering occurs later in the mutant then in the wild type. When plants are grown in the noninductive short

days, flowering does not occur at all in the mutant, unless exogenous GA is applied. These results indicate that GA is an important component in the multifactorial control of flowering in *Arabidopsis* & is a limiting factor for flowering under short day conditions.

Genetic Expression

The ultimate target of the signals that promote flowering are the genes that define the identity of floral meristems, particularly –

1) LEAFY (LFY) 3) CAULI FLOWER (CAL)

2) APETALA 1 (AP1) 4) TERMINAL FLOWER 1 (TFL 1)

LFY : LEAFY, a gene for floral meristem identity, is necessary and sufficient to convert indeterminate shoots into flowers in *Arabidopsis*.

TFL 1 : It regulates flowering and indeterminary. Its wild type shows increased number of terminal flowers while TFL-1 mutant flower early, the primary inflorescence branches & produces terminal flower.

Three other genes influence the determinary of the floral meristem : AP1, APETALA 2 (AP2) and CAL. For example the ap1 and ap2 mutations enhance the lfy phenotype, cal ap1 mutants are more highly indeterminate and markedly over produce floral meristems, resulting in cauliflower like inflorescence.

LFY, AP1, AP2 and CAL have distinct functions that overlap at several stages of early flower development, possibly acting to enhance each other's activity.

Genetic and Molecular Analysis of Flower Development

When specific analysis of floral development was done then it was observed that genetic mutation can change this process at molecular level. For the genetic and molecular analysis of flower development, ABC model is given.

The ABC Model Describes the Specification of Floral Organ

The genes, which affect the development, are identified by mutation. As a result floral organs are reduced altered and misplaced.

Mutation is done in four genes, which causes misspecification of organ. Mutation in these genes generally affects the development in two adjacent whorls. These genes are following:

1) AP2 2) AG 3) PI 4) AP3

If AP2 is mutated then it affects development in the first and second whorls. Due to mutation in ap2 sepal is replaced by carpel as well as stamens replace petals.

If AG is mutated then it affects the flower development in third & fourth whorls. Sepals replace stamens and gynoecium is replaced by second ag flower, so sepals, petals and sepals are developed.

PI and ap3 phenotypes are similar. If they are mutated then second and third whorls are affected. So AG AP2, PI & AP3 genes are called homeotic genes. Four original ABC mutants are ap^2, ap^3, pi & ag. Each mutatnt shows the homeotic transformation due to which one organ is replaced by another organ.

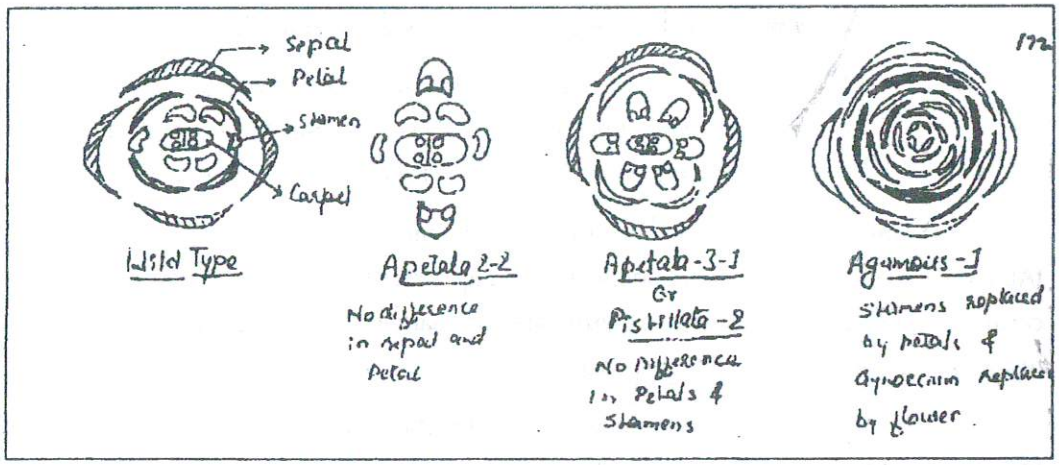


Figure 3.3

A) Floral whorls :

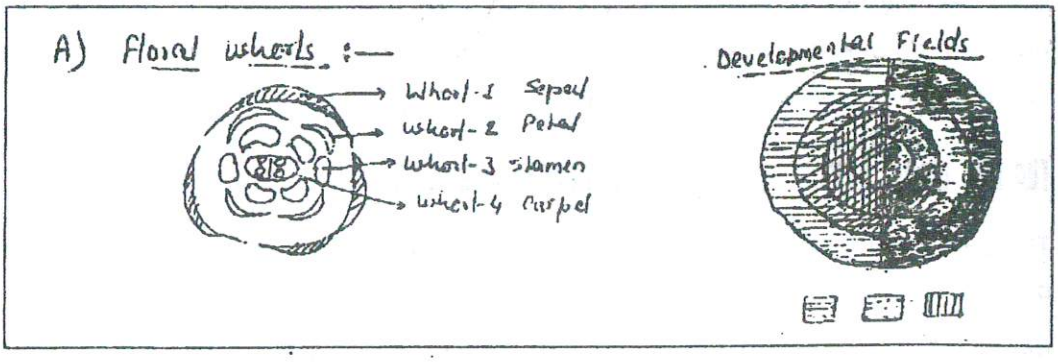


Figure 3.4

Combination ABC model : This is a combinational model where the function of A is sepal development. When A is combined with B then A+B formed the petals, B+C formed stamens while alone C formed the carpel. According to this model the function of A and C are Antagonistic.

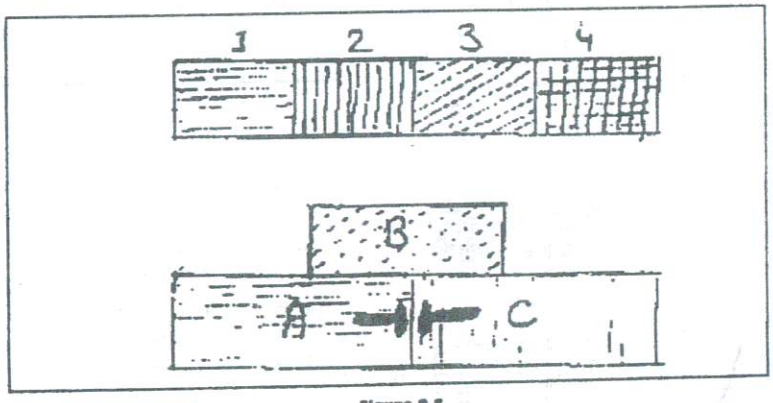
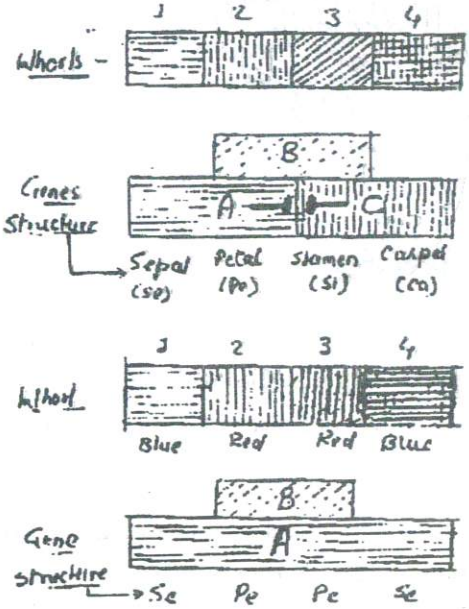


Figure 3.5

Original ABC Genes and Their Proposed Function

A gene (ap2) when mutates then it increase the function of C. In the C gene if ag is mutated then the function of A is increased. AP3 & PI gene when mutate then loss of function of B gene occurs.

The proposed antagonistic functions of the A and C leads to the following predictions :



Wild type : Sepal – Blue
Petal – Red
Stamen – Green
Carpel – Black

If the function of C is removed or mutated then A becomes functional throughout the meristem & forms only sepal and petals.

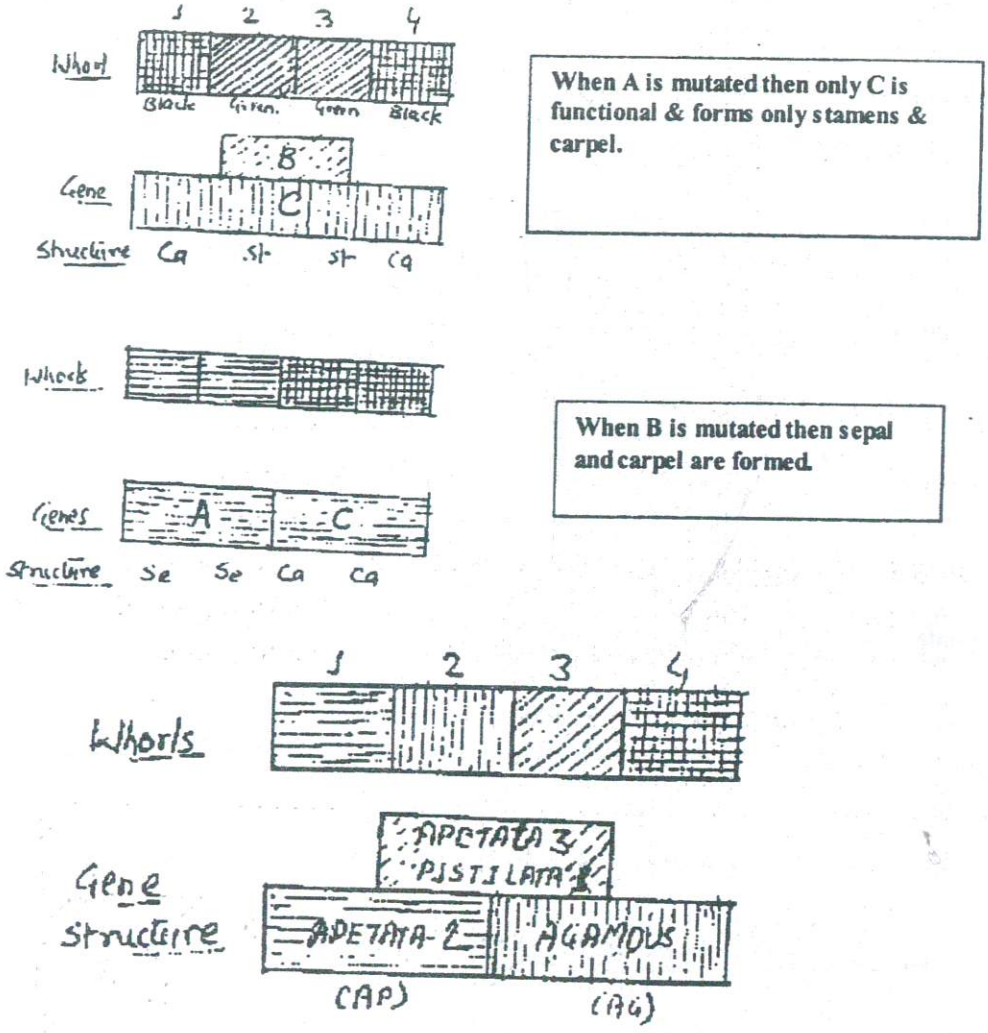


Figure 3.6

Homeiotic Mutant in Antirrhinum

Flower formation in a function of floral Meristem. During flowering, the SAM switches from vegetative to inflorescence development. Flowering may be involving a change from indeterminate to determinate growth. The vast number of flower mutants in *Antirrhinum majus* (snapdragons) and Arabidopsis have been found out.

The mutants that led to the idea that flower development was a pattern formation process were the homeotic selector mutants. In homeotic mutants, the right organs developed in the wrong place. ABC model was proposed to explain how the organs in the whorls are

normally identified and why the organs were misidentified in mutants. It (ABC model) posits three classes of homeiotic genes that determine the identity of various floral organs: class A, B &C genes.

In *Antirrhinum*, the floral homeotic genes are as follows: —

Class A: — SQUAMOSA (SQA): —Ortholog of AP1, AP2 of *Arabidopsis*.

Class B: — DEFICIENS (DEF), GLOBOSA (GLO): — Ortholog of AP3, P1 of *Arabidopsis*.

Class C: — PLENA (PLE): — Ortholog of AG of *Arabidopsis*

In each whorl, a different combination of one or more homeiotic genes is expressed, and it is the particular combination of functions that determines organ identity in each whorl. Class A genes function in Whorls 1 and 2, class B in whorls 3 and 4, and class C in whorls 2 and 3. Thus, in whorl 1, the production of sepals is determined by the class A function alone; in whorl 2, petals are formed as a result of the action of both class A and B functions; in whorls 3, stamens are identified by a combination of class B and C functions; and in whorl4, carpels are the product of the class C function acting alone.

1. **Floricula gene (FLO gene): —**

FLO – If wild type, then,

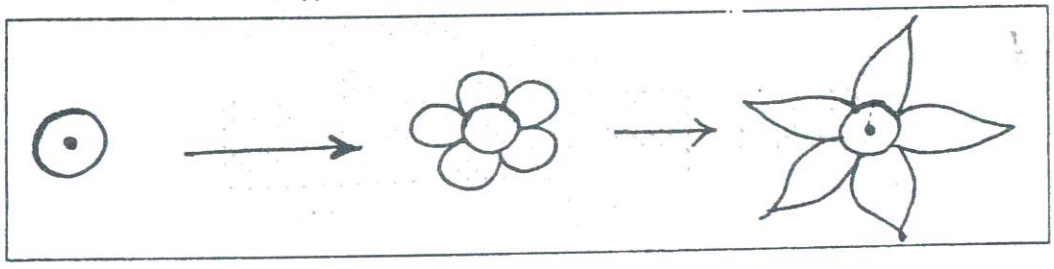


Figure 3.7: Dorsal / Ventral / Lateral Bracts Form

If flo (mutant):

Then, dorsal / lateral / bracts are formed and no ventral bracts are formed. No petals are formed.

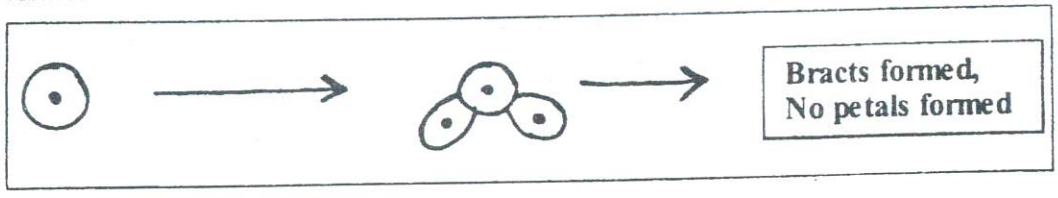


Figure 3.8

2. SQUIA gene: —

If SQUA (wild), then synchronous growth takes place, i.e. dorsal bract, then ventral bract and then lateral bract will be formed.

But if squa (mutant), then firstly lateral bract, then ventral bract will be formed.

Sequence of inner floral/outer floral organs is determined by SQUA gene. FLO gene is epistatic, while, SQUA gene is hypostatic.

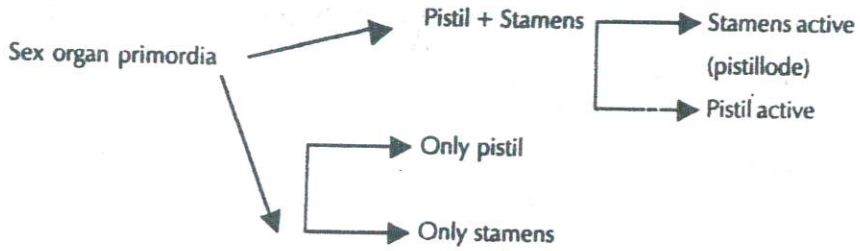
3. Centroradialis gene (CEN) gene: —

It is Ortholog of TFL gene. If CEN (wild) then cymose inflorescence will be there, but cen (mutant) cause racemose (terminal) inflorescence. CEN actually determines the position of flower on floral apex.

Sex Determination

Higher plants are hermaphroditic, bearing perfect flowers with both male and female organs. In other plants, unisexual flowers of both sexes are found on the same plant (monoecious plants) or on different plants (dioecious plants). Unisexuality promotes out breeding, which creates genetic variability. Although only about 10% of plants are known to be strictly monoecious or dioecious, unisexuality arose many times during plant families. Because unisexuality arose many times during plant evolution and is found in 75% of plant families. Because Unisexuality arose independently many times, there is no consistent genetic basis for sex determination in plants.

Unisexuality usually arises instead by arresting or aborting the development of sex organ primordial once they have formed.



The development of unisexuality has been extensively studied in *Zea mays* (maize), if it is a monoecious plant. The male flower is tassel (staminate inflorescence) and the female flower is the ear (pistillate inflorescence). Unisexuality in maize results from the selective arrest in the development of the organs of one sex or the other. Immature inflorescence meristems

are bisexual and virtually identical. In the primary florets of the ear, the stamens initial arrest and the gynoecium continues to develop. In secondary ear florets, both stamens and gynoecia initial abort, leaving a single pistillate floret in each ear spikelet. Just opposite occurs in gynoecium.

Sex determination in maize tassel and ear development has been analyzed genetically, and mutations have been identified that block the feminizing genes in the ear or masculnizing genes in the tassel.

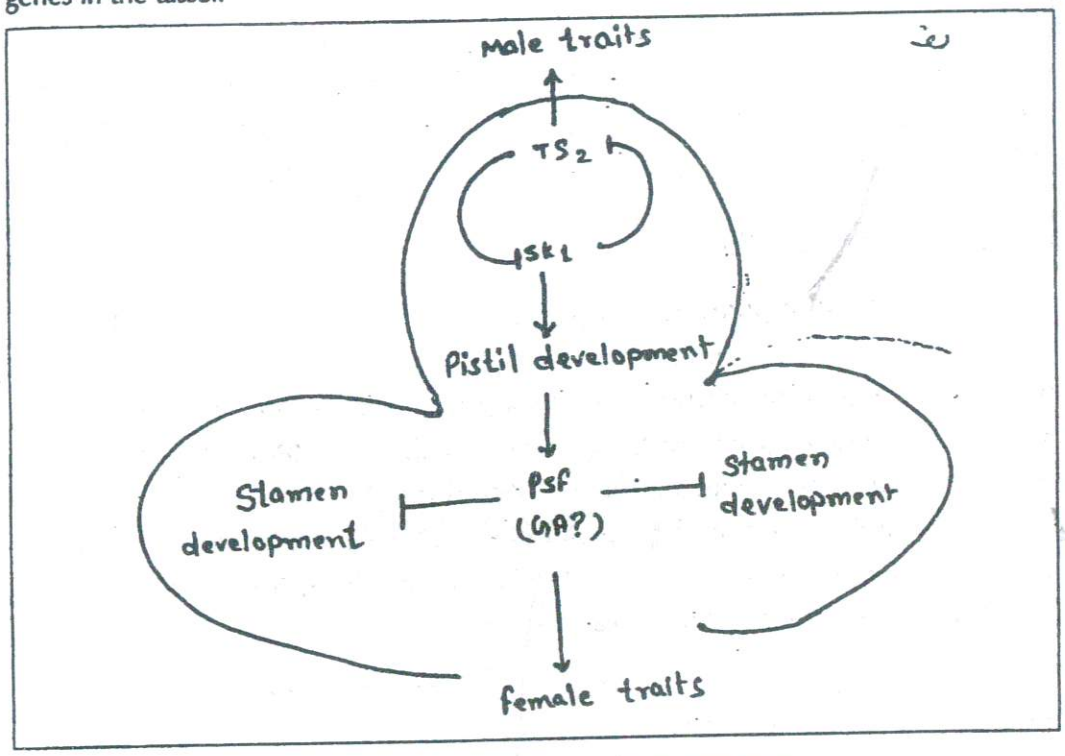


Figure 3.8: Development of Florets on Tassel Inflorescence

SK1 (silk less) promotes development of pistils that produce a pistil specific factor, PSF, which blocks stamen development and promotes the feminization of floral tissue. Sk1 may also prevent the action of TS2 in aborting gynoecia development in primary ear florets. TS2 is thought to block SK1 action in promoting pistil development and PSF production. Ts2 also promotes stamen development and masculinization of floral tissue.

Male Gametophyte

Anther Structure and Micro Sporogonesis

Pollen grains develop into the anther, which are tetrasporangiate. Each anther lobe has 2 sporangia, which are separated by sterile tissue. On maturation, the sterile tissue of mid portion gets destroyed, due to which 2-2 sporangia gets sticked together. In monothecous plants, there is only one anther lobe. Ex.: — *Moringa, Wolfia.* In the young anther, large cells are found below the epidermis, which act as sporogenous tissues. These cells divide periclinal on a plane and forms parietal cell towards outside and sporogenous cells towards center. Parietal cells divide by many periclinal and articlinal division and forms 2-5 anther wall layer. Primaty sporogenous cells take part in mitosis and form microspore mother cells.

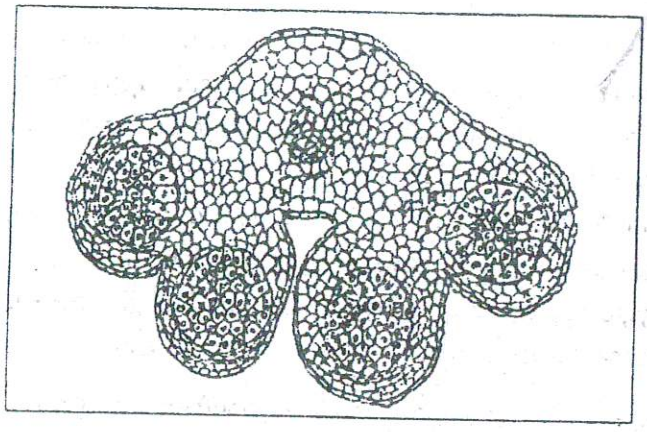


Figure 3.10: T.S. of tetrasporangiate Anther

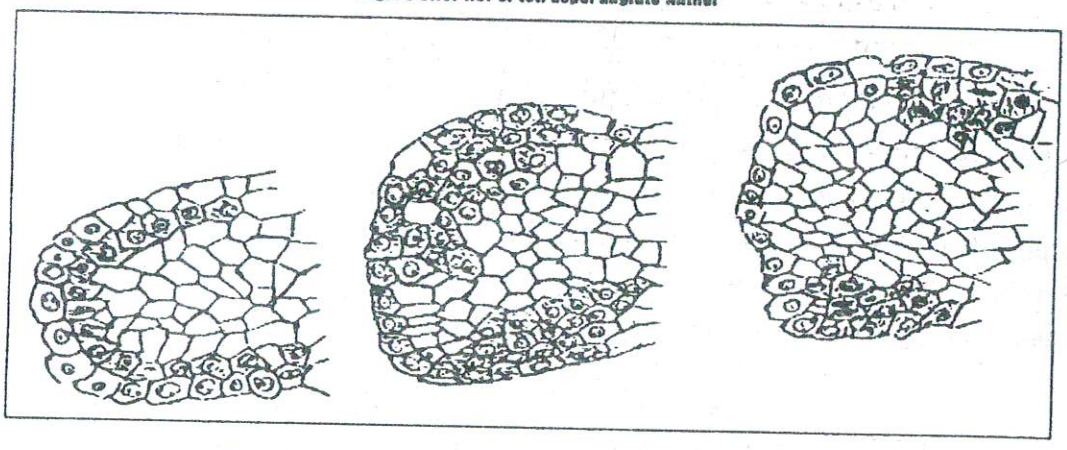


Figure 3.11: Microsporogenesis as seen in Transverse Section of Anther

A. Some hypodermal cells have differentiated into archesporial tissue

B. Primary parietal layer and primary sporogenous tissue have differentiated

C. The cells of the primary parietal layers are in the process of cutting wall layers

Structure of microsporangium

Anther Wall

Anther wall consist of epidermis, endothecium, 2-3 middle layers and 1-layered tapetum.

(a) **Epidermis:** — During the anther development, epidermal cell articlinal divide because internal tissues continuously increase. These cells are flat and flexible. It protects internal tissues.

(b) **Endothecium:** — it is in the form of single layer, but in some plants, it may be multilayered. Endothecium development from parietal layer. These cells are having fibers band. Endothecial thickening is made up of a- cellulose. Lignin is not found. According to Vasil, thickening has only cellulose and a very small amount of lignin. In *Pisum* and *Lens*, small amount of pectin and lignin have also been seen. A fibrous band shows expansion of inner and outer wall and these, being hygroscopic, help in pollen dispersal. Though, these bands are absent in hydrocharitaceae and some cleistogamous flowers.

(c) **Middle layers:** — Cells of middle layers are ephemerals and these are flat. These start destroying on meiosis. In some plants, middle layer can survive for a long time and it develops fibrous thickening. In many species, middle layers act for starch storage, which enters into the anther sac during pollen development.

(d) **Tapetum:** — It is the innermost layer of anther wall, and most of its development occurs in tetrad stage of Microsporogenesis. These keep on surrounding the sporogenous tissue completely, because food materials reach upto the spores by passing through only this layer. Tapetum is made up of 1 layer, which has dense protoplasm and large nucleus. It develops by parietal cells and forms homogeneous layer. But in some species, like- in *Alectra thomsoni*, tapetum is made up of large cells and smaller cells. In *Antirrhinum majus* and *Impatiens glanduliferous*, tapetum is made up of peripheral cells (of sporogenous). According to Maheshwari, tapetum always forms from parietal cells layer. On the basis of behavior, tapetum can be divided into 2 kinds:

(i) **Amoeboid type:** — It is also called as invasive or periplasmodial tapetum. In this, early breakdowns of the inner and radial walles of its cells occur. The protoplast masses move into the anther cavity, which forms a tapetal periplasmodium. These get invested all around the pollens. This tapetum has been seen in *Alisma, Butomus, Tradescantia, Typha* etc. In *Tradescantia,* Mepham and Lane saw a detailed structure of it. During development, its cell organelles get rearranged. The hydrolytic enzymes are released by dictyosomes in the tapetal cells themselves, due to which breakdown of cell wall takes place in meiotic stage. Often these enzymes digest the walls of some sporogenous cells also. During meiosis, the periplasmodium surrounds each microspore mother cell. After meiosis the callose wall around the spores is degenerated, due to which spores get separated and get surrounded in periplasmodial tapetum. The close association between the developing microspores (pollen) and the tapetal cytoplasm (periplasmodium) suggests that it (the latter) transports material into the pollen.

(ii) **Secretory type:** — It is also called as partial or glandular tapetum. This tapetum remains in the original position throughout the microspore development. This tapetum is more common among angiosperms Echlin and Godwin have studied tapetum in *Helleborus foetidus.* At the stage of sporogenous tissue, the tapetal cells possess mitochondria, plastids, a number of spherical bodies (pro-ubish bodies), and dictyosomes with only a few vesicles. The cell walls are relatively thin at stage. During meiosis, the cytoplasm appears denser and cell wall also becomes thick. During meiosis, there is further increase in the number of pro-ubish bodies and the size of the nuclei. These bodies are more aggregated near to the anther cavity at the tetrad stage, the number of ribosome further increase and that of microtubules and plastids decreases. Ribosome surrounds the pro-ubish bodies. The dictyosomes get associated with a large number of vesicles at the periphery soon after the microspores have separated, ribosome become more prominent, and the inner region of the cytoplasm contains abundant dictyosomes. Now, the pro-ubish bodies eventually pass out through the tapetum and into the space between the cell wall and the membrane, they rapidly become coated with Sporopolenin, and are now called ubish bodies. At this time, cell wall disappears and the cytoplasm has completely disorganized. These ubish bodies take part in the organization of exine of pollen.

In *Nigella sp.* (Secretory type of tapetum), at the tetrad stage, the inner tangential walls of the tapetum disintegrate and a new membrane, called tapetal membrane, is formed around the tapetal protoplasts. In compositae, where the membrane is Plasmodial, these form a new layer near to the middle layer. Tapetal and extra tapetal membrane are aceto lysis resistant. These membranes enclose the developing pollen grains, in the

Nuclear Behaviour in Tapetal Tissue

In the tapetal cells, the total DNA content of the tissue increases rapidly during the meiotic prophase. This is achieved in one more of the following ways: —

(I) **Multinucleate condition:** — Such a situation arises, when during mitosis, nuclear division (karyokinesis) is not accompanied by wall formation (cytokinesis). Ex: — *Mimusops elengi.*

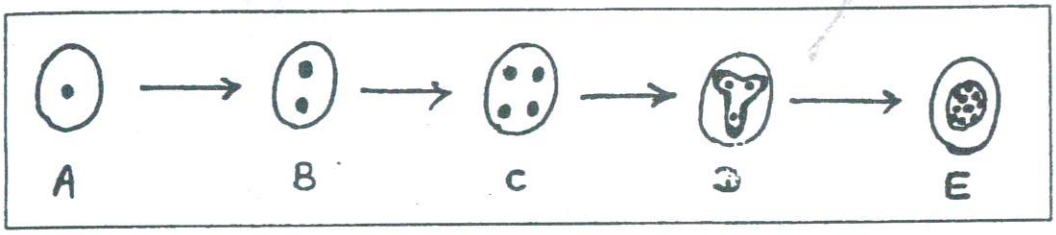


Figure 3.12: Tapetal Cells: Mitosis and Fusion of Nuclei

(II) **Endomitosis:** — This is a type of mitosis, in which the chromosome duplication and chromatid separation takes place within the intact nuclear membrane and without the formation of a spindle and chromosome separation.

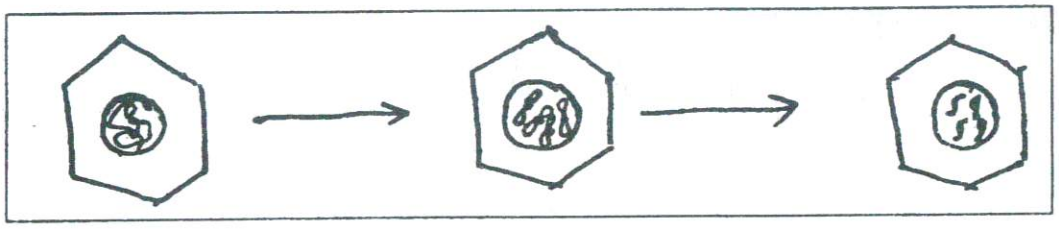


Figure 3.13: Absence of Spindle and Cytokinesis

(III) **Formation of restitution nuclei:** — In this process, mitosis goes on normally upto the early anaphase stage but the two sets of chromosomes finally get incorporated into a common nuclear membrane to form a restitution nucleus. It has the chromosomes doubled.

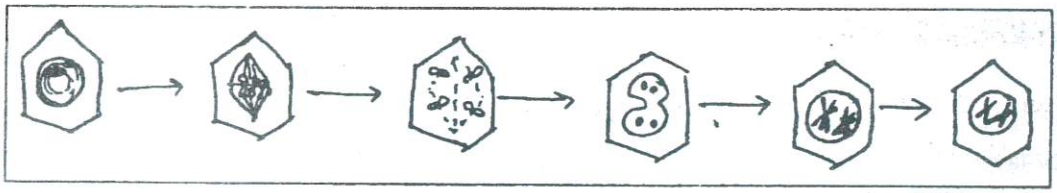


Figure 3.14

(IV) Polyteny: — This refers to an increase in the number of chromonemata per chromosome. This mode of DNA increase does not alter the number of chromosomes per nucleus.

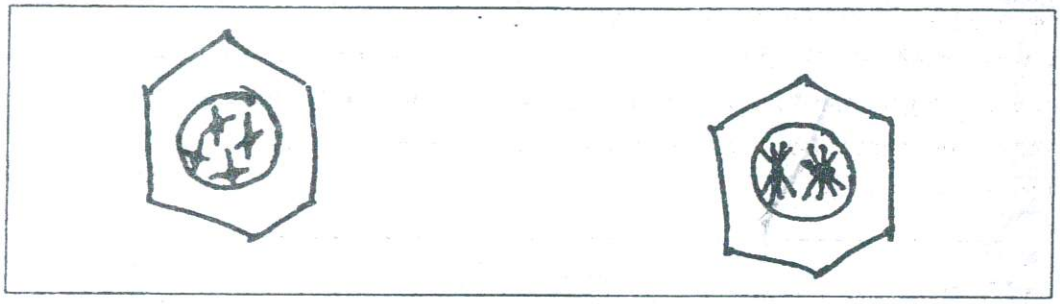


Figure 3.15

A. One chromosome is having only 2 chromatids

B. Each chromosome possesses 4 chromatids

In this process, number of chromonemata gets increased in chromosomes, but number of chromosomes becomes constant.

Role of Tapetum

1. It transports nutrients upto spore tetrad during meiosis.
2. Plasmodial tapetum shows callose activity, which separates spore tetrad.
3. Sporopolenin controls pollen compatibility.
4. In post meiotic period, tapetum forms pollen wall.
5. Tapetum transports pollenkitt and tryphine. Pollenkitt shows hydrophobic nature and tryphine shows hydrophilic nature.
6. In pollen wall, both types of compounds: — of tapetal cells and of gametophytic are present, which shows allergy because pollens also act as allergens.

Sporogenous Tissue

Sporogenous tissue directly acts as pollen mother cell. In it, meiosis division takes place, which changes diploid cells into haploid cells. Meiosis-I is a reduction division, in which number of chromosomes is reduced to half, while meiosis-II is a mitotic division. Thus, from one mother cell, 4 microspores are formed. Upto the beginning of meiosis, plasmodesmata connections are found in between tapetum and PMC (Pollen Mother Cell), but after the beginning of meiosis in PMC, these connections are lost callose starts depositing in between plasma membrane and original wall. Plasmodesmata connections charge into channels, which shows the maximum development upto pachytene. Due to these channels, cytoplasm can easily move within the cells. In such conditions, these show synchronous relation. But during mitotic division, synchrony is not seen, because channels get blocked. But at the last of meiotic prophase, callose wall destroys channels. Hence, new channels get formed in microspores.

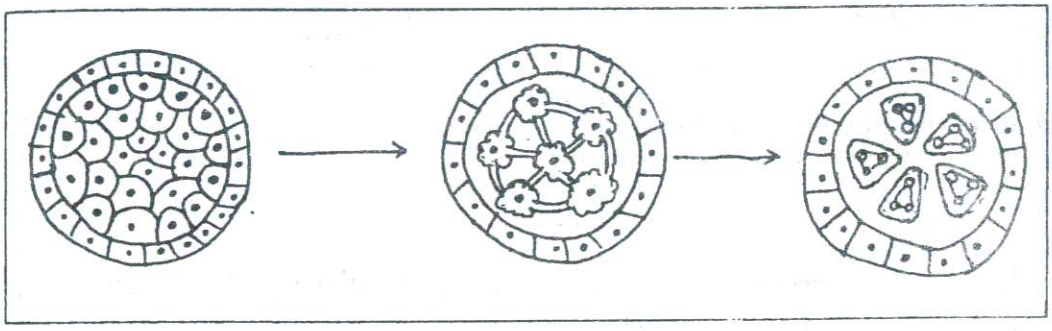
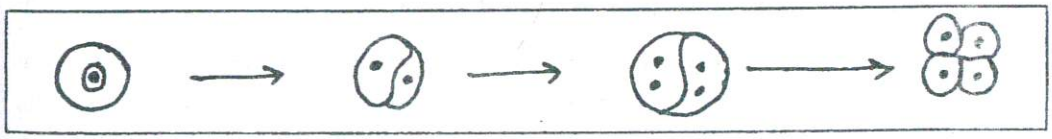


Figure 3.16: Formation of Spore Tetrad

In this, cytokine sis is either successive or simultaneous type: —



Successive type cytokinesis

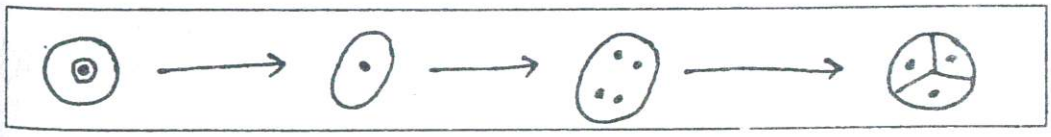
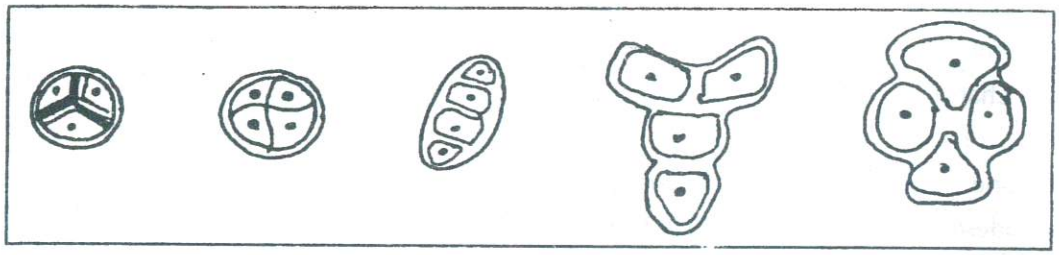


Figure 3.17: Simultaneous type Cytokinesis

These spores get arrangement in different manners:—



Figur 3.16: Types of Tetraspore Arrangmnt

A. Tetrahedral

B. Isobilateral

C. Linear

D. T-shaped

E. Decussate

Thus, different structures of microsporangium are formed as follows: -

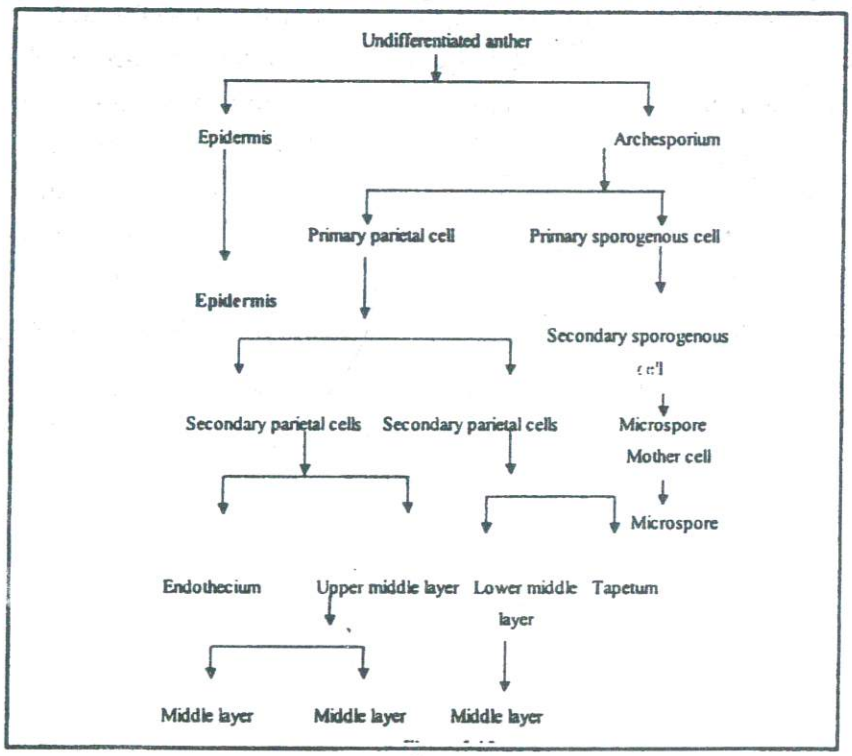


Figure 3.18

Pollen Development and Gene Expression

In the anther, uppermost layer is epidermis, which is followed by wall layer and tapetum. In the center, sporogenous cells are present, which are known as archesporial cells. These are diploid cells. Later, archesporial cells give rise to microsporocytes. It is interesting that the genetics of sporocytes is different than after somatic cells. Dawe and Freeling studied cell lineage system in maize. They studied that pigmented sectors created by Ac-ds transposon; when ds elements are removed from specific loci, then pigmentation disappears. These genes are formed, and then they form tetrads by dividing mitotically. During pollen development, tapetum secretes callose enzyme. This enzyme digests callose cell wall, so microspores separate. Actually, microspores are interconnected through callse wall. After separation of microspores, these divide through asymmetric mitotic division, which is called as pollen mitosis- I. It produces 1 small generative cell and 1 large vegetative cell. Vegetative cell surrounds the generative cell. The vegetative cell has tube nucleus and metabolites that power the pollen germination and tube elongation. Later, generative cell again divides and produces 2 sperm cells. The tube nucleus is transported through elongating pollen tube, and the sperms are delivered when pollen tube enters micropyle. Eady explained that generative and vegetative cells are differentiated due to a gene lat 52. It is studied in tomato. This gene is activated only in vegetative cell. They observed that the transfer of lat 52 gene, in vegetative cell, is the result of unequal mitotic division. They produced transgenic tobacco plants, which is having lat 52 GUS promoter and cultured the microspore of these transgenic plants. The high level of colchicines is provided to block the pollen mitosis-I. They observed that it does not prevent lat 52-GUS expression, and this gene expressed at the same time as it expressed in the untreated cells. It means unequal division is required for asymmetric expression. At lower colchicines level, division occurs, but it is not symmetric, because both the cells have large vegetative nucleus, and lat52-GUS was expressed in both the cells.

It means, for the formation of sperms, lat52 should not express in generative cells. Similarly, after formation of microspores, the exine is formed through tapetum secretion, which is formed of sporopollenin polymer. It means all the spores have similar natured exine, and shows same pattern determinants. This sporopollenin is made up of flavonoles and lipids. It is also responsible for interaction between pollen and stigma. In the tobacco, male sterility is the result of pollen suicide transgene. So, pollens are formed, but become inactivated later on. It is not clear that which signal can transfer it from phase first to phase second, but TA56 gene is isolated, which is expressed in non-sporogenic tissue and synthesize

thiolendopeptidase. It starts cell specific degeneration. This gene expression is transcriptionally controlled. The formation of pollen grains is also related with gene expression in gametophytic tissue; like- LAT 52 synthesizes trypsin inhibitor, which play an important role in pollen development. It by using antisence RNA, lat 52 expressions is inhibited, and then pollen development gets inhibited.

Male Sterility

In the plants, nuclear or cytoplasmic genes control male sterility. This process damages the sperms and in that case, either germination does not occur or pollens do not get formed. Many causes are giving for male sterility, which are:

i. Anther suppression, abortion, phyllody, petallody and pistillody.

ii. Abnormal meiosis or gametophytic development, which is indicated by meiocyte or gametophyte. In this condition, meiosis fails and genetically imbalanced spores are formed, or the development of gametophyte may be abnormal. It may be determined by sporophyte.

iii. Pollens are normal, but anther dehiscence failure.

iv. Premature dissolution of callus.

v. Fungal or viral infection.

But, here, we are concerned with second type of male sterility, in which non-viable pollens are formed. The gametophytic pollen sterility is generally due to meiotic abnormality and Sporophytic due to cytoplasmic/genic.

1. Genetic male sterility: — This type of male sterility is controlled by single gene'M'. The 'M' shows fertile male. In this case, F_1 generation will indicate male fertility, but after selfing, in F_2 generation, 3:1 ratio is obtained in fertile and sterile.

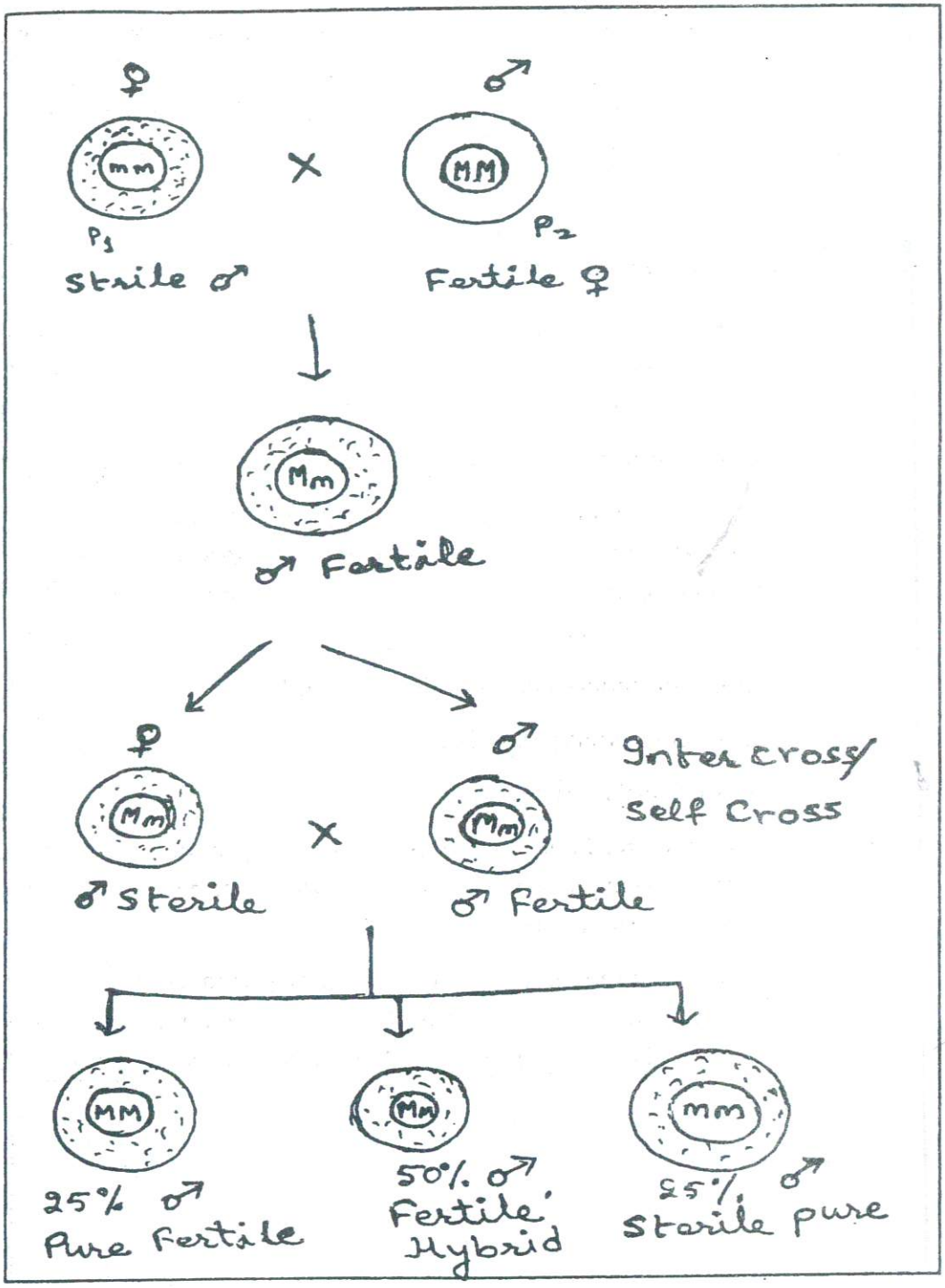


Figure 3.20: Inheritance of genetic male sterility in plants

Cytoplasmic Male Sterility

First time, it was determined by Rhoades in maize. He proved that male parent controls male sterility only and nuclear gene couldn't affect it. During the cross, F_1 generation is sterile (cross between sterile and fertile male). When the back cross is carried out with male fertile, then also in the next generation, sterile males are obtained. It shows that nuclear genes cannot control the fertility.

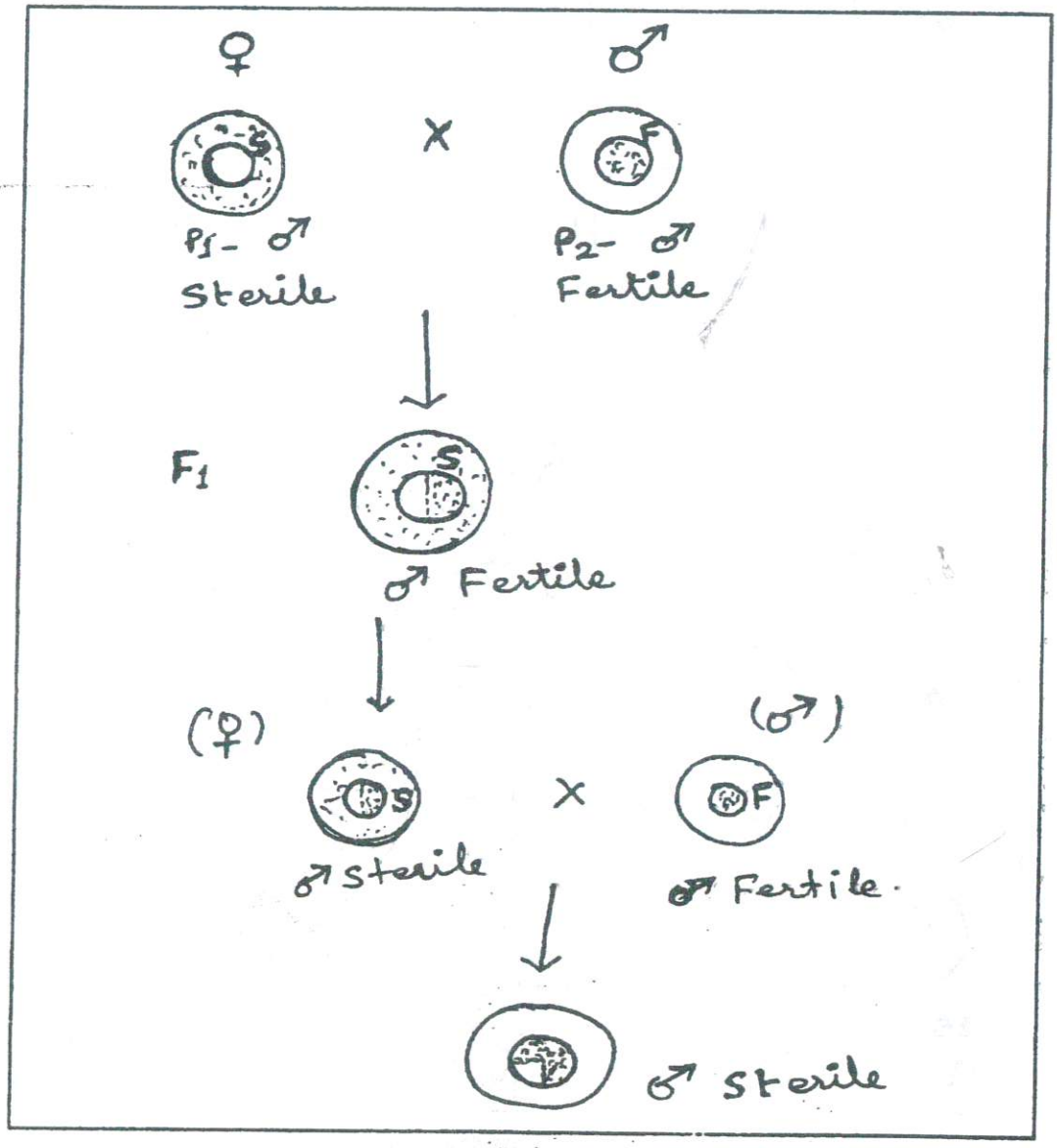


Figure 3.21: Maternal Inheritance of Cytoplasmic Male Sterility in Plants

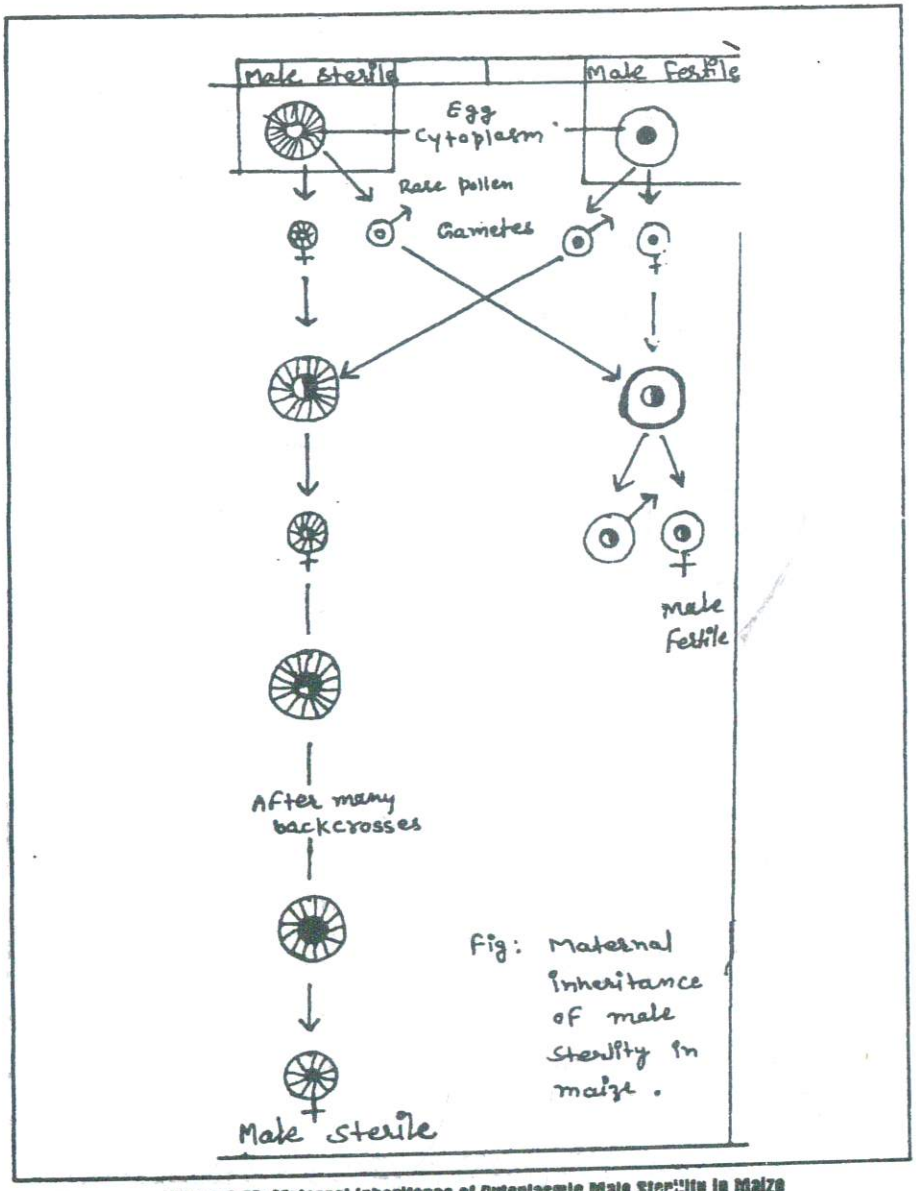


Figure 3.22: Maternal Inheritance of Cytoplasmic Male Sterility in Maize

Cytoplasmic-genetic Male Sterility

In some cases, it is observed that male sterility is controlled by cytoplasm, but in the nucleus, restore gene is also present, which can restore the fertility. If female parent is male sterile, then nucleus of male parent will determine the phenotype of F_1 because a fertile male is having the restore gene 'R', then in F_1 generation, although cytoplasm is sterile in F_1 generation, although cytoplasm is sterile in nature, but due to 'R' gene, it will be fertile, but if male

parent is having 'r' gene, then during the test- cross rr, the 1:1 ratio is obtained for fertile and sterile.

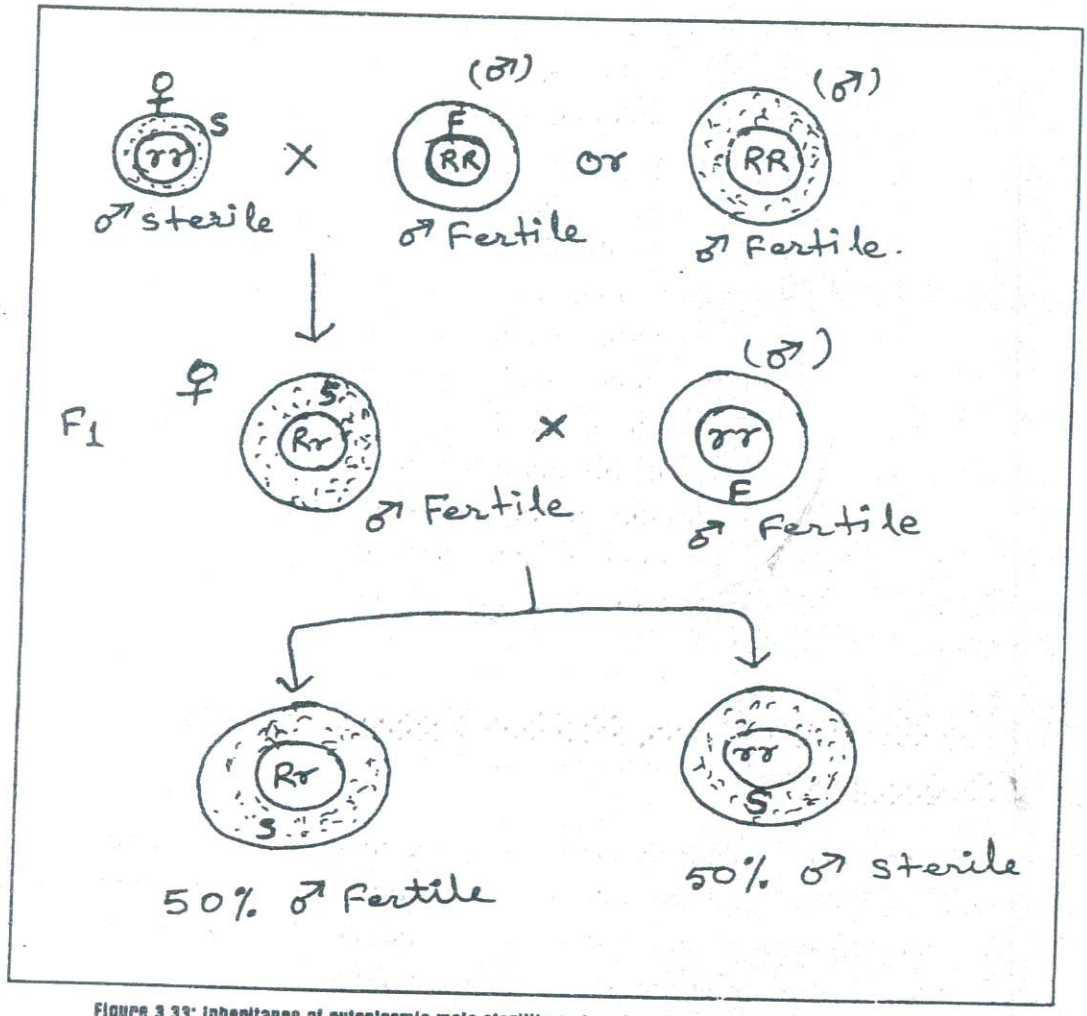


Figure 3.33: Inheritance of cytoplasmic male sterility and restoration of fertility due to restorer gene

Sperm Dimorphism and Hybrid Seed Production

In the pollen tube, generative nucleus divides and forms 2 male gametes. Both male gametes interconnected with each other, and later CaSS indicated that vegetative nucleus is also present in contact with male gametes. So, these 3 cells more with each other in pollen tube, which is known as male germ unit. On the basis of MGU (Male Germ Unit), it is indicated that the fate of 2 sperms are morphologically different. It is proved with the help of computer-assisted 3D-structure of MGU. So, the phenomenon through which in the same pollen tube, morphologically differentiated sperms are formed is known as "sperm

dimorphism". When its 3D structure was reconstructed, then in the *Plumbago*, it has been seen that the 2 sperms of a pair do not differ only in cell size and shape, but they also differ in size of the nucleus and number of cytoplasmic organelles. The smaller sperm, which is not associated with vegetative nucleus, is having approx. 24 plastids and 40 mitochondria, while larger sperm, which is closely associated with vegetative nucleus, is not having the plastids, but approx 256 mitochondria present. It confirms that sperm dimorphism is the important character of angiosperm. Even in those plants, where plastids are absent, also show sperm dimorphism. But in grasses, this dimorphism is absent. In the barley, both the sperms are essentially absent. In the barley, both the sperms are essentially isomorphic. Actually, the formation of MGU is an important character of plants, because it allows the targeted fusion of female gamete and polar nuclei. Sperm to sperm connection is also important, because it reduces chance of heterofertilization, which is common in maize and barley, where sperms are isomorphic. The dimorphism can be applied for hybrid seed production, because if the individual sperm is recognized, which is going to fuse with female gamete, then in this sperm, any gene can be transferred through genetic engineering, and through in vitro fertilization hybrid seeds are produced, which will be helpful in the improvement of crop plants.

Pollen Germination, Pollen Tube Growth and Guidance

When pollen mature in the anther, then these are released from anther, and transferred at stigma through pollination. At stigma, pollen germinates and forms the pollen tube. It penetrates stigma, and elongates in the style. It is transferred upto embryo sac and release the male gamete in the embryo sac, which initiates fertilization.

Pollen Germination

Normally, when pollen transfers at the stigma, then develops single pollen tube. The emergence of pollen tube from pollen is known as pollen germination. Generally, pollens produce single pollen tube, but sometimes it shows many pollen tubes, which is called as polysiphonous condition, like: - In *Althaea rosea*, 10 pollen tubes are seen, but later, growth is indicated by only one tube. In such plants, where pollen gains are present in tetrad form or Pollinia, many pollen tunes are produced. In the Amentiferae, pollen tube may be branched. If suitable moisture is available for pollen, then the germination is initiated;

due to hydration of cytoplasm, it is activated, and nature of cellular organelles change. Some important changes noted in *Lycoprsicon* are as follows:

1. The ER, which is present in stacks, becomes free.
2. Many vesicles are formed. Small are related with pectocellulosic wall and large with callosic wall.
3. Thin callose layer is formed. It is the dictyosomes activity, which is formed below germ pore.
4. Ribosome aggregate and form polysomes.
5. Lamellae are formed in the plastids.

But it does not change mitochondria, generative cell and vegetative cell. It is also observed that during pollen maturation, germination specific RNA transcripts, and stored in inactive form. At the time of germination, it is activated, and germination takes place. In *Lycoprsicon*, the small pollen tube emerges out, then generative cell and vegetative nucleus transfer in it, and pollen tube shows 4 zones: —

(i) Apical zone: — It is swollen and having many vesicles, but cell organelles are absent.

(ii) Sub-apical zone: — Here, cytoplasm is cell organelles rich.

(iii) Nuclear zone: — Here, generative cell and vegetative nucleus is found.

(iv) Vacuolar zone: — It shows transition between active and inactive cytoplasm.

Pollen Tube Growth

Pollen tube comes out through germ pore, where the exine layer is very thin. The tip region rapidly grows. In a growing tube, cytoplasm is present in the apical region, and then a large vacuole is present. To restrict the entry of cytoplasm in the apical region, callose plugs are formed at regular distance. So, the pollen tube divides into many compartments. Plugs originate at the inner side of the wall and grow towards the center and finally, it seals the tube and the cytoplasm is left behind. The plug is gradually degenerated. This partition prevents the backflow of cytoplasm and transparent, which is called cap block. It is present during the tube growth. Behind the cap block, cell organelles are present, like mitochondria, Golgi body, ER, amyloplast etc. The pollen tube wall is 3 layered. Outer is made up of pectin, middle pectocellulosic and inner is callosic layer. Pectose is rich in β-1,4 glucans and callose is rich in β-1,3 glucans.

Pollen tube is not having proper plasma membrane. For the pollen germination and pollen tube growth, following factors are there:

(i) **Carbohydrates:** It is needed to control osmotic press and used as respiratory substrate if pollen are placed in H_2O, then they may burst, but if they are kept in sugar solution, then bursting is prevented.

(ii) **Boron:** Generally, pollens are Boron-deficient, while stigma are Boron-rich. So, stigma initiates the pollen germination. Actually, Boron reduces bursting and enhances sugar transportation. It also activates enzymes.

(iii) **Calcium:** It is seen that when many pollens are kept with each other, then pollen germination gets increased, because pollens secrete the Ca^{+2} ion, which is absorbed by other pollens. It is known as population effect.

(iv) **Flavinoid:** Pollens having higher amount of Flavinoid. If Flavinoid deficiency is created, and then pollens cannot germinate.

(v) **Enzymes:—** Pollens having cellulase, pectinase and callase, these increase the rate of tube elongation.

(vi) **Plant hormones:—** Auxins may promote the pollen tube growth.

(vii) **Physical factors:—** The 20-30°C is best temperature for tube growth.

Pollen Tube Guidance

Pollen tube when develops, then this pollen tube enters in the style, and then enters in ovule. Then, it takes part in fertilization. So, the growth of pollen tube towards unfertilized eggs occurs in the presence of some guiding signal and how pollen tube shows response? The pollen tube which continue, to grow, is having polysaccharides, glycoprotein and glycolipids. In the style mainly, arbinogalactin is present. This compound is playing important role in guidance. Cheung proved that in vitro condition, this compound stimulates the pollinated pistil and kept in agar medium, then pollen tubes grow in all the directions. When this glycoprotein extracted from style and transferred in medium in one direction, then pollen tube grows in a particular direction. It shows that this glycoprotein is responsible for guidance. When this glycoprotein is deglycosylated, then the direction is not demonstrated. It shows that glycosyl group is responsible for direction. It is also reported that in the style, high concentration of glycoprotein is present, where pollen tube forwards. Its highest concentration has been observed in the base of style and least at the top. It

shows a gradient from top to bottom. It means pollen tube moves from lower to higher concentration of glycoprotein. When through genetic engineering, antisense RNA was transferred in the style cells, which interfere in the production of glycoprotein, then in this condition, the pollen tube growth was slowed down in the style, and fertility reduced. [In the ovule, pollen tube guidance occurs through Ca^{+2} ions.] In the pollen tube slowly, the amount of Ca^{+2} ion increases. It shows the influx of Ca^{+2} ions. Supplying Ca^{+2} ions can reorient the pollen tube growth. As cytosolic Ca^{+2} level increases slowly, pollen tip bends towards the direction of Ca^{+2} ion. It has been seen that if mutants are taken, then pollen tube does not show preference, while wild type shows preference. If ovule cannot develop the embryo sac, then it cannot attract pollen tube. It means embryo sac itself attracts pollen tube. Similarly, PoP2 and PoP3 mutants also indicate sterility. It means these are also related with synthesis of attracting factors. PoP2 and PoP3 functions in both male and female parents. It means it codes such molecules, which is present in pollen tube and pistil both.

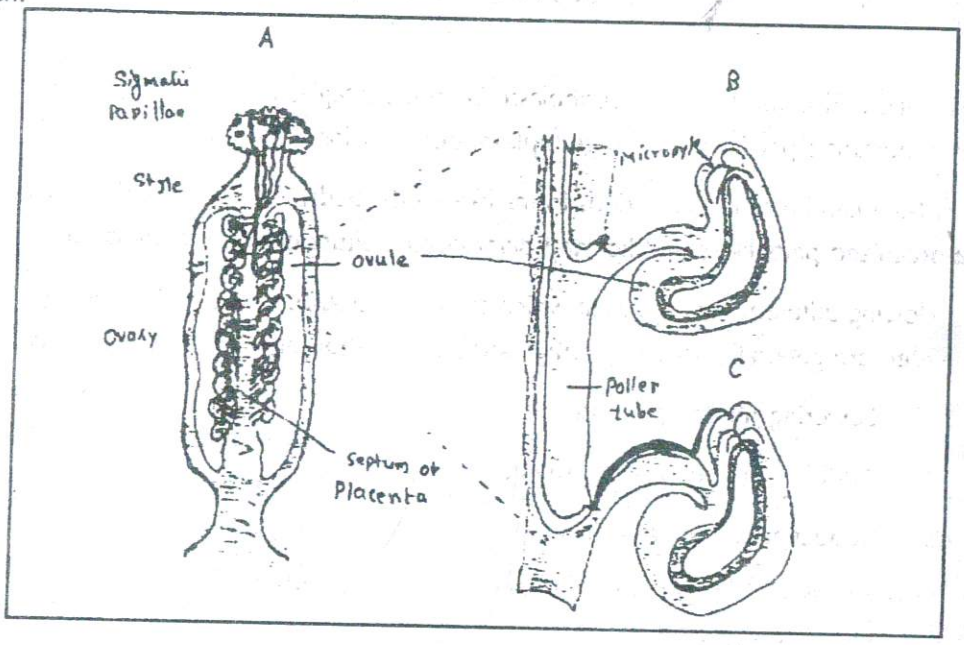


Figure 3.34: Pathway Followed by Growing Pollen Tube in Arabidopsis

Pollen Storage

For the artificial pollination, pollens are collected and stored. For the storage, following processes are adopted:—

Dry and Cold Storage

Controlling temperature and humidity can increase the longevity of pollens. For the storage of viable pollen, the optimum temperature is -5°C to -10°C and humidity is 25-50% like the pollen of mango remain viable for 8 days, but at 5°C and 25% moisture, viability is maintained upto 5 months. In the apple, it is maintained upto 9 years at 17°C. If some anhydrous substances are mixed with pollen, then it is beneficial, like powdered Lycopodium spores and egg albumin. King proposed freeze- drying method for storage, in which after freezing, water is removed from pollen and kept in vacuum.

Cryogenic Storage

Germ plasm and plant genetic resource conservation is an important thrust area of biotechnology. Several national and international institutes are involved in it like – NBPGR (National Bureau of Plant Genetic Resources), IBPGRI (International Plant Genetic Resources Institute).

For storage and conservation of germplasm various strategies have been developed. The most important aspect here is that germplasm does not lose its viability.

Germplasm can be stored in various forms like seeds, buds, protoplasts, cells, tissues etc. Well-organized parts like shoot lips and plant lets in culture can also be used for storage.

The following different techniques in which the growing stage and growth of germplasm is suspended are generally used for storage and conservation of germplasm. These are:

1. Lowering the temperature.
2. Addition of chemical retardants or hormones.
3. Reduction in oxygen concentration.

In such methods limited growth is allowed, so they are effective for about a year and periodic renewal is required.

But the most popular and most effective method of long-term storage of cell cultures is cryopreservation. This storage is done at low temperature using liquid N_2. By this method storage can be done virtually for infinite periods.

The steps in typical cryopreservation procedure for cell culture are shown below:

Cell culture under standard conditions

↓

Pre grow for 5 days in medium containing mannitol

↓

Cryoprotect with 0.5% to 10% DMSO + 1M sucrose (or glycol) in culture medium

↓

Transfer cells and cryoprotectant solution in ampule : Freeze at 1°C for 1 min to -35°C : Hold for 40 min, transfer to liquid N_2

↓

Store the ampule in liquid N_2 can. To revive the germ plasm.

↓

Thaw ampule in warm water.

↓

Pour into semisolid medium.

↓

Transfer recovered cells to liquid medium.

Storage of plant protoplasts is recommended in many cases and survival level is upto 75%.

The steps for the cryopreservation of shoot tips are described below:

Plants under standard conditions

↓

Dissect shoot lip

↓

Pre grow for 2 days on medium containing 5% DMSO.

↓

Cryoprotect with 10% DMSO in culture medium.

↓

Pick shoot tip by a hypodermic needle & dip into liquid N_2 immediately transfer to ampule containing cryoprotectant : Freeze at 0.5°C, 1 min in liquid N_2.

Thaw in warm water.

↓

Pick tip and dip into medium at 25°C → place on semisolid medium.

↓

Revived

Pollen needed for experiments can also be cryopreserved. The best stage of cells for cryopreservation is early exponential phase. Lag phase & stationary phase cells are susceptible to freezing injury, callus cultures, cell suspensions, zygotic embryos and somatic embryos can all be cryopreserved.

Organic Solvents

The pollens can be kept in organic solvents, like:— acetone, benzene, ethanol, ether, chloroform and phenol. It is observed that these are not toxic for pollen.

Some practical application of pollen storage are as follows:—

(i) To hybridize plants that flower at different times and locations or show non-synchronomous flowering.

(ii) To provide a constant supply of short- lived (recalcitrant) pollen.

(iii) To facilitate supplementary pollination for improving yield.

(iv) To eliminate the need to grow male lines continuously in breeding programmed.

(v) To ensure the availability of pollen throughout the year without using nurseries or artificial climate growth rooms.

(vi) To obviate the variability incidental to the daily collection of pollen samples.

(vii) To study pollen allergens and the mechanism of self-incompatibility.

(viii) To provide material for international exchange of germplasm.

(ix) Long-term germplasm storage, especially of unique genotypes.

Pollen Allergy

Pollens are found in free state in the atmosphere, which take part in reproduction by anemophily. But it has been seen that these pollens present in air cause allergy in many people. Therefore, these have also been kept in the category of allergens. These enter into the respiratory system with breathing and thus they show allergy. Actually, proteins are found on the exine of pollen, which are deposited on the exine of pollen by tapetum. Their main functions are to control incompatibility. But these proteins in human act like external proteins and show antigenic reaction, due to which immune system becomes active. In human body, immune system depends on IgG, IgA, and IgE, which form antibodies. Actually, IgA shows primary defense line, which are capable of destroying proteins

like pollens, but if due to any reason, pollen proteins enter inside the body by crossing primary defense line, then IgE becomes active acting like secondary defense line and for pollen proteins, active site is formed on IgE forever. When again these pollens enter into the body second time, then pollen proteins attach with IgE by which IgE starts secreting histamine which starts mucous secretion, sneezing, destroying cells, and many other processes. This is an example of pollen hypersensitivity. These activities start just after a few seconds of pollen entrance.

Anti-histamine medicines are taken for the treatment of this allergy. Sometimes, pollen allergy may lead to death. Therefore, it is better to prevent from pollens.

Pollen Embryo

Pollen embryogenesis can be defined as production of a haploid individual by development of pollen containing the male nucleus. The elimination or inactivation of egg nucleus occurs before fertilization. This occurs in vivo. On the other hand in vitro haploids can be induced by using anther androgenesis (also called androgenesis) or from cultures of individual pollen grains. These in vitro methods are very successful for haploid production. Guha & Maheshwari published the first report of anther culture from Datura innoxia.

Technique of Androgenesis

For androgenesis important requirements are:

(1) Healthy plants grown in controlled environment.

(2) Knowledge of pollen ontogenesis.

(3) Temperature treatment – arrests exiting metabolism & shifts to new pathway of embryogenesis.

(4) When androgenic embryos are formed, then their development into plants depends on various factors like – media composition, light, temperature, differentiation of embryo primordial & transfer to green house.

Thus, young plants grown in controlled environments are used to select bud of right stage. According to some workers best anthers are ones in which uninucleate microspores midway between release from tetrad and first pollen grain mitosis are present.

The selected buds are surface sterilized and anthers are removed from them along with their filaments. There are dipped in ethyl alcohol and tested for correct stage of pollen. If

the stage is correct then other anthers from bud are removed and placed horizontally on medium. Care should be taken not to injure anthers.

In different approach pollen from anthers are used to prepare pollen suspension for cultures.

The cultures are maintained in alternating periods of light (12-18 hr, 5000-10,000 lux/m^2) at 28°C and darkness (12-6 hr).

The wall tissues turn brown & burst after 3-8 weeks due to pressure of callus or plants. Individual plants or shoots are transferred to rooting medium & rooted plants are transplanted to pot with soil in green house.

Factors Affecting Pollen Embryo Formation

Number of factors influence anther culture :

(1) **Genotype of donor plant:** Thus, highly responsive genotypes should be selected. The genotype can be improved by breeding.

(2) **Anther wall factors:** Wall factors effect anther culture. So many workers use "Nursing effect of whole anthers for androgenic development of isolated pollen of number of species.

(3) **Culture medium:** The requirement of medium is different for different genotypes & for different age of anther. For most solanaceous species complete nutrient medium of Nitsch or MS medium is required. Iron plays important role in pollen- embryo development. For non-solanaceous plants addition of growth adjuvant (growth regulators, complex nutrient mixtures coconut milk or yeast extract) is required N6 medium of Yu-Pei is also a good androgenesis medium. Sucrose is essential & activated charcoal stimulates androgenesis in some systems.

(4) **Stage of microspore or pollen development:** The stage varies with the species.

(5) **Effect of temperature and light:** Temperature shock enhances androgenesis (cold at 3-5°C for 72 hours). Frequency of haploids is better in light. But isolated pollen are sensitive to light.

(6) **Physiological state of donor plant:** Anthers of plants grown in short day and high light intensity perform better. Seasonal changes also change the physiology of donor plant.

Differentiation of Pollen into Gametophytic or Sporophytic Tissue

Mechanism of Androgenesis

1. **Morphological:** The pollen which form haploid is smaller and stains less. Such embryogenic pollen are present in low quantities so frequency of haploid formation is low.

2. **Physiological:** Elaborate ER, abundant ribosomes and normal mitochondria are present in gametophytic pollen. Quiescence and repression of organelles in microspores leads to differentiation of embryogenic pollen. The differences between two are shown below:

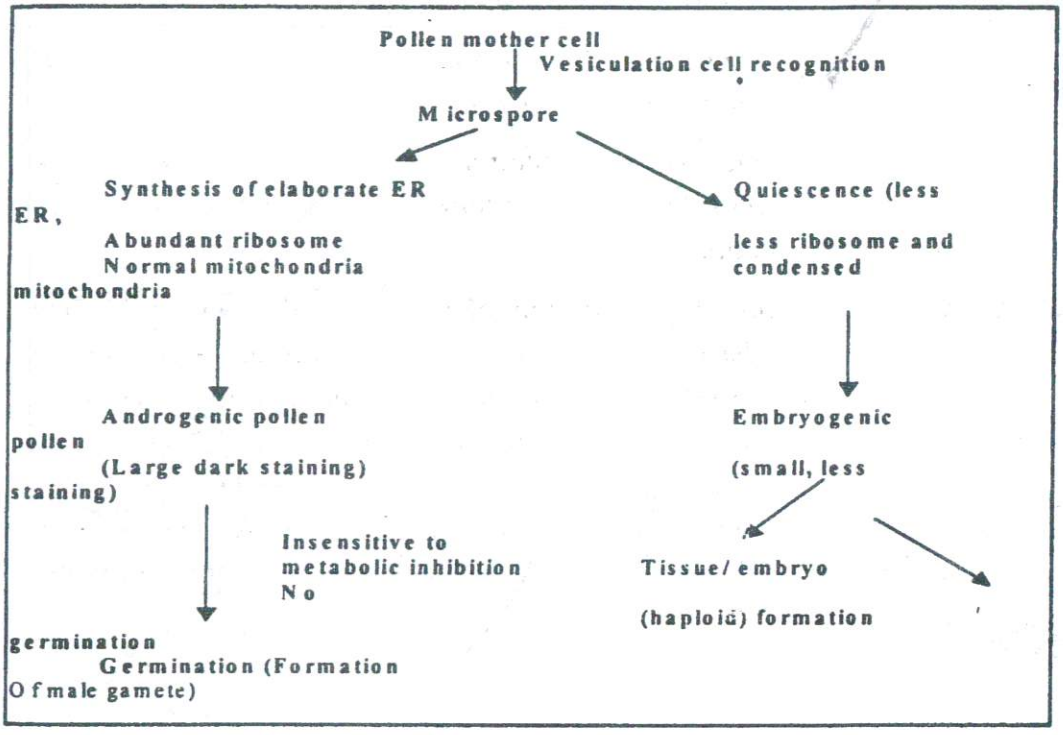


Figure 3.35

Pathways for development of androgenic haploids are following:

The four pathways based on initial divisions in microspore have been identified which lead to in vitro androgenesis.

Pathway I : Microspore divide equally to form two identical daughter cells. Both contribute to sporophyte development. Eg. *Datura innoxia*.

Pathway II : Microspore divide to form unequal vegetative and generative cells. Vegetative cell forms sporophyte & generative cell degenerates eg. *Triticum*.

Pathway III : Microspore divides to form unequal cells but embryos develop mostly from generated cells eg. *Hyoscyamus niger*.

Pathway IV : Microspore divides unequally but both vegetative and generative cells contribute to sporophyte eg. *Datura innoxia*.

Later development : After any of the above pathway, the embryogenic pollen becomes multicellular and bursts open. It assumes globular shape & after normal stages form plant.

All stages & development is shown in Figure 3.36.

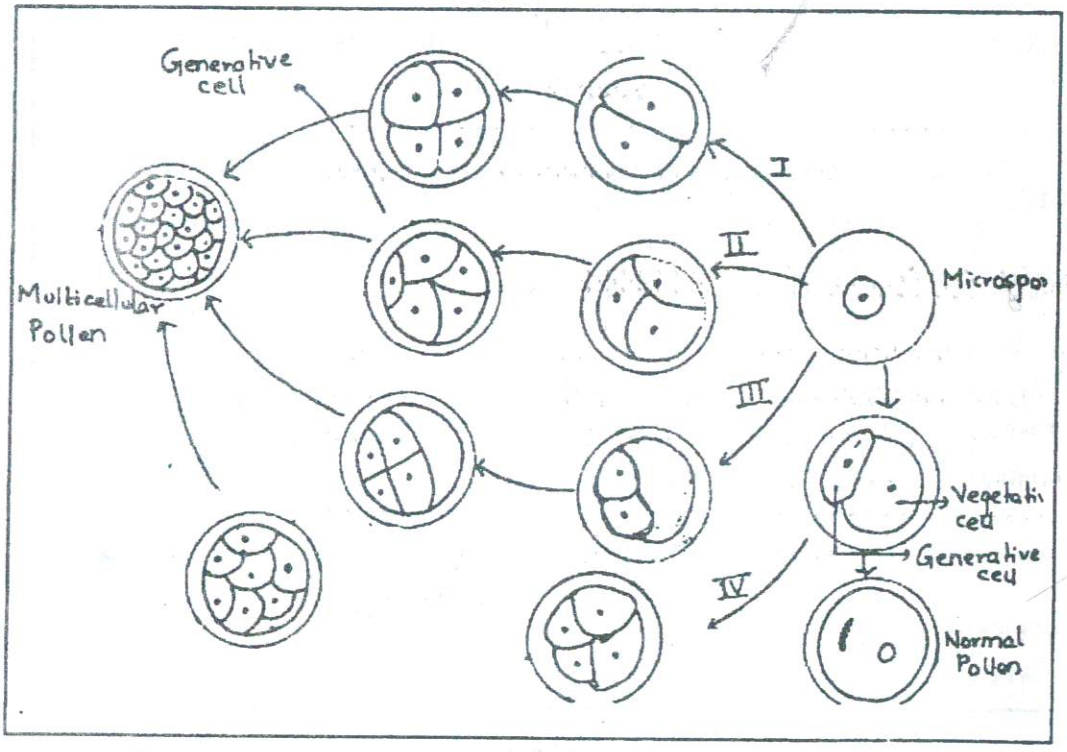


Figure 3.36

Formation of Multicellular Pollen

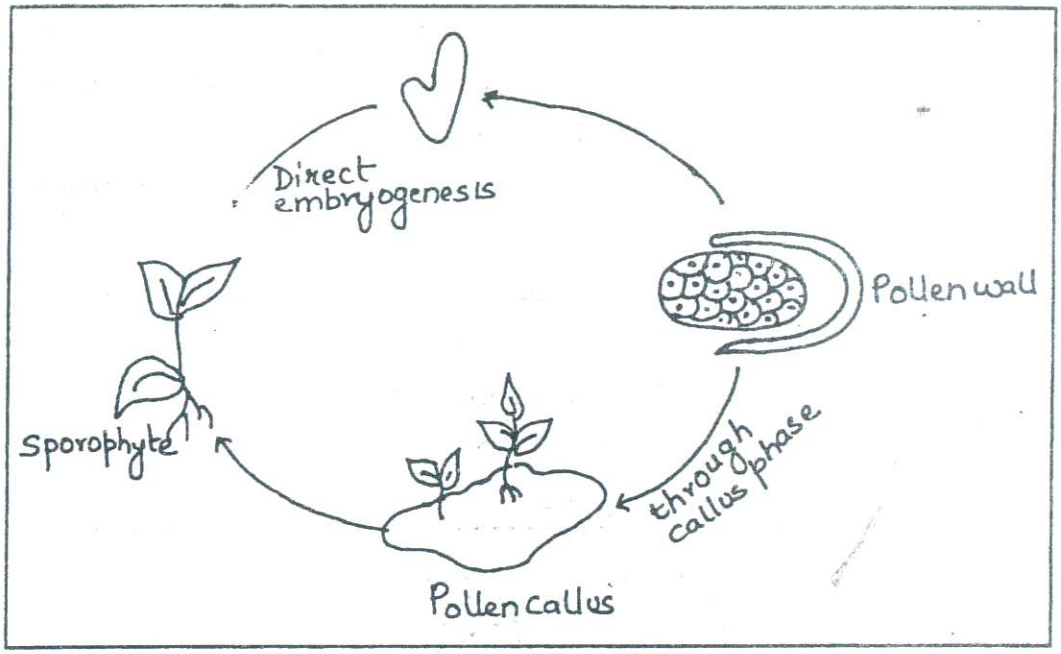


Figure 3.37: Development of Multicellular Pollen into Sporophyte

Haploids from Pollen or Microspore Culture

Anthers contain heterogenous population of pollen grains. Thus, development of haploids from isolated pollen has advantage as only plant of single genotype will develop. This was first reported by Tuleche in gymnosperms & later Kameya & Hinata in angiosperms. Nurse culture technique is suitable for pollen culture. This is shown in figure below.

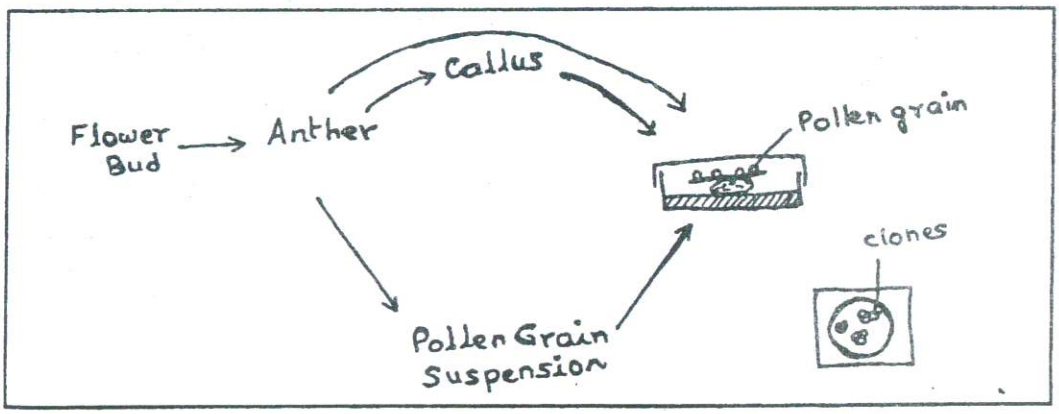


Figure 3.38

Diploidization of Haploid Plants

Haploid plants can grow normal but do not produce viable gametes & no seed. So its necessary to diplodise the haploids. This is done by using colchicines (0.4%).

Applications of Androgenesis

1. Use in Plant breeding
    a. Hybrid sorting in plant breeding.
    b. Production of diploid hybrids for breeding.
    c. Releasing new varieties through F1 double haploid system.
    d. Selection of mutants resistant to diseases.
    e. Development of asexual lines of Trees/perennial species.
    f. Transfer of desired alien genes.
    g. Establishment of haploid & diploid cell lines of pollen plant.
2. Other uses
    a. In mutation research.
    b. Gametoclonal variation.
    c. Cytogenetic research.
    d. Evolutionary studies.
    e. Genetic studies.

Female Gametophyte

Ovule Development

Ovule is the fertile part of the gynoecium. It develops from homogenous tissues of placenta, and it looks like orthotroups. Then, around this tissue, integument arises, which forms nucellus in the mature ovule. Integument shows dermal origin. Outer integument is initiated by dermis or subdermis, with the differentiation of integument, ovule becomes curved and it shows megaspore tetrad stage, and in this condition, final shape occurs. The integument grows faster and surrounds the nucellus. At upper end, integument is not formed, which is known as micropyle.

The development of ovule was described by Reisner (in 1993). Ovule consist of a nucellus, in which megasporangium develops. This is also having 1 or 2 integuments and funiculus. It develops from primordial cell. The subdermal primordial cell is present below nucellus. It enlarges and known as archesporium. In many species, it behaves like megasporocyte, or it divides and forms multicellular archesporial, but only one cell is changed into megasporeocyte. During enlargement of ovule primordium, it is having 3 different regions:

1. Nucellus containing megasporeocyte.
2. Chalaza flanked by integument.
3. Funiculus, which connect ovule with ovary wall.

The ovule is polyclonal in origin. The megasporeocyte is derived from L_2 layer and integument is derived from tissues surrounding nucellus. Inner integument is derived from L_1 and L1 and L2. Integuments grow during embryo sac formation. In many species, nucellus gets destroyed during development of embryo sac. Their integument comes in contact with embryo sac. The polarity of ovule is described as micropyle and Chalaza. During growth, ovule bends and micropyle comes near to placenta, so pollen tube can easily enter in. Megasporocyte divides through megagametogenesis embryo sac is produced. The number of nuclei in embryo sac is different in different species. In majority of species, chalazal megaspore enlarges and the other three degenerate. It is seen that functional megaspore has thin callose wall and non-functional is having thick wall. Generally, functional megaspore becomes 8-nucleated and 7 cells are formed, in which 2 synergids 1egg cell form egg apparatus, which are present at mycropylar end, 3 antipodals present at chalazal end, and 1 central cell is having 2 nuclei.

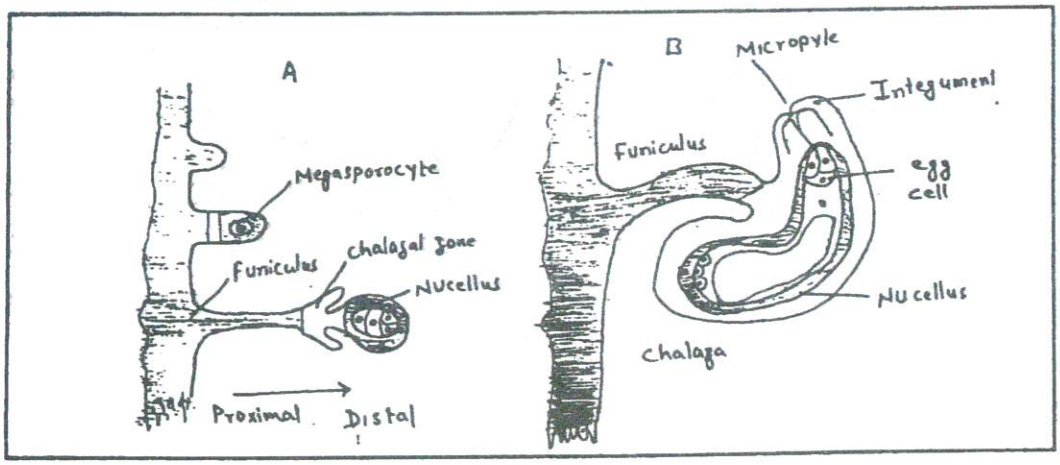


Figure 3.38

A. Different steps of ovule development

B. Mature and unfertilized ovule structure

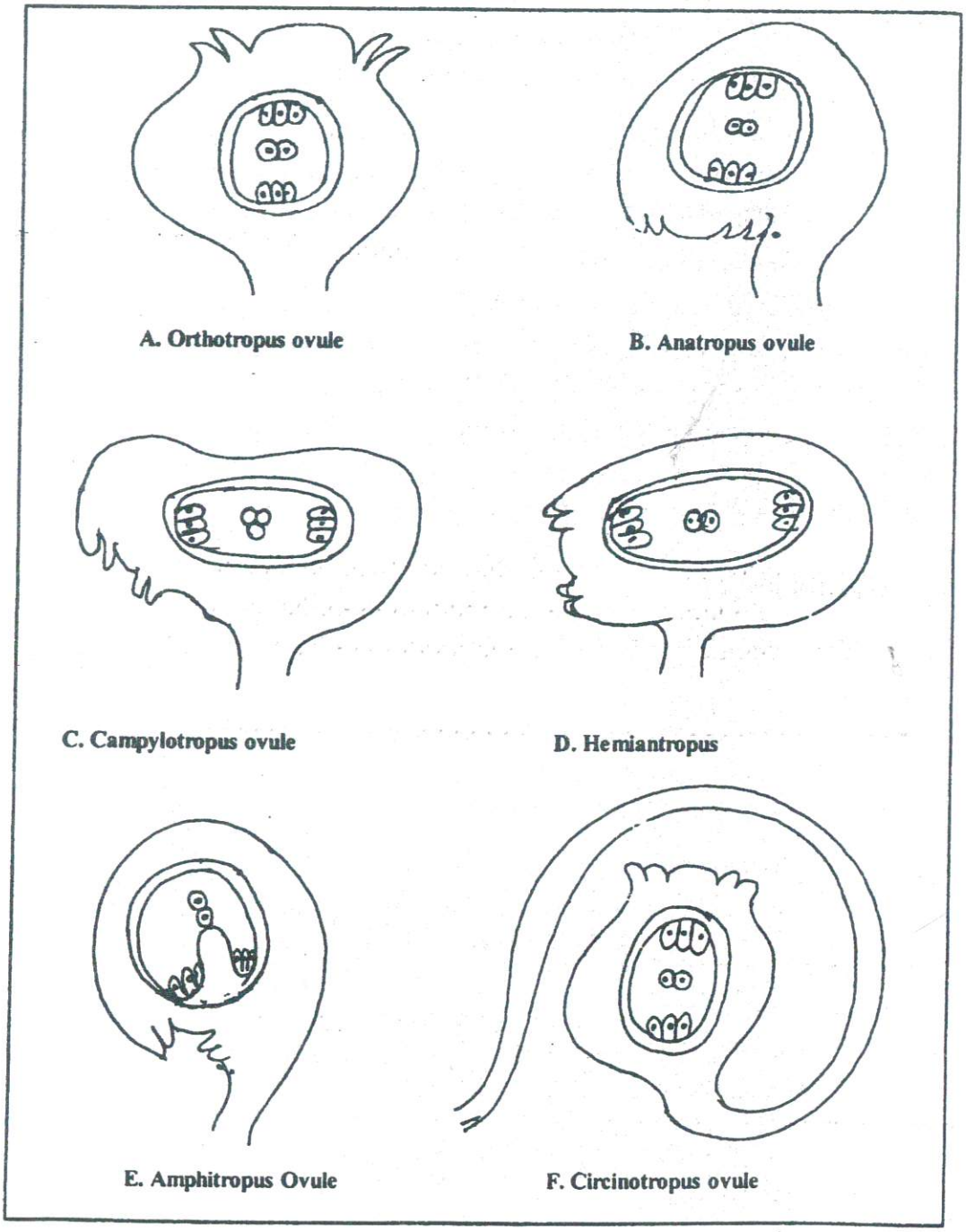


Figure 3.40: Types of ovules in angiosperms

Megasporogenesis

Introduction

Female gametophyte is also called as embryo sac. It is a main characteristic of angiosperm and in dicots and monocots, its development is same. Embryo sac develops from megaspore mother cell, in which first of all, meiosis takes place and 4 megaspores are formed. It is called as megasporogenesis. After it, in many cases, 3 megaspores degenerate and in 1 megaspores degenerate and in 1 megaspore, post- meiotic division takes place, due to which 7-celled and 8- nucleated embryo sac is formed. This type of division is called as megagametogenesis.

Structure of Typical Embryo Sac

Main structures of embryo sac are as follows:

Embryo Sac Wall

In cotton, it is rich in pectic substances. Diboll and Larson believe that the innermost layer (inner) wall is active megaspore wall and the outer layers are the remains of crushed nucellar cells. Plasmodesmata are not found in the outer wall, while in the inner wall, plasmodesmata are found.

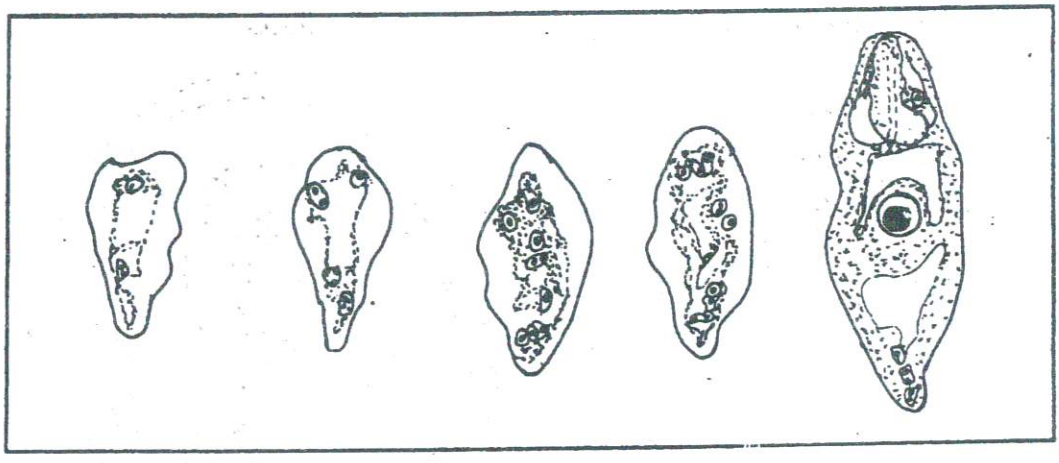


Figure 3.41: Development of Embrysac

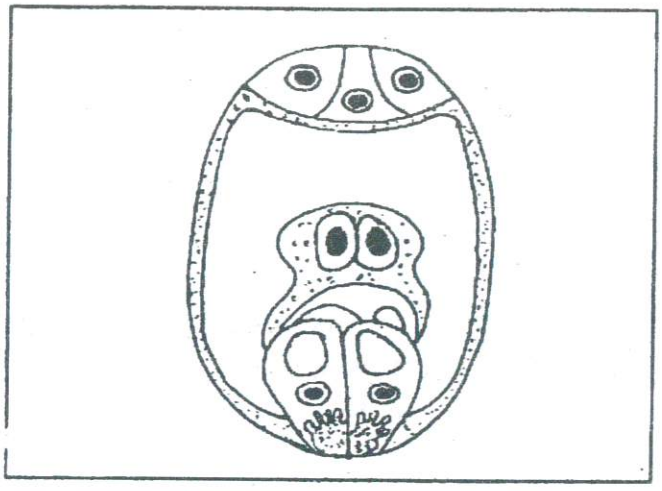


Figure 3.42: Structure of Embryo Sac

Synergids

These cells are present at the mycropylar end of the embryo sac and their number is two. They are pointed or hooked towards the micropyle. The wall around the synergids is incomplete. The upper chalazal one-third of the cell lacks a wall. (In this region, the protoplast of the synergids is separated from that of the central cell by double membranes; one of the synergids and the other of the central cell. In some species, like: — *Epidendrum scutella,* the wall extends all over the synergids cells. Filiform Apparatus (FA) is present at the mycropylar end. Habermann first described it. He explained that this apparatus is a mass of finger like projections and each projection has microfibrils enclosed by a non-fibriller sheath. These are also called as "transfer cells". One large or many small vacuoles, which have calcium salts and carbohydrates, occupy the chalazal region of the cell. The cytoplasm is rich in mitochondria, Endoplasmic reticulum and dictyosomes. When pollen tibe enters into the embryo sac, then one synergids degenerates. The other synergids degenerates after pollen discharge. In some species, like *Cotula australis, Sedum sempervivoides, Quinchamalium chilense,* Synergid haustoria's are also present.

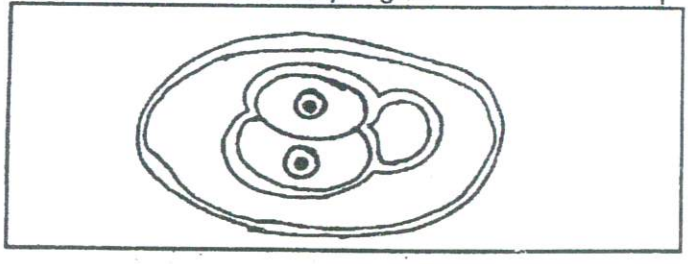


Figure 3.43: Synergid and Egg Position

Synergids Mainly Control the Following Functions

1. They play an important role in directing the pollen tube growth by secreting chemotropically active substances.
2. The degenerating synergids forms the seat for pollen tube discharge in the embryo sac.
3. Filiform apparatus may help in the absorption and transportation of materials into the embryo sac from the nucellus.

Egg

The egg shows common walls with 2 synergids and the central cell. The wall is thicker in the mycropylar region, but becomes thinner towards the chalazal side. It is absent at the chalazal end in cotton, maize and *Torenia*. Actually, the end wall of the egg cell is the wall of the central cell. The egg cell becomes highly polarized early in its development because at the chalazal end, all the cytoplasmic elements get aggregated. The mycropylar end has a large vacuole. Number of organelles in a mature cell gets reduced. Number of cristae in the mitochondria reduces. Dictyosomes becomes either inactive or it is absent. Amount of starch is more in the egg. Ribosomes are more in number. In *Plumbago capensis*, many finger- like projection have been seen. Due to the absence of synergids in it, the egg cell forms haustoria's.

Antipodals

Usually they degenerate before or soon after fertilization, without any appreciable enlargement. Normally, their number is 3, but it has some variations. In the sapotaceae and thismiaceae, the antipodal nuclei degenerate even without organizing intocells. In grasses, a large number of antipodal cells are formed. In the *caltha palustris,* they persist upto the octant stage of the proembryo. The highest number of antipodal cells known is 300, recorded in *Saja paniculata*. *Zea mays* have about 20 antipodal cells, each with 1-4 nuclei. These show the formation of multinucleate protoplasm or syncytium. These show endopolyploidy or polyteny. The antipodal cells in maize have abundant mitochondria, plastids and multicisternal dictyosomes. In some species, antipodals form projections, as in poppy. The antipodals are rich in ascorbic acid and oxidases compounds. Starch, lipids and proteins also occur in abundance. In many plants, antipodals form haustoria also.

Central Cell

It is the largest cell of the embryo sac, and mother cell of the endosperm. It has large central vacuole, which is the reservoir of sugars, amino acids, and the inorganic salts. The nuclear of the central cell is called polar nuclei. Their number is 2, which, before or during double fertilization, fuse to form secondary nucleus. Its cytoplasm indicates more number of cell organelles, which continuously takes part in photosynthetic activities. Wall of central cell is highly variable. It is thick near to the nucellus and thin near to the chalazal and egg apparatus. The central cell is connected with the egg, synergids, and the antipodal through plasmodesmata connections.

Types of Female Gametophyte

Monosporic Embryo Sac

Its chief characteristic is that it is derived from only one of the four megaspores, and the remaining 3 degenerate. These are of 2 types: —

I. **Polygonum type:** — The embryo sac is formed by the chalazal megaspore, which is a 7- celled and 8- nucleated stricture. It has 1 egg, 2 synergids, 2 polar nuclei and 3 antipodal.

II. **Oenothera type:** — This type of embryo sac is derived from the mycropylar megaspore of the tetrad and is 4- nucleated. It has an egg apparatus, 2 synergids and 1- polar nuclei. Antipodals are absent.

Bisporic Embryo Sac

In this, the first meiotic division is accompanied by wall formation, so that a dyed (2-celled) is formed. One of the dyed cells degenerates and the second one undergoes the second meiotic division. Both the megaspore nuclei contribute to the formation of the embryo sac. Bisporic embryo sacs are of two types: —

I. **Allium type:** — the embryo sac is derived from the chalazal dyed cell. It is 7-celled, 8-nucleated embryo sac.

II. **Endymion type:** — The embryo sac is formed by the mycropylar dyed cell. It is also 7-celled 8- nucleated embryo sac.

Tetrasporic Embryo Sac

In this group, meiosis occurs in megaspore mother cell and 4 nuclei are formed. It is not accompanied by wall formation; all the 4-coenomegaspore nuclei take part. Hence, it is heterogenic type of embryo sac. The arrangement of the four nuclei in the coenomegaspore is of types:

A. **1+1+1+1 arrangement:** — In this, one nucleus is at the mycropylar end, one is at the chalazal end and two placed laterally, one on each side. These are of following types:

I. **Peperomia type:** — In this type, embryo sac is 16 nucleate. It has an egg apparatus (1egg and 1 synergids), 6 peripheral cells and a central cell with 8 polar nuclei.

II. **Penaea type:** — It is 16- nucleate embryo sac, which consist of 1 egg, 2 synergids, 4 polar nuclei, 3 chalazal and 3+3 lateral cells.

III. **Plumbago type:** — It is 8- nucleate embryo sac. It consists of an egg cell and 4- nucleate central cell (4 polar nuclei) and 3 peripheral cells.

B. **2+2 arrangement type:** — In this, 2 nuclei are at the mycropylar end and 2 at the chalazal end.

I. **Adoxa type:** — Embryo sac is 8 nucleate 7-celled. It has 1 egg, 2 synergids, 2 polar nuclei and 3 antipodals.

C. **1+3 arrangement type:** — 1 nucleus is present at the mycropylar end and 3 nuclei are at the chalazal end. These are of following types:—

I. **Drusa type:** — It is 16- nucleated embryo sac. It has 1 egg, 2synergids, 2 polar nuclei and11 antipodals.

II. **Fritilaria type:** — In this type, at the chalazal end the three nuclei fuse together to form triploid nucleus and it again divides to from 4 haploid and 4 triploid nucleus, which forms 1 egg, 2synergids, 2polar nuclei (1 haploid+ 1triploid) and 3 antipodals (triploid).

III. **Plumbagella type:** — It is similar to fritillariria type, but it is 4- nucleate embryo sac. It has 1 egg, 2 polar nuclei (1haploid+ 1triploid) and 1 antipodals (triploid). Synergids are not found in it.

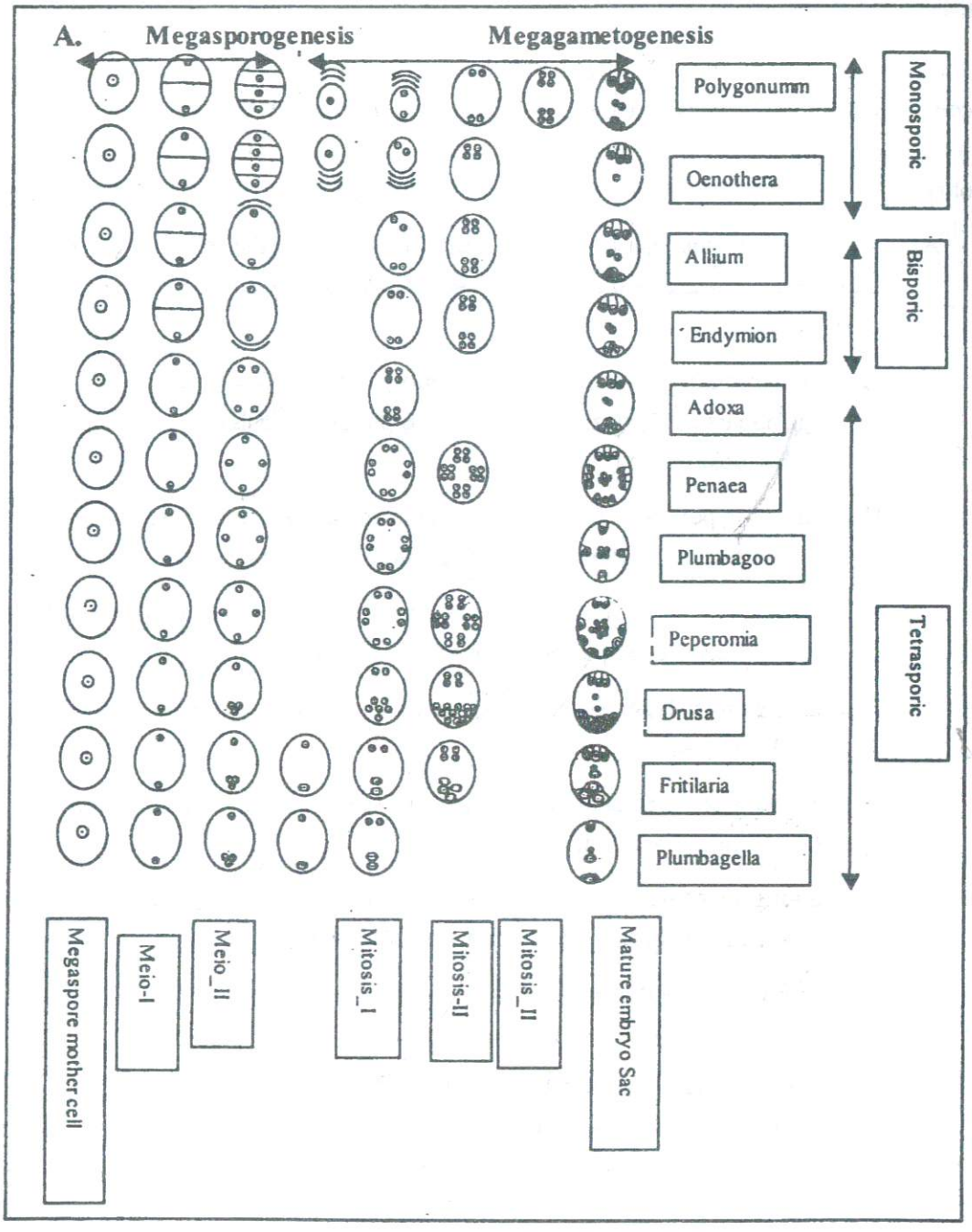


Figure 3.44: Types of embryo sac

Special Embryo Sac

It is the end of meiosis, the four nuclei in the coenomegaspore are arrangement in the 1+2+1 fashion, one at the mycropylar end, one at the chalazal end, and two in the center of the cell. Based on the behavior of the 2 central of the center of the chalazal end, and two in the center nuclei, there are 2 variations within this type of embryo sac:

(i) In this, mycropylar and chalazal cell form 4-4 cells by 2-2 mitosis, due to which embryo sac becomes 10- nucleate. All the 4 nuclei in the chalazal quartet organize into 4 antipodal cells. 3 polar nuclei, 1 egg and 2 synergids are present.

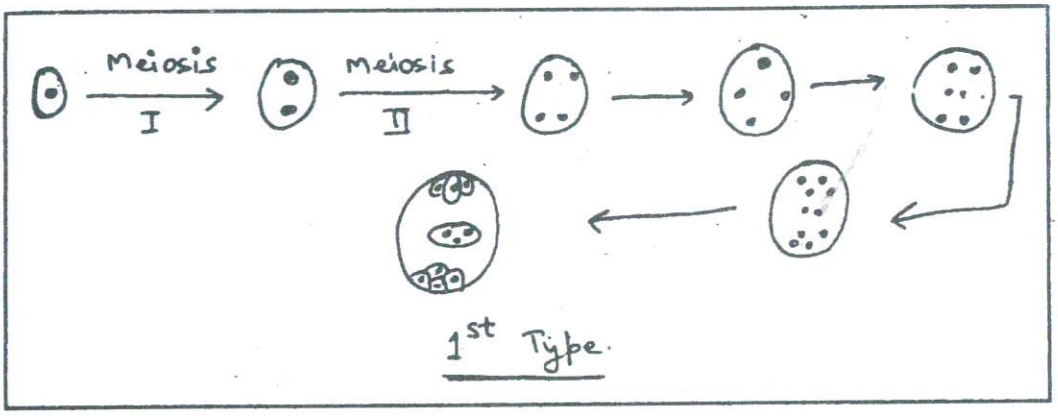


Figure 3.45

(ii) The two central nuclei fuse to form a diploid nucleus. After fusion, all the 3 nuclei in the coenomegaspore undergo two mitotic divisions. In this way, their groups of 4 nuclei each are formed. 1egg and 2 synergids form egg apparatus, 2 polar nuclei (1 haploid+ 1 diploid) and 7 antipodals (3 diploid + 1diploid) and 7 antipodals (3diploid + 4 haploid) are formed.

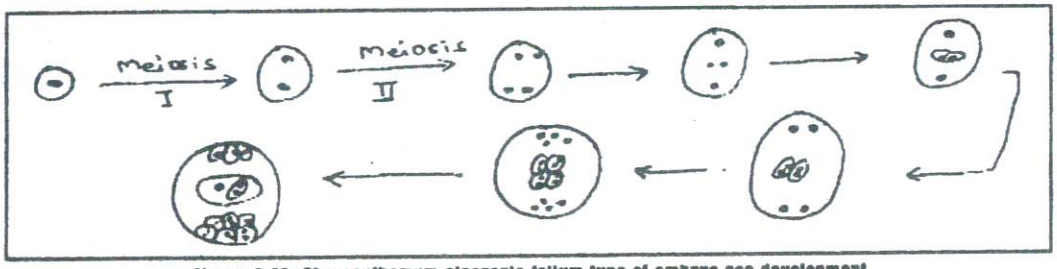


Figure 3.46: Chrysanthemum cineraria tellum type of embryo sac development

Haustorial Behavior of Embryo Sac

Embryo has haustoria also, which absorbs food from the nucellus. Ex:— In Santalaceae, mycropylar haustoria are found. In *Utricularia*, in 2-celled stage, apex of embryo sac grows and directly comes in contact with placenta. In *Mida* and *santalum*, the embryo sac grows beyond the ovule at the 4-nucleate stage whereas in *Leptomeria*, it occurs at the mature embryo sac stage. In the Loranthaceae, embryo sac enters into the style. They reach the base of the style in *Macrosolen cochinchinesis*, upto 1/5 the length of the style in *Leptostegeres germmiflorus*, half the length of the style in *Dendrophthoe falcata* and upto stigma in *Helixanthera ligustrina*.

Most of the loranthaceae and santalaceous members show the extension of the chalazal end of the embryo sac in the form of a lateral caecum. Some members form haustoria on the mycropylar end, as in *Nuytsia*, *Atkinsonia* and *comandra*. In *Exocarpus stricus*, middle of the embryo sac forms haustoria.

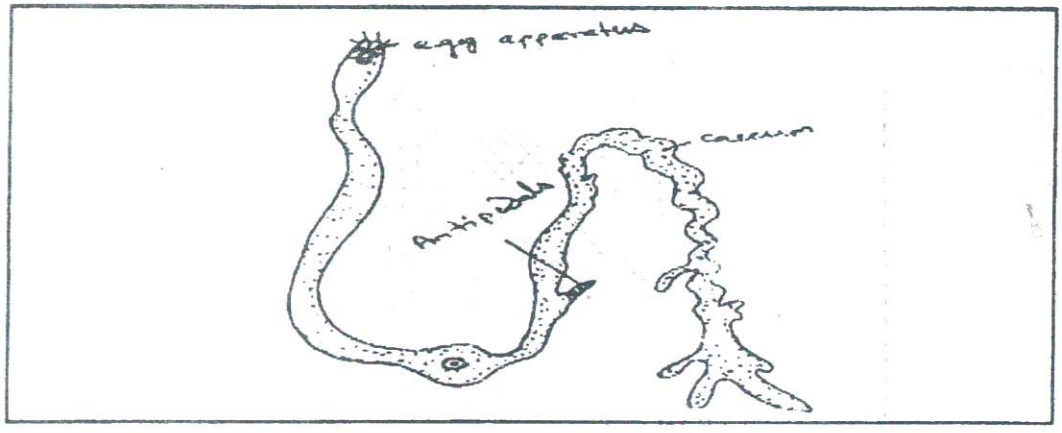


Figure 3.47: Chalazal Haustoria in Santalum album

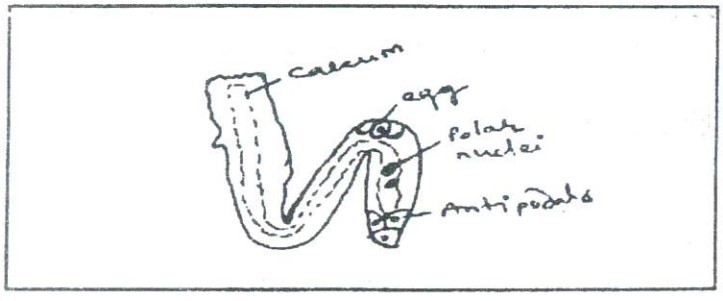


Figure 3.46: Lateral Haustoria in Embryo Sac of Comandra umbellata

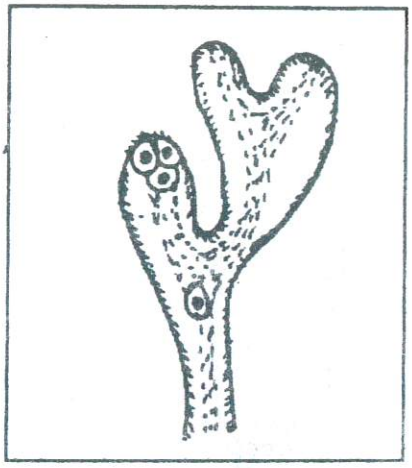


Figure 3.49: Micropylar Haustoria of a Mature Embryo Sac of Nuytsia floribunda

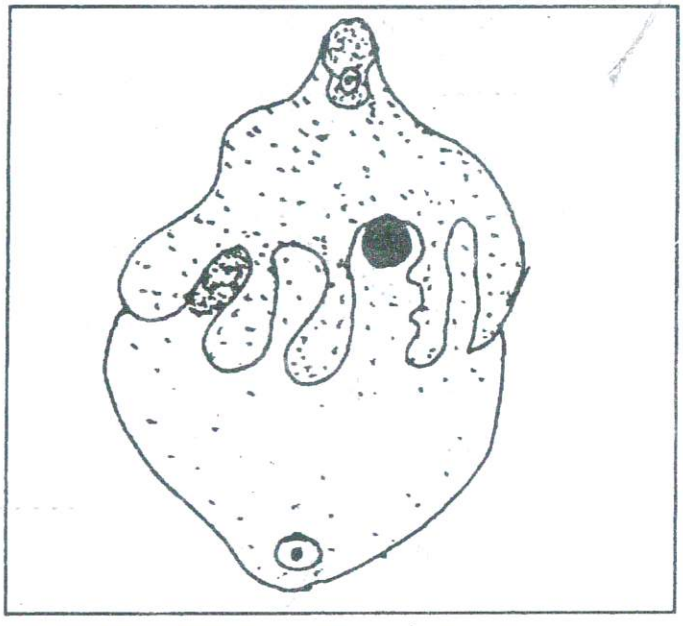


Figure 3.50: Finger like Haustoria in Exocarpus strictus

Nutritional Behaviour

In general, nucellus is the obvious pathway for nutrients to enter the embryo sac. The morphology of the ovule suggests that the main pathway of nutrients into the embryo sac is the chalazal end. Coe saw this in *Zephyranthes* byexperiment. He proved with the help of C^{14} that the nutrients enter into the embryo sac through chalazal end. It is due to this reason, antipodals, in the form of projection, becomes active and in end absorb the food. In *Quercus gambelii*, Mogensen suggested that in this plant, the most likely pathway of

food transport within the ovule is from the outer integument to Chalaza, and through the postament to the embryo sac.

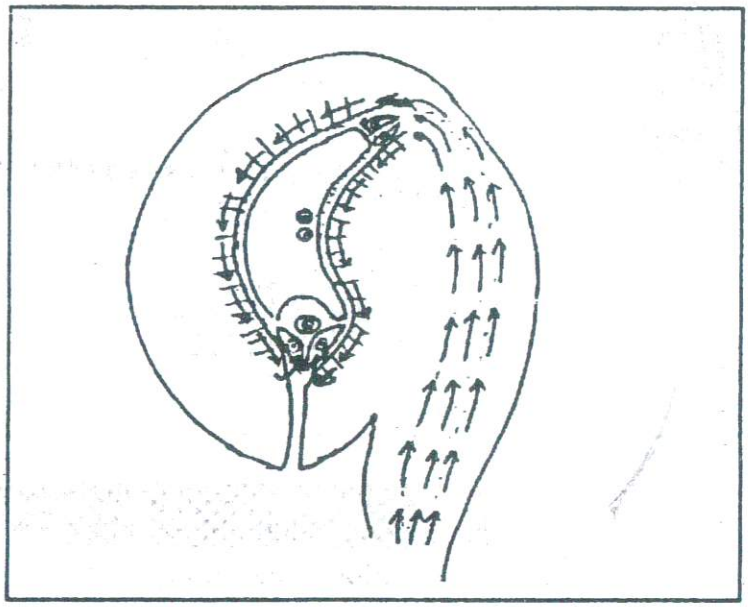


Figure 3.51: Flow of Nutrient in the Ovule of Linaria bipartita

CHAPTER 4

POLLINATION, POLLEN-PISTIL INTERACTION AND FERTILIZATION

LEARNING OBJECTIVES

- Pollination, Pollen-pistil Interaction and Fertilization
- Mechanism of Pollination
- Breeding System: Commercial Considerations
- Structure of the Pistil
- Pollen-stigma Interactions
- Sporophytic and Gametophytic Self-Incompatibility
- Double Fertilization
- Seed Development and Fruit Growth
- Embryogenesis, Ultrastructure and Nuclear Cytology
- Dynamics of Fruit Growth
- Polyembryony
- Apomixis
- Somatic Embryogenesis

Pollination, Pollen-pistil Interaction and Fertilization

Pollens develop in the anther and after maturation of pollen, anther dehiscence occurs and hence pollens become free in air. Then, pollens transfer at stigma through any mode, which is known as pollination and the mode, which pollens are transferred, is known as pollen vectors.

Anther Dehiscence

When anther matures, then desiccation of anther tissues occur. The temperature and humidity should be suitable. In the anther, at particular point, the tissues are thin, which is known as stomium. During the desiccation, mechanical layer becomes active and the anther rupture along with stomium. In the endothecium's, fibrous thick cells are present, which behave like mechanical tissue. In the cleistogamous flowers, endothecium's reduced, so anther dehiscence occurs. Pollen germinates in the pollen chamber, and the pollen tube comes out through anther wall.

Mechanism of Pollination

Pollen transfer: On the basis of pollen transfer, pollination is of two types:

Self Pollination

It is possible only when flowers are bisexual, and the stigma and pollen mature at the same time. The pollination time is proper and no genetic barriers are available. The transfer of pollens from anther to stigma within the same flower is called self-pollination. It is carried out in following sequence:

(a) Opening of flower.

(b) Maturation of anther and stigma.

(c) Dehiscence of anther.

(d) Transfer of pollen at the stigma.

But, in some species, flowers never open, which are not cleisto-gamous flowers. These are pure self-pollinated plants and they don't need the opening of flower.

The flowers which open are called as chamogamous flowers in which pollens transfer through any agency, but in cleistogamous, vectors are not needed. Some plants are having both types of flowers, like: -- Commelina benghalensis.

Cross-pollination

When pollens are transferred from anther of one flower to stigma of other flower, then it's known as cross-pollination. If the pollens are transferred between two such flowers, which are present on the same plant, then it is known as Geitonogamy, and when pollens transfer between such flowers, which are growing on 2 different plants then it is known as xenogamy. The cross-pollination is maintained in plants by following mechanism:

a. **Self- sterility**

Actually, in many plants, pollen are transferred at stigma, but either pollen does not germinate at stigma or pollen tube cannot enter style, or pollen tube is prevented by style, then it creates self sterility and fertilization does not occur.

b. **Dichogamy**

When the maturation time of male and female sex organs differ, then known as dichogamy, like Saxifrega sp. when anthers dehiscence occur before maturation of stigma, then known as protondry, Eg. Saxifraga impatiens, but if stigma matures earlier than anther, then known as protogamy. Like- Aristolochia, Brassicaceae and Rosaceae.

c. **Herkogamy**

When flowers are bisexual, but the structure of male and female sex organs show barrier for self pollination, then known as herkogamy, like- Gloriosa.

d. **Heterostyly**

When flowers are bisexual, but the sex organs show polymorphism, then known as heterostyly, like in the Primula, distyly occurs. In first type of the flowers, style is long and anther filament is smaller. It is pin tyed plant, while in second typestyle is short and anther filament is long. It is known as thrum eyed plants. Similarly, in Lythrum and Oxalis, trystyly is observed, in which styles are short, middle length or long and they are differentiated in stamen short style flower is having middle and long filament stamens which prevent pollination or fertilization.

Pollination Vectors

The transfer of pollen is carried out through some agencies, which are known as pollen vectors. Only in exceptional cases, agencies are not required, like, in Velisnaria, male flower is self transferred near the female flower. These agencies are following:

Anemophily

When pollens are transferred with the help of air, then it's known as anemophily. It is non-directional and wasteful process, because less number of pollens is transferred at the target. So wind is known as Hit or misaffair. So, such plants produce plenty of pollens. These pollens are small, smooth, light and dry. The female flowers must have specific structure, like-stigma is feathery or brush like, and projected towards outside. In anemophily plants, number of ovules is less.

Hydrophily

It shows the transportation of pollen with water. When plants are pollinated inside the water, then known as hyphydrophilly, like -Zostera and Ceratophyllum. In these plants, plants are long and needle like. While when male flower is transferred up to female flower, then known as ephydrophily, like-Vallisnaria. In this condition, at the time of pollination, male flower is detached from plant, while female flower is projected at the surface of water. So male flower reach to female flowers and pollination occurs.

Entomophily

When pollination occurs through insects, then known as entomophyllous. It needs same specific devices like- Salvia shows lever mechanism. When any insect sits at first plant, then its sex organs bonds downward side. The anthers present at inner side, so it touches the surface of insect and pollens transfer at insect. Then, these insects when sit at other flower, then outer side style touches to insects surface. So, pollens are transferred at stigma. Similarly, another important mechanism is observed in Yucca, in which flowers are cleistogamous, but cross-pollination occurs. Some insects, lay down eggs in this flower for which they transfer their transpositor in the flower and through this transpositor, pollens are transferred.

Ornithophily

In the tropical region, birds are important pollinators, which is known as ornithophilly process, like: Humming birds, sunbirds, honey eaters. Generally, such flowers have tubular or cup shaped structure, like: --Nicotiana or Callistemon.

Chiropterophily

When bats carry out the pollination, then it's known as chiroptero-phily. Such flowers are having long stalks, like: —*Mucuna gigantiea*.

Breeding System: Commercial Considerations

Introduction

The process through which characters of next generations are manipulated through selection is known as breeding system. This system played important role in green revolution. Following aims can be fulfilled with the help of breeding system:

1. We can obtain the more yield from the same area. Which will be helpful to fulfil the increasing demands due to overproduction.
2. The quality of the crops can be improved and economically more potential crops can be obtained.
3. The major part of the crop is destroyed due to diseases and insects; and disease and insect-resistant plants can be obtained through breeding.
4. The crops, according to specific climate, can be obtained.
5. Stress tolerant plants can be produced.

Breeding Method of Self-pollinated Crop

Mass Selection Process

In the self pollinated crop, the group of plants is grown, among which best quality plants are selected, which is based on disease resistance quality of seeds, amount of seeds. This processis completed within 7crop cycles.

(i) **Ist year:** Seeds from desired plants are collected.

(ii) **IInd year:** The seeds which are obtained from Ist year plants, are grown with local species and selection in made after comparison with local species.

(iii) **IIIrd to VIth year:** In these years, the adaptability of crops in the local area is tested.

(iv) **VIIth year:** In this year, the final selection is carried out.

Its merits are that plants are selected from natural groups, and due to selection, undesirable characters are separated. But many times, undesirable characters are present in them, which may appear in the next generation.

Pure Line Selection

Mendel and Johansson started it, and Johansson proposes the theory. This process is completed in 9 years:

(i) **Ist year:** 200- 1000 plants are selected from mixed progeny.

(ii) **IInd year:** The selected plants are grown in a row and the superior plants are selected from this group.

(iii) **IIIrd year:** The seeds, collected from second year plant, are grown in replicated plants and again the selection is made.

(iv) **VIth to VIIth year:** In these years, the yield testing is done, and the quality of crop is studied.

(v) **VIIIth to IXth year:** In these years, the final selection is carried out and the seeds are released in market.

Following precautions are carried out during this process:

1. The seeds are collected from different sources.
2. Collected seeds are numbered and preserved.
3. Different characters of the plants are compared.
4. Time of flowering, ripening and harvesting are recorded.
5. Only superior plants are selected, which are stored in dry and insect free places.
6. From the collected seeds, 50% are stored and 50% are used.
7. Seeds are grown in the rows and the rows are recorded.
8. The inspection is carried out time to time.
9. Comparision of local and high yield plants is carried out.
10. Best seeds are selected on the basis of characters.

Breeding Method of Cross -pollinated Crops

In the cross - pollinated crops, easily the hybridization is carried out. So, the characters from two parents can be transferred in the progeny. This method is based on the following points:

Determination of Male and Female Plant

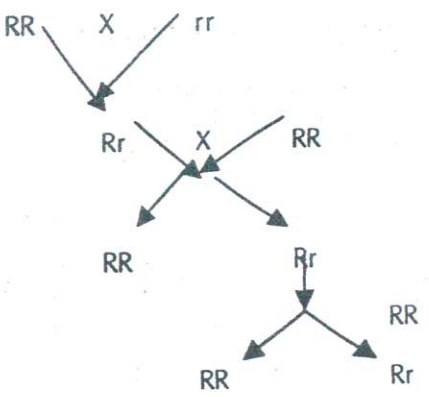
It is the important aspect because if such male and female plants are taken, which show desired characters as cytoplasmic inheritance, then, it cannot be transferred. That's why; in this case, female plant should be selected. Plants should be homozygous. The plant which is having more good characters should be taken as female.

Cross Technique

The cross techniques is based on detail knowledge of a particular crop, because breeders must know the budding time, flowering time, pollination time and fruiting time. The study of opening of flower bud to fertilization is called anthesis. So, breeders study that when stigma will mature, when pollen will mature and when will pollination take place. If the flowers are bisexual, then the chance of self -pollination may be there. So, if we are going to use this plant as female plant, then the anthers of the stamens are removed. This process is known as emasculation, while the plant that is used as male is bagging. Bagging is also done in emasculated plant.

Generally, 2 types of crosses are used for hybridization

1. **Back cross:** In this process, where F1 generation is obtained, then it is crossed with dominant parent. So, as the back cross is repeated, the amount of dominant genotype increases and at last, it becomes 99.975%. Then final selection is carried out. It takes approximately 10 years.



50% F1

75% F2

87.5% F3

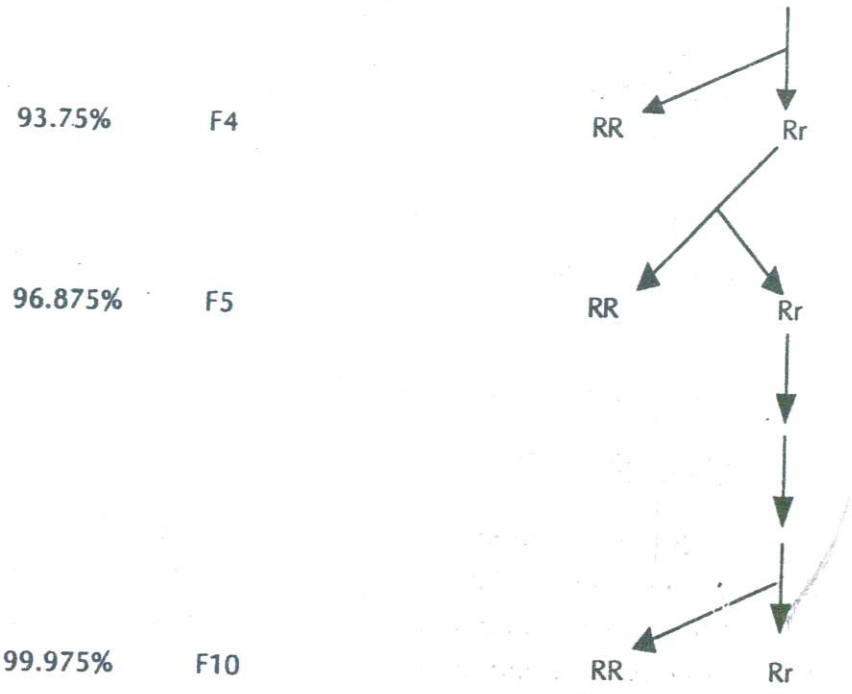


Figure 4.1

2. **Multiple Cross:** If different crops are having different characters, then these crops are combine grown, which will take part in multiple crossing. In this condition, after some generation plants will have all the characters, like: some plants are having different characters, A, B, C, D, E, F, G and H, then the crossing pattern will be as fallowing:

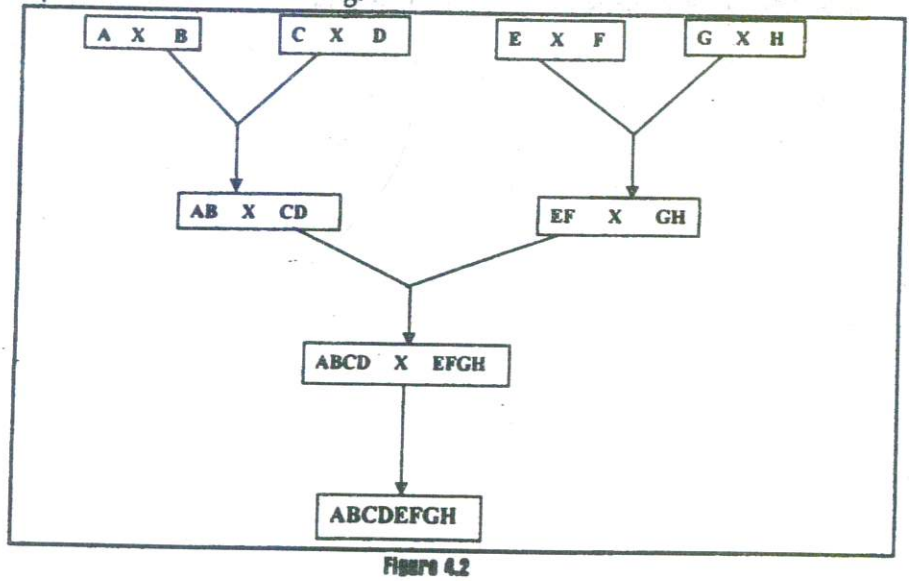


Figure 4.2

During the hybridization work following precautions are carried out:

(1) Male and female plants are carefully grown.

(2) Male or female for hybridization.

(3) From each generation, pollen grains are preserved.

(4) Emasculation is carried out at proper time.

(5) Stigma is preserved at proper time.

(6) If once the plants pollinated, then its pollination is prevented from other pollens.

Structure of the Pistil

In Angiosperms, the female organs in the flower are referred to as gynoecia, or pistils, which can be composed of one or more carpel that are usually fused together in higher plants. At anthesis (i.e., the time when the flower opens and fertilization occur), the pistil has 3 functional parts, the ovary, which contain ovules; the style, through which pollen tube grows and the stigma, which is at the top of style where pollen grains land and germinate.

The ovary is an enclosed space that is often divided into separate locules and is formed by tissue fusions at the margins of the carpals. In arabidopsis, the carpels grow as a primordial cylinder and two locules are formed by the extension and fusion of tissues from either side of the cylinder. The space defines the ovary and is enclosed by fusions at the margins of the extending cylinder, and by the development of inner wall layer called the placenta. In many other plants, carpals fuse after they are formed (in a so- called post genital fusion). Styles are short in Arabidopsis, longer in tomato and very long in maize. Tissues at the top of the style differentiate into stigma. Stigma formation involves the proliferation and extension of papillary cells that secrete compounds to which pollen adheres and which promote or prevent the growth of pollen tubes.

Pollen-stigma Interactions

Hydration of pollen is the usual triggers for pollen germination in species with dry pollen. Stigma surfaces can either be wet or dry depending on the species and environment conditions. On wet and sticky stigma surfaces, such as in Tobacco, there are no barriers to pollen hydration. On dry stigmas, pollen germination can be controlled by pollen-stigma

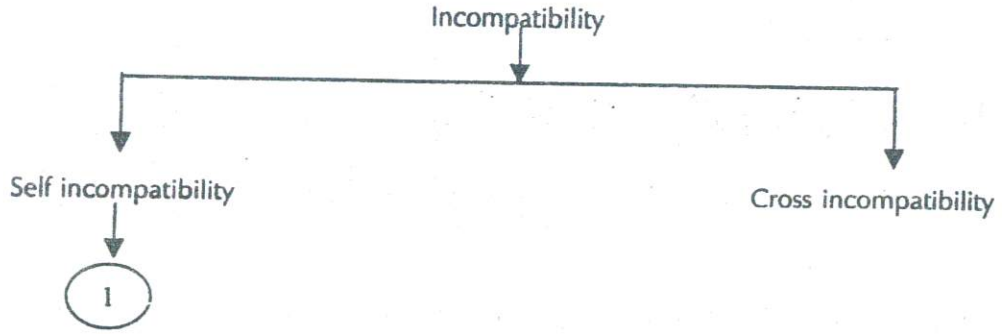
interactions and may involve the delivery of water and other substances from the stigma. A conditional male sterile mutant in Arabidopsis was found that prevents hydration by interfering with pollen stigma interaction 1 (pop1), insights callose production in stigmas of Arabidopsis flowers (usually a defense response to foreign pollen). Pop1 pollen germinates in vitro, but it fails to germinate on the stigma surface because callose deposits block access to the stigma fluids that are needed for pollen germination. The mutations conditional because in humid conditions, pop1 pollen will take up sufficient moisture to germinate despite the formation of the callose barrier. The defense response in the stigma to pop1 pollen occurs because pop1 pollen lack extra cellular lipids in their tryphine coat (i. e., the coat deposited by the breakdown of the tapetum).

Sporophytic and Gametophytic Self-Incompatibility

Introduction

In nature, sexual reproduction is a specific process, which is based on the regular and sequential steps. In plants, pollens are found as male gamete and eggs are found as female gamete. This pollen reaches onto the stigma by pollination. But, on stigma it germinates by a specific process and forms male gametophytes. It fertilizes female gamete. It is called as fertilization. But each pollen does not germinate on stigma. Only that pollen germinates which are compatible. While the other pollens are incompatible, which cannot fertilize eggs, it is called as incompatibility. Hence, the process that restricts pollen to fertilize the egg is called as incompatibility. This process helps in continuing the actual characters from generation to generation.

Types of Incompatibility



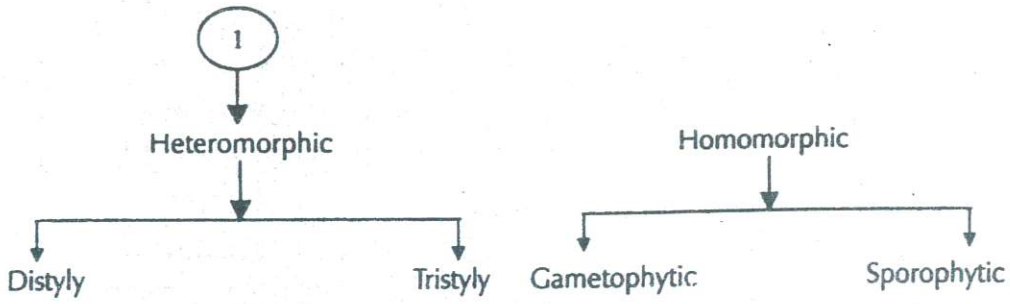


Figure 4.3

Self- Incompatibility

When the pollens of the same species are not viable on the same plant, then It's known as self-incompatibility. In this process, self-pollination is prevented and cross- pollination gets successes morphological. It is of two types:

(a) Heteromorphic

When, morphologically there is a difference in stamen and style, due to which the self pollination gets stopped, then it is called as heteromorphic self incompatibility. Ex. As in Primulla and Lythrum, Heterostyly condition is found. 2 types of such situation has been seen:

(i) **Distyly:** In such condition, dimorphic flowers are found. In some flowers, style is long and in some flowers, style is dwarf. Stamens are smaller in long styly flowers and in dwarf styly flowers, stamens are longer. Their development is controlled by a gene allele S, which is as followes:
SS or Ss–Long style, Dwarf stamen
ss — Dwarf style and Long stamen

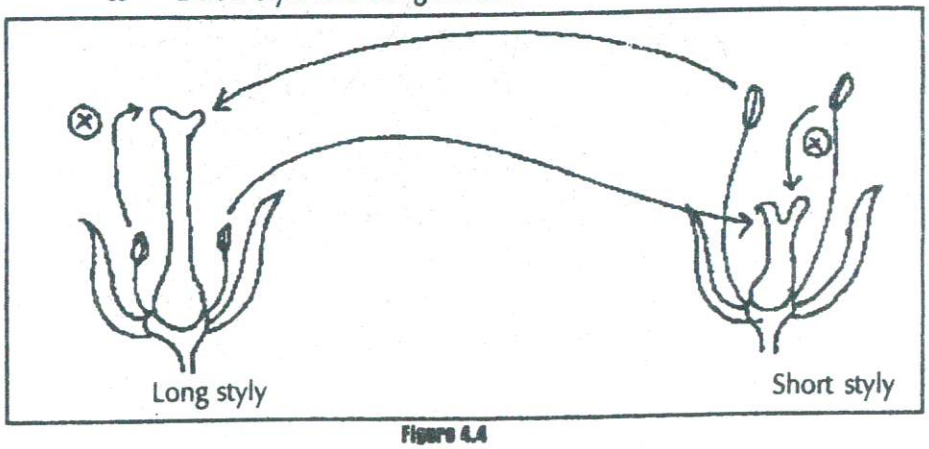


Figure 4.4

ii. *Tristyly:* These type of flowers show 3 morphological forms, which are controlled by allele S. It one pair of allele is effective, and then style shows medium size. It 2 pairs of alleles are there, and then style is dwarf. It both the pairs are recessive, and then long style is there. Among these, maturation time differs, and so self- pollination is not useful. $S_1S_1S_2\ S_2$ or $S_1S_1\ S_2.s_2$ or $S_1s_1\ S_2\ S_2$ or $S_1\ s_1\ S_2.s_2$ — Dwarf style $S_1S_1s_2\ s_2$ or $S_1\ s_1\ s_2\ s_2$ or $s_1\ s_1\ S_2\ S_2$ or $s_1\ s_1\ S_2\ s_2$ — Medium style $s_1\ s_1\ s_2\ s_2$ — Long style

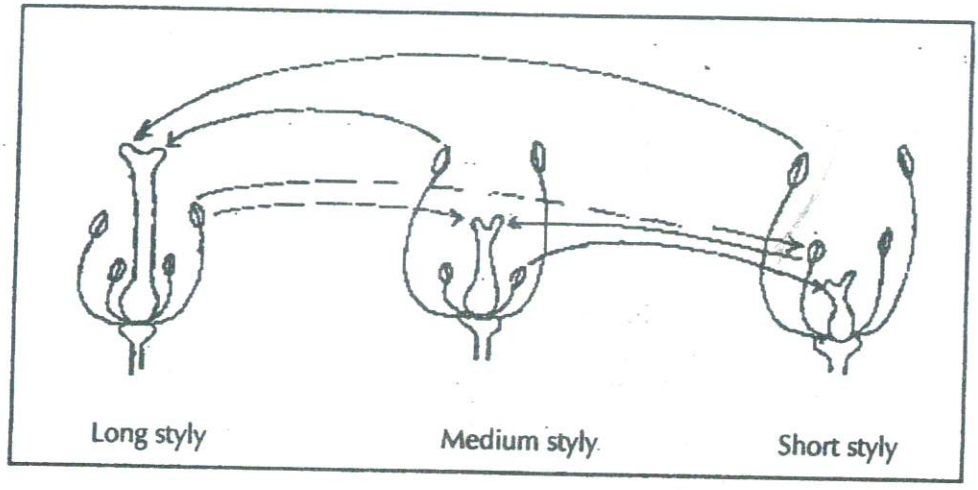


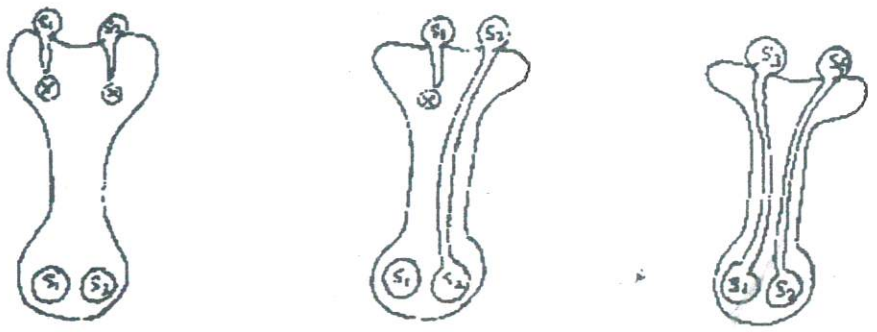
Figure 4.5

(b) ***Homomorphic*** **:** All mating types of this type are morphologically similar. Hence, for identifying them, breeding test is done. Mainly this incompatibility is of 2 types:

i. Gametophytic self incompatibility

This type of incompatibility is controlled by the genotype of pollen, like- Graminae, Liliaceae, Solanaceae, Trifolium. East and Mangelsdorf proposed opposite 'S' allele Hypothesis for it. According to it, if pollens have been formed on S_1S_2 plants, then these pollens will be having S_1S_2 allele. In such situations, pollen tube shows styler inhibition and these pollens will not be viable. But among S_1 and S_3 pollens, the pollens having S_3 genotype will be fertile. In other words, 50% compatibility will be shown. If pollens are S_3 and S_4 type, then these will show the full compatibility on the S_1S_2 plant

Male		Female		
S_1S_2	X	S_1S_2	⟶	100 % Incompatibility
S_1S_3	X	S_1S_2	⟶	50% Incompatibility
S_3S_4	X	S_1S_2	⟶	0% Incompatibility



100% Incompatibility 50% Incompatibility 100% Compatibility

Figure. 4.6: Mechanism of Gametophytic Self Incompatibility

ii. Sporophytic Self Incompatibility

When incompatibility is controlled by the genotype of sporophytic tissue, and then it is called as sporophytic self- incompatibility. Due to this reason, all the pollens formed on it are either incompatible or compatible. As if S_1 allele is dominant, then all the pollens will behave like S_1 irrespective of this that they are of S_1 or S_2 genotype. Similarly, if S_2 is effective allele, then all the pollens will show the behaviour as S_2 type. Hence, sporophytic genotype of S_1 S_2, S_1 S_3 or S_1 S_4 type will be fully incompatible on S_1S_2 plant, but will show full compatibility with S_3 S_4.

Male		Female		
S_1S_2	x	S_1S_2	⟶	100% Incompatible
S_1S_3	x	S_1S_2	⟶	100% Incompatible
S_3S_4	x	S_1S_2	⟶	100% Incompatible

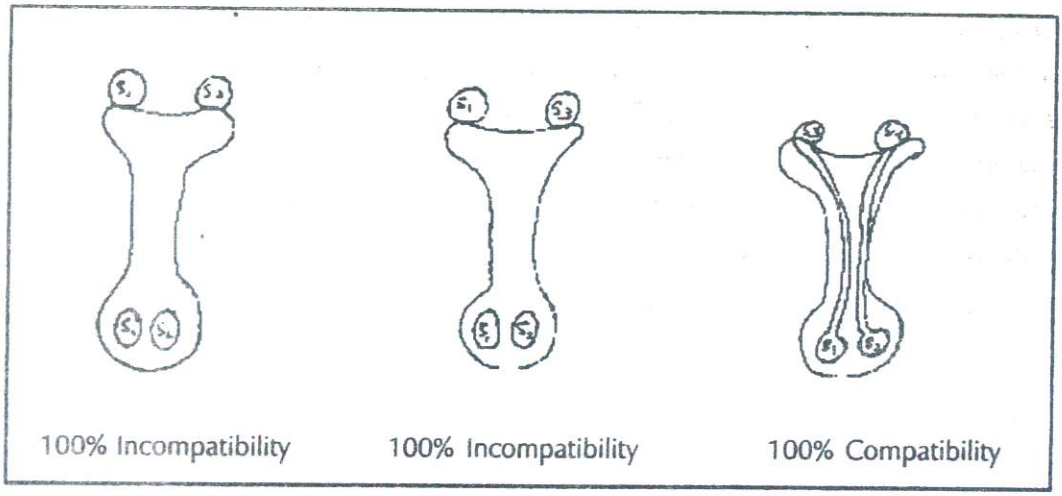


Figure 4. 7: Diagram showing Sporophytic Self Incompatibility

Cross Incompatibility

Its main reason is the different nature of the pollen proteins.

Physiology and Biochemistry of Incompatibility

The two reactions are important for incompatibility:

(i) Recognition Reaction

In this process, stigma or style tries to recognize the pollen. In SSI system, just after the pollen on stigma, this reaction starts.

(ii) Rejection Reaction

When stigma or style restricts the pollen tube from extending in forward direction then (onwards), this reaction is called as Rejection reaction.

Green has shown that after hydration, pollens release hydrolytic enzymes, which shows interaction with stigma. If these enzymes dissolve the proteins of stigma, then stigma becomes soft, due to which germination tube penetrate stigma and enters inside. This process is called as recognition reaction. But if these enzymes show callose deposition by interacting with proteins of stigma, then stigma becomes harder. In this situation, pollen tube cannot enter inside. Since this process is controlled by stigma, so it is called as stigma inhibition. Generally, SSI system shows stigma inhibition.

Other inhibition is at styler level, which is shown by GSI-system. Actually, in this process, pollens germination to form pollen tube, which germinates and by penetrating stigma, it enters style. Here, pollen tubes secrets hydrolytic enzymes. If these enzymes soften styler tissue then pollen tubes go ahead. It is called as styler tissue, and then pollen tube goes ahead. It is called as recognition reaction. But if these enzymes show callose deposition, then rejection reaction will occur. In this situation, pollen tube cannot proceed.

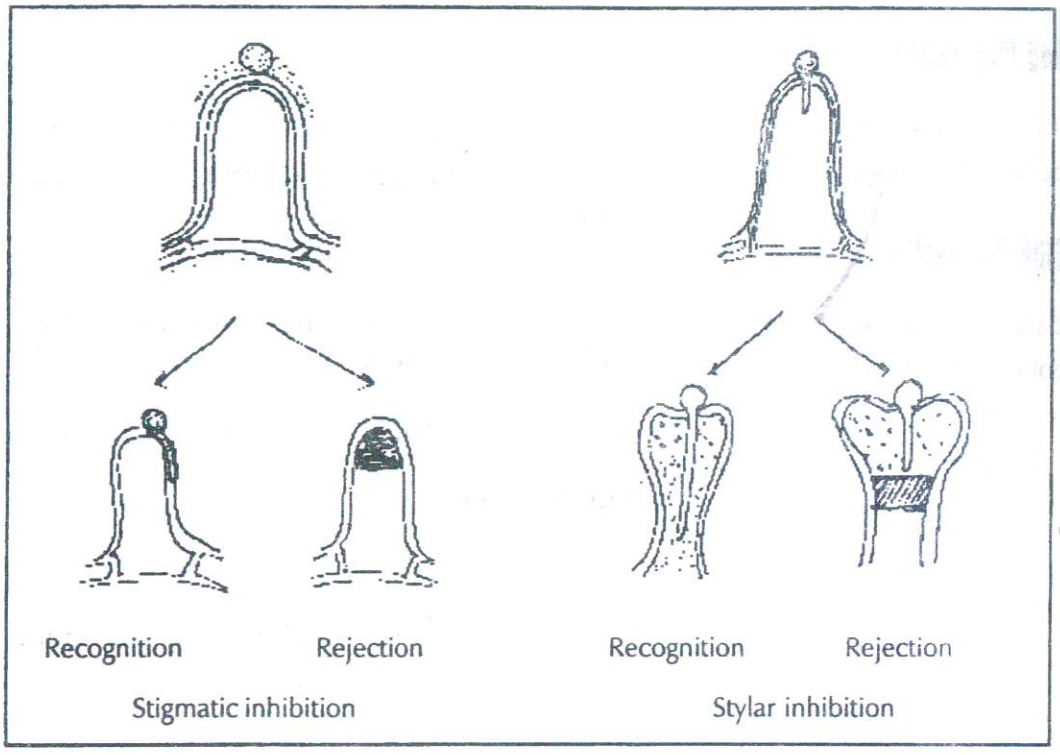


Figure 4.8: Mechanism of Recognition and Rejection at Stigma and Stylar Level

Methods to Overcome Incompatibility

Mixed Pollination

If the mixture of pollens is sprayed on the stigma, then stigma inhibition can be controlled. In this, live pollen can be inactivated by radiation, which is called as mautor pollen. By mixing it with incompatible pollen, and germ tube enters inside the other's style.

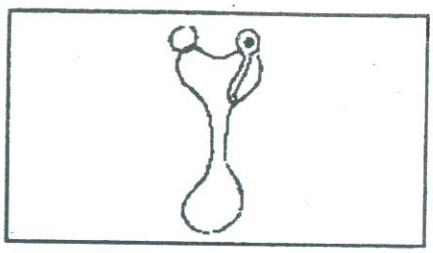


Figure 4.9: Manter Pollination

Bud Pollination

It has been seen that rejection reaction is set only after the formation of flower. Hence, if pollination is done in the bud from itself, then pollens show compatibility.

Stub Pollination

If stigma shows rejection, then stigma is cut and pollen is poured into style, due to which pollen will show compatibility.

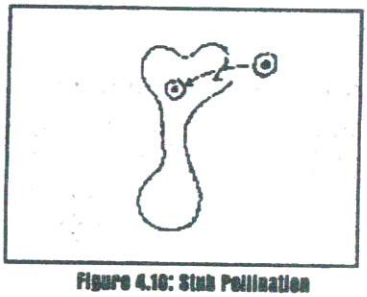


Figure 4.10: Stub Pollination

Intra Ovarian Pollination

If inhibition is shown on stigma or style then pollen is directly entered into the ovary, due to which fertilization occurs. But only the plants having the large ovary adopt this process.

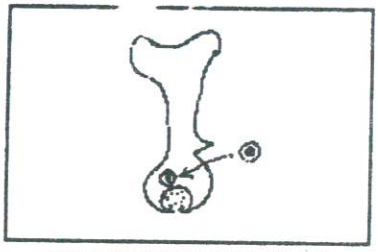


Figure 4.1: Intraovarian pollination

Test Tube Pollination

It is an easy method. In this stigma, style and ovary wall have been removed and fertilization has been made to occur by bringing ovule in contact with pollen.

Heat Treatment of Style

If style is kept at medium temperature (50°C) for sometime, then styler inhibition stops and pollen shows fertility.

Irradiation

Due to radiation,'S' allele gets mutated. In this condition, pollens show compatibility.

Chemical Treatment

Choudhary performed successful fertilization in plants by taking 100mg /liter 2,4-D.

Parasexual Hybridization

In this process, protoplast of pollen and egg is isolated and these are fused to obtain the zygote by protoplast culture. By this process, pollens become compatible.

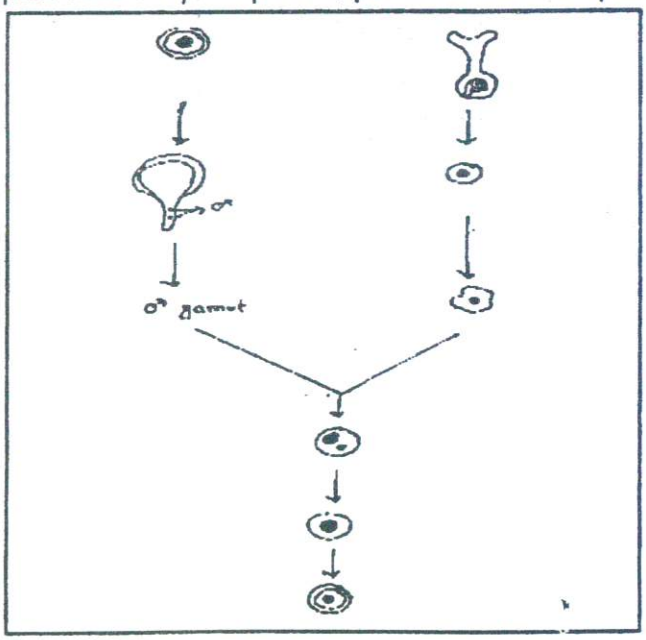


Figure 4.12: Diagram Showing Parasexual Hybridization

Polypoidy

It has seen that if pollen is diploid, then they don't show incompatibility.

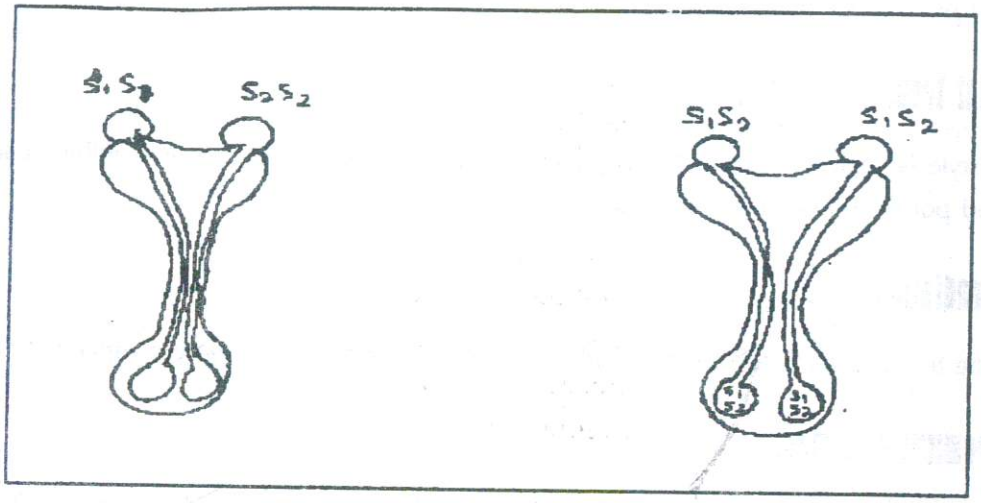


Figure 4.13: Polyploid Gametes Indicating 100% Incompatibility

Significance of Incompatibility

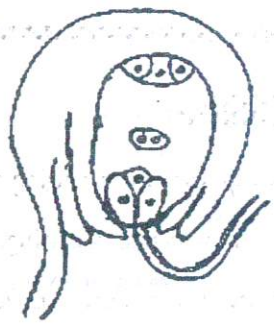
1. In nature, plants inbreeding and out breeding are balanced by incompatibility.
2. These controls inter and intraspecific breeding.
3. Intraspecific incompatibility shows reproductive isolation.
4. Incompatibility is an obstacle in plant improvement programme, because intraspecific fertilization is not possible.
5. Knowledge of incompatibility is helpful in understanding the method of preventing it effectively.

Double Fertilization

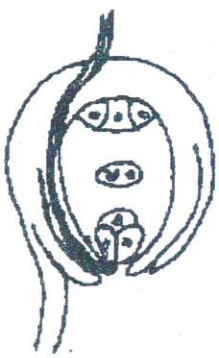
First time, Nawaschin reported that both the sperm that are related by pollen take part in fertilization. It means they fertilize 2 different components of the embryo sec. This phenomenon is an important characteristic of Angiosperm, is named as double fertilization. Actually, the nucleus one sperm fuses with the egg nucleus, which is known as syngamy, while the second sperm migrates in central cell and fuses with polar nuclei. Generally, plants are having 2 polar nuclei, and the third nuclei are of sperm. These 3 nuclei are fused

in the central cell, which is known as triple fusion. So, the double fertilization and triple fusion is the character of Angiosperm and it produces triploid central cell. Later, it produces endosperm. Although in plants only one polar nucleus is present, or in some plants, more than 2 polar nuclei are present.

During the fertilization when pollen tube enters in the synergids, it enters through filiform apparatus and then release sperms in the synergid, and only male nuclei is migrated out of it, but some evidences suggest that sometimes male cytoplasm is also involved in fertilization. Russel observed it in plumbago, while Cass observed it in Barley. It shows that cytoplasmic virions are transferred up to eggs, and it shows biparental inheritance of plastids in some plants. According to Jensen, the first sperm comes in contact with plasma membrane of egg, and second contacts the plasma membrane of central cell. At the point of contact, the membrane dissolves and the sperm nuclei enters the egg. Now, this nucleus is passively transferred upto egg nucleus and second male nucleus enters in the central



A. Porogamy



B. Chalazogamy

C. Mesogamy

Figure 4.14: Types of Entry of Pollen Tube into Ovule

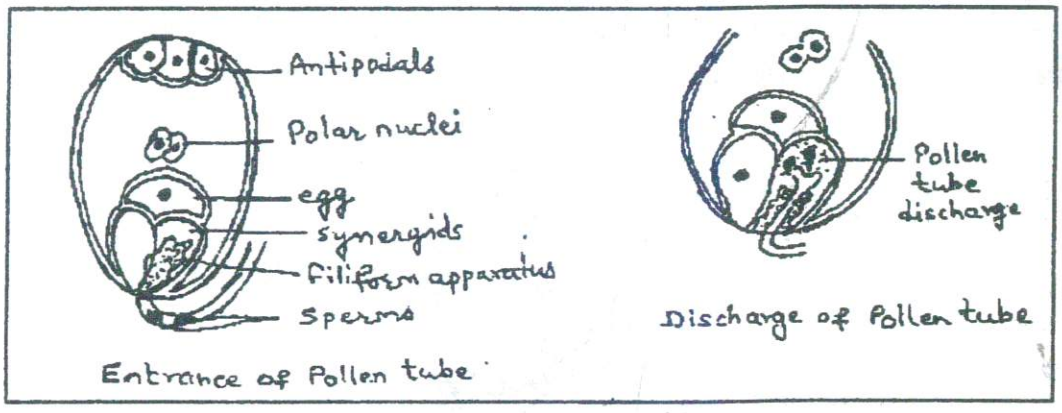


Figure 4.15: Entry of Pollen Tube into Embryo Sac Through Filiform Apparatus of Synergid

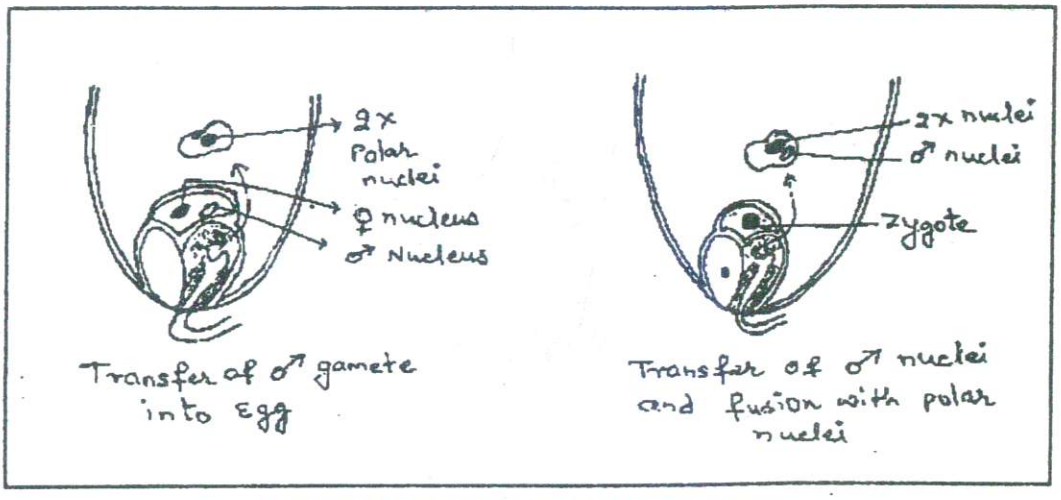


Figure 4.16: Formation of Zygote and Double Fertilization cell

It means, always first sperm attaches with egg. Although the fusion time of egg and sperm nuclei is longer, because the egg is present in inert stage, and central cell is in active state.

Fusion of egg nucleus and sperm nucleus is called syngamy, which is of three types:

1. Pre- mitotic

In this case, sperm nucleus immediately fuses with egg nucleus and the zygotic nucleus starts division like- Poaceae and asteraceae.

2. Post- mitotic

The sperm and egg nuclei remain in contact, but firstly they enter into division phase and afterwards fuse. Like- Lilium and fritillaria.

3. Intermediate

In this condition, sperm and egg nuclei fuse after completion of mitosis. So, contents of both nuclei shows incomplete mixing, like- Impatiens.

Significance

- The result of double fertilization is triploid nucleus and this nucleus produces endosperm. Genetically, it is strong. Its synthetic activities are high. So, it can accumulate more food, and it provides more nutrition to developing embryo.
- Due to double fertilization, endosperm always develops after fertilization, and it is fuse that zygote is there, which will develop the embryo. That's why, the energy which is accumulated by endosperm will be surely used, otherwise endosperm develops before fertilization (as in Gymnosperm) and if fertilization does not occur, then energy of endosperm will be wasted.

Seed Development and Fruit Growth

Endosperm Development During Early, Maturation and Desiccation Stages

Nawaschin reported that 3X nucleus is formed from double fertilization. Later, it develops as endosperm, which is integral part of seed. It means, in Angiosperms, endosperm develops after fertilization. Although in the Gymnosperms, it develops before fertilization. In Angiosperm, the endosperm is developed through a sexual process. In the Monocots,

endosperm is the main nutrient storage tissue in the seeds. While in many monocots, endosperm can be absorbed before seed maturation. So, the food is reserved in cotyledons.

Endosperm Development

Generally, endosperm develops by 3 means :

(a) **Cellular type**

In this condition, after each nuclear division, cell wall formation occurs and after new division, multicellular embryo gets formed. Like- Lycoprsicon and Centranthus.

(b) **Free nuclear type**

In this condition, cell enlarges and nuclear division takes place continuously. But, cell wall is not formed. Later, nuclei are migrated in the peripheral region and then cell wall formation occurs. Like- Cereals (Maize)

(c) **Helobial type**

In this condition, primary nucleus divides unequally, and unequal cells are produced. Larger mycropylar cells divides through cellular development, while smaller cells become multinucleated. So, it is intermediate condition between free- nuclear and cellular type, like- Ixolirion.

In the endosperm, the pattern of cell division is studied in maize. In the waxy locus, amylase accumulation does not occur. But, if AC transposon is removed, and then amylase is secreted. In the seed development, maternal tissue, embryo and endosperm show different genetic makeup. Endosperm is generally triploid, because it is having 2 female nuclei and 1 male nucleus. In the monosporic plants, genetic makeup of endosperm and embryo are similar, because these are having same meiotic products. Although quantitatively it is different, because embryo is diploid and endosperm is triploid. The ratio of maternal and paternal gene, is 2:1 in endosperm and in embryo, it is 1:1. It is also observed that if the gene balance in embryo, endosperm and maternal tissue is disturbed, then seed development is arrested, which is known as seed breakdown. The success of embryo is based on endosperm development, because failure in endosperm development shows embryo abortion. The genetic differences between parents show incompatibility.

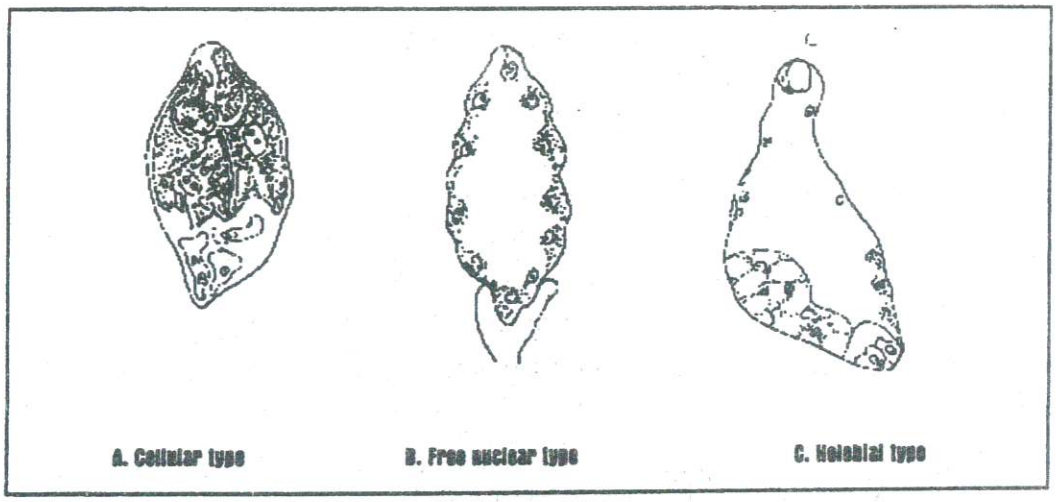


Figure 4.17

Types of Endosperm Development

Actually, in the different species, ploidy different occur, like- in the potato, when diploid and tetraploid plants are crossed, then embryo does not develop due to failure of endosperm. It is known as endosperm balance number, which indicates that successful cross occur between the species when endosperms maternal- paternal ratio is 2:1. Linn studied the maternal- paternal ratio in Maize. With the help of mutation, he created variations in the number of polar nuclei, by using diploid and tetraploid paternal plant and paternal genome variation was created. But always endosperm is found when maternal -paternal ratio was 2:1.

	Maternal	Paternal	M: P ratio	Endosperm (pre.andAb.)
Normal	Polar =2x	x	2:1	Endosperm
Mutation	Polar =4x	x	4:1	No endosperm
	Polar =2x	2x	1:1	No endosperm
	Polar =4x	2x	2:1	Endosperm

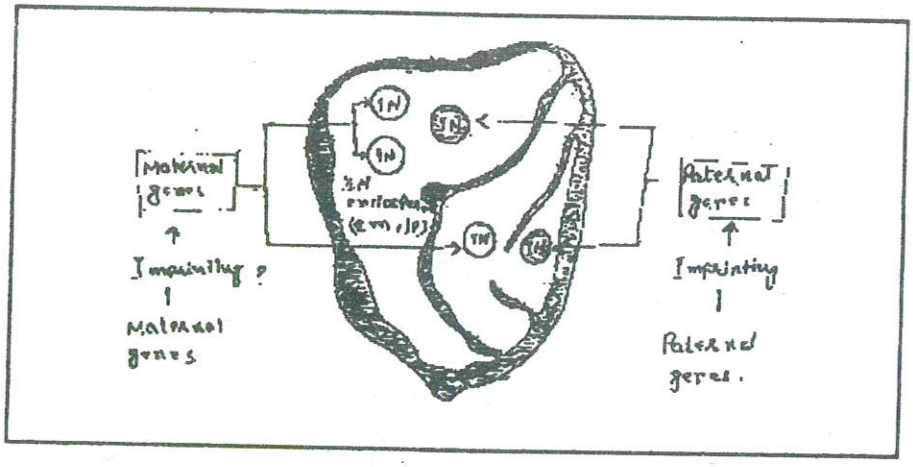


Figure 4.10: Diagram Showing Balance Between Maternal and Paternal Genomes in the Production of the Triploid Endosperm and Diploid Embryo in the Maize

It proves that for the endosperm development, proper balance should be there in M: P genome. Many genes are expressed only in endosperm, in which many genes are duplicated and show endosperm specific expression. They code the products that control the synthesis of stored nutrients, like- starch. If these genes mutate, then endosperm development gets affected, like- shrunken is a starch deficiency mutation, which inhibits synthesis of phosphorylase enzyme. So, starch biosynthesis gets inhibited. In the embryo and endosperm, direct symplast connections do not occur, which prevent virus transmission. It means the transfer of nutrient takes place through apoplast method. Firstly, the nutrients accumulate in the base of endosperm, and then transferred in other cells.

Maturation Stage (Storage Protein)

During this stage, seeds accumulate large quantities of storage macromolecules, like- proteins, lipids and starch. Some proteins are used as food source for embryo, while others are related with biosynthesis or protection. In all seeds, the major storage protein is globulin and albumin, while prolamines occur only in cereals. These storage materials are major source of food for man and animals. Large families of genes encode these storage proteins, and these seed storage protein genes are expressed during seed maturation. Its high level expression indicates the higher amount of protein accumulation. This protein synthesis is transcriptional regulated. According to Dure, on the basis of expression, genes are divided into two groups.

a. The first group expresses at mid maturation stage and synthesizes seed storage protein

b. The second group expresses at late maturation and early post abscission stages and synthesize late embryonic abundant (LEA) protein. These proteins behave like protectants. It protects the embryo from desiccation. It means the different genes are active in different maturation stage. It shows hierarchical control. The important regulator factor of gene is ABA. It enhances seed storage protein synthesis and suppresses the germination. In the wheat, the ABA level increases during seed development, while after maturation, level comes down. It also controls seed germination in immature condition. Like- in Arabidopsis, ABA mutants were studied. In the normal seeds, seed storage protein is napine and cruciferin, but in the mutants, the mRNA for these proteins is not synthesized, while mutants are related with ABA synthesis. It means ABA controls the synthesis of these proteins. But all protein synthesis genes are not based on ABA.

Desiccation Stage

Dormancy is the unique process that makes surviving the seed in unfavorable condition, although it is broken down during germination. At the late maturation stage of seed development, embryos are able to germinate, but its germination is prevented, in some seeds, instant germination can be obtained. It embryos are explanted at mid- maturation stage and placed in culture medium, then it does not show normal development, but continuour development occurs. It synthesizes the seed protein, while if normal embryo is taken in culture medium, then it switches on such gene, which are helpful to reserve food material. The main problem is when this switching condition occurs. The embryos switch off the protein synthetic genes and initiate the switch off protein breaking genes. Comai and Hendra indicated that this shifting occurs during desiccation. They also compared pattern transcription in maturing embryos and dry seed embryos. They also compared germinating seedlings .Dry seeds are not actively engaged in m-RNA synthesis. While in the germinating stage, gene expression is observed. They observed that the pattern of gene expression in dry seeds were similar maturing embryos. So, they concluded that during desiccation stage, no significant change occur in gene expression, but the new pattern is initiated during seed germination.

Embryogenesis, Ultrastructure and Nuclear Cytology

Cell lineages during Late Embryo Development

During double fertilization, the first cell will be zygote. It is diploid cell, which is developed into embryo. This cell passes through many developmental stages, which is known as embryogeny. The plant embryos are simple and made up of axis and cotyledon. Axis is related with seedling growth, which is made up of epicotyls, SAM, hypocotyls, radical and RAM. When the zygote is formed, then it shows dormant period. After syngamy, large vacuole gates reduced. Due to reduction in size, cytoplasm is deposited at chalazal end, where first division takes place. The number of active dictyosomes decreases. After fertilization, complete cell wall develops around zygote, so it becomes isolated cell and then it starts division.

Embryogeny

In the Angiosperm, the first division is transverse, which form apical and basal cells. In some plants, like- Lauranthaceae, this division is vertical and oblique in Triticum. This 2-celled stage is known as embryo. Through transverse division, these show 4- celled stage. Now, 2 upper daughter cells get divided through vertical division and form octant. In the angiosperms, 2 type of octants occur. In first, the component cells are arranged in 2 tiers of 4-4 cells, like- Capsella; while all 8 cells are present in 1 tier, like- Lactusa. In dicots, on the basis of division planes, following types of embryogeny have been recognized:

ii. Apical cell divides longitudinally:

   a. Embryo is developed only from apical cell: Crucifer or Onagrad type

   b. Basal and apical, both cells from embryo: Asterad type

iii. Apical cell divides transversely:

   a. Basal cell plays minor role or no any role (I) Basal cell forms suspensor: Solanoid type. (II) Suspensor is formed from apical cell: Caryophyllad type.

   b. Basal and apical, both cells from embryo: Chenopod type

When embryo becomes octant, then continuous division occurs and multicellular globular embryo develops. In this stage, embryo becomes 3- layered. L1 is protoderm layer, which

forms epidermis; L2 is ground Meristem. It is having storage proteins and forms cortical parenchyma cells, and L3 is procambial cells and form vascular tissue. These cells can be identified by cell division pattern. Like, in 16- celled proembryo, articlinal division takes place in protoderm. At the end of globular stage, cotyledons and axis system gets formed and later the embryo becomes heart - shaped. Then, it becomes torpedo- shaped and at last, cotyledons bend down. These changes in shape occur due to cotyledons. Another important aspect is formation of RAM and SAM that is post- embryonic centre of plant development. RAM is developed at the beginning of heart stage, when hypocotyls elongate. Its origin is dual. It is derived from proembryo and upper cell of proembryo, while SAM is formed at the late heart stage. It develops between emerging cotyledon. Its cells are small and with dense cytoplasm. In the organization of SAM , cells are taken from all the 3 layers, which indicate cell lineage in 3 layers.

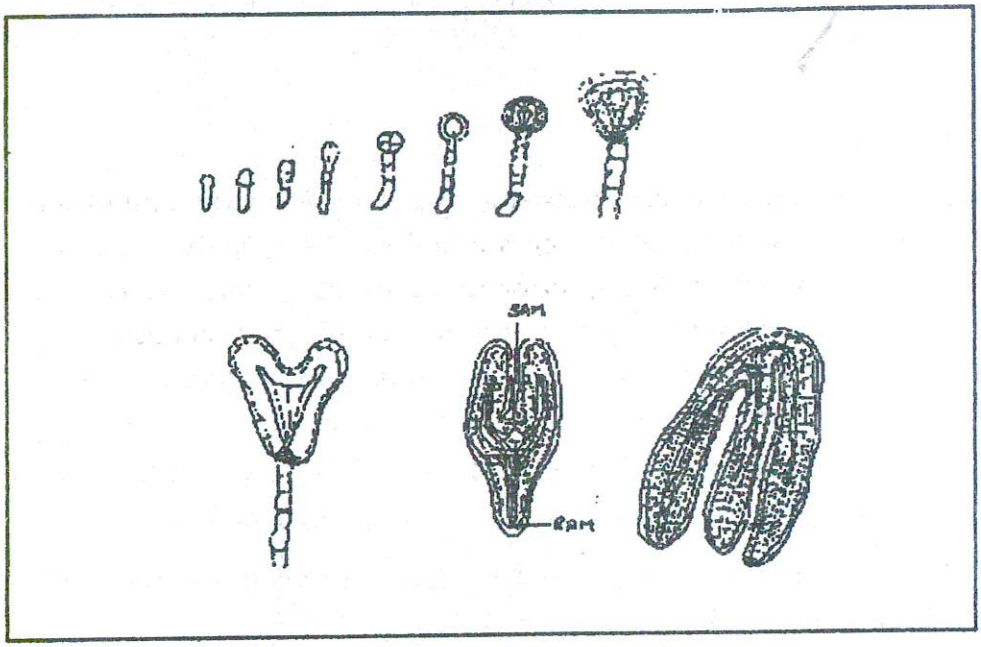


Figure 4.18: Different Stages of Embryo Development

Ultrastructure and Nuclear Cytology

Plant embryos are morphologically simple, but molecularly complex. In the embryo, many genes express at a time. The complexity of RNA is studied with the help of RNA- DNA hibridigation. In the Nicotiana, 20,000 types of RNA have been isolated. Some genes express in stage- specific manner, like- seed storage protein genes. So, the cDNA of stage specific genes can be used as marker gene. The RNA accumulated in ground tissue Meristem

at mycropylar end. Then, cell differentiation occurs. So, the gene expression is first molecular indicator of polarity.

For the study of genetics of Embryogenesis, embryo lethal mutants and pattern affecting mutants are used. Embryo lethal mutants can block embryo viability and development, while pattern formation mutant is related with different patterns in embryo. Its important genes are master regulators, which are present at the top of regulatory pyramid. Master regulators are less in number. With the help of this study, it has been seen that 4000 genes are required for normal Embryogenesis, while 40-50 genes affect pattern formation. On the basis of pattern mutant, it has been indicated that apical basal axis can be divided into 5 segments, which produce different organs in embryo or seedlings, which are SAM, cotyledon, hypocotyls, radical, and RAM. This differentiation can be seen in early stages. In the 4- celled stage, upper and lower tiers are present. Upper tier produced cotyledons and SAM, while lower tier produced RAM and hypocotyls.

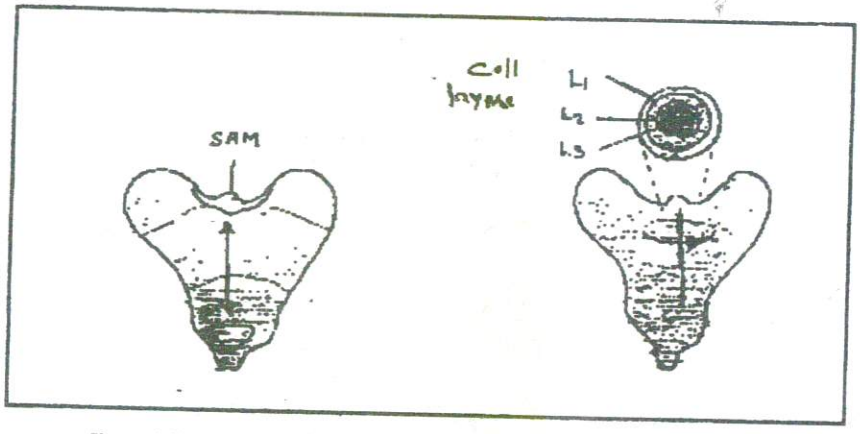


Figure 4.26: Body Plan and Segmentation Pattern Proposed for Arabidopsis Embryos

Apical- basal axis mutants also indicate it. Like- gurke mutants lack the apical segment while fackel mutants lack the middle region. So, the embryo develops from 3 layers- L1to L3 and in the mutants, when any layer is missing, then, deficiency is created.

Cell Fate Maps and Lineage System

In the maize embryo, regular pattern of cell division has been observed. In Arabidopsis, this organization is regular. The fate of cells can be studied in heart shaped embryo, and in Arabidopsis, root and hypocotyls cell lineage has been observed. But, it is not clear that root and hypocotyls were developed in compartment; to carry out their cell lineage analysis, CaMV35 promoter GUS is used as a marker, which is interrupted by AC transposon, and

the GUS- marker is activated where the events occur. In the heart stage embryo, 4 tiers of ground Meristem cells occur, which develops root and hypocotyls. In the globular stage, upper and lower tiers of cells are present, which are derived from initial proembryo cell. Then, lower tier divides by transverse division, which produces ground Meristem and protoderm cells. At the late heart stage, lower tier is divided into 4 tiers and the lowermost protoderm cell becomes RAM initial, which is divided by periclinal division and forms lateral root cap. Its other tiers produce hypocotyls and intermediate zone. The largest embryonic sector is changed into cotyledon. Second largest sector is root and hypocotyls. In such a way one by one tiers are formed and the organs in the embryo differentiate.

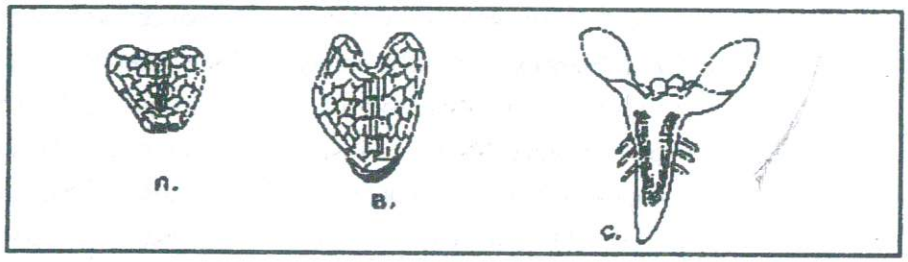


Figure 4.21: Fat Map of Arabidopsis Embryo Focusing on Root and Hypocotyls Regions. Lower tier it of cells is divided by transverse division at an early heart shaped stage.

A. An upper tier It and lower tier IIt

B. Late heart shaped stage

C. Seedling

Fate map of Arabidopsis embryo focuses on root and hypocotyls regions. Lower tier, 1t, of cells is divided by transferase divisons at an early heart -shaped stage.

(A) Into an upper lower tier and a lower tier (2t).

(B) Late heart-shaped stage,

(C) Seedling.

Dynamics of Fruit Growth

In the angiosperm, fruit development is an important character. True fruits are developed after fertilization, but sometimes, fruits are developed without fertilization, which is known as parthenocarpy.

Fruit Growth

Generally, fruits are made of epicarp which is single layer in dried fruits, while 3-layered in succulent fruits, which are epicarp, mesocarp and endocarp. Generally, epicarp is hard, mesocarp is spongy and endocarp is membranous. Mainly, fruit is derived from L1 and L2 . Which signal is responsible for fruit development is not known, but it is concluded that the clicking of fruit growth is result of pollination or fertilization, because sometimes, without seed formation, fruits develop, and the parthenocarpy is genetically controlled by hormones, like: - GA and Auxin. In the tomato, fruit development is divided into 3 steps.The phase I is related with ovary development, fertilization and fruit setting, which is known as anthesis, while during phase-II, cell division occurs in fruits. So, seeds are produced and embryos are developed. In phase III, fruit cells are expanded and embryos mature. After development of fruit, ripening occurs, in which fruit becomes soft and colour changes. During fruit growth, cell division occurs in pericarpand placenta. Its rate is affected by seed development. In the tomato the rate of fruit growth is proportional to number of seeds in tomato, and the fast growth is observed in phase III. At this time, cell division is inhibited, so growth is result of cell expansion. During the fruit formation, metabolic capacity of plant increases. Fruits collect sugars and starch, because they behave like metabolic sink capacity, is genetically determined but indirectly, cell numbers in ovary controls it. The cells of green tomato having chloroplast and pericarp are similar to palisade layer of leaf. Many genes control fruit weight, which may be 5-25.

Fruit Ripening

During fruit ripening, colour, softness, aroma and flavor get changed. Although in the different species, different biochemical pathways are involved, but characters are common. Fruits may be climacteric and nonclimateric fruits, respiratory burst does not occur and ethylene is not involved as: -- orange and strawberry. It means ethylene is important factor for ripening of climacteric fruits. It is initiated after cell division and cell expansion destroyed. At that time, respiration suddenly increases and higher amount of ethylene is produced. If we use ethylene inhibitors, like: -- silver ion, then ripening of fruits is inhibited. The treatment of tomatoes delay it's ripening. For explanation of its mechanism, tomato mutants have been discovered, which are insensitive to ethylene. The ethylene is a triple response hormone. Its fruit-ripening mutant is known as never ripe (Nr) mutant. It cannot show triple response of ethylene, and it shows the ETR1 mechanism.

The precursor of ethylene is 1, aminocyclopropane1, carboxylate(ACC) synthase. It is the rate-limiting step of ethylene biosynthesis. This enzyme is coded by one enzyme, when its antisence RNA was transferred, then ethylene production inhibited and it prevented ripening. In this condition, if ethylene is provided from outside, then ripening occurs. Actually, the softening of fruits occurs due to dissolution of calcium pectate of middle lamellae, which is dissolved by ethylene. So cells become loose and fruits become soft.

Polyembryony

Introduction

Presence of more than one embryo in a seed is called as Polyembryony. Additional embryo does not mature always. These remain arrested in early stages or these get degenerated during seed development. Therefore, if in any species, mature seeds are found out on the basis of percentage of polyembryony, and then they will be lesser in comparison to actual frequency. Hence, in developing ovule, actual presence of two or more than 2 proembryo or embryos indicates polyembryony. In some texa, like- Citrus, mangifera, occurrence of polyembryony shows abnormal feature. Antone Von Leeuwenhoek (in1719) first time reported occurrence of polyembryony in orange seed.

Polyembryony in Angiosperm

- Cleavage polyembryo
- Formation of embryos by cells of embryo sac in comparison to egg.
- Development of more than one embryo sac in the same ovule.
- Activation of same sporophyticcells of ovule.

1. Cleavage polyembryony

1. In Angiosperms, in orchids, commonly cleavage polyembryony is found. Swamy (1943) reported supernumerary modes of embryo formation in Eulophia epidendraea.
   (a) Eulophia epidendraea
   (b) Zygote divided irregularly and mass of cells gets formed, which simultaneously grow at chalazal end and forms many embryos.

(c) Small bud grows out from the proembryo, which acts as embryo.

(d) Branches get formed from filamentous embryo and each branch forms embryo.

In orchids, cleavage polyembryony develops during seed development. In Vanda, during seed germination, plural embryo gets formed. In this genus, apical promeristem of embryo divides to form 3 to 9 primordial, which develop and form embryos. In genus- exocarpus, suspensor polyembryony is found. Six embryos get developed in the ovule by the proliferation of suspensor cells. In Zygophyllum fabago, suspensor embryo develops in heart shaped stage.

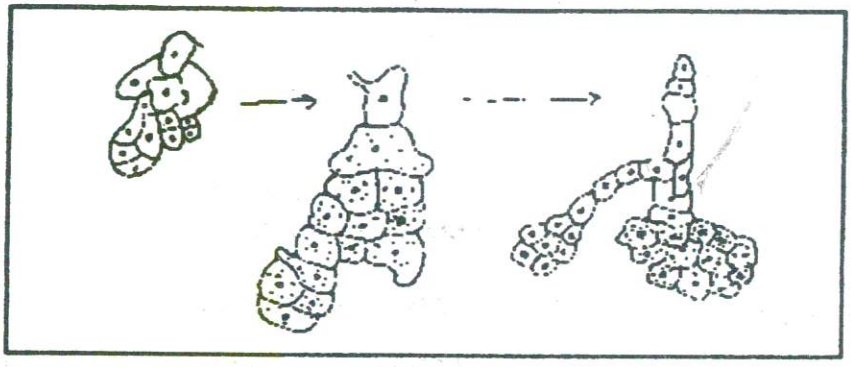


Figure 4.22: Cleavage Polyembryony in Eulophia epidendraea

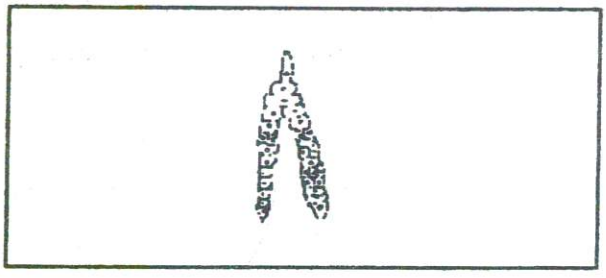


Figure 4.23: Suspensor Polyembryony

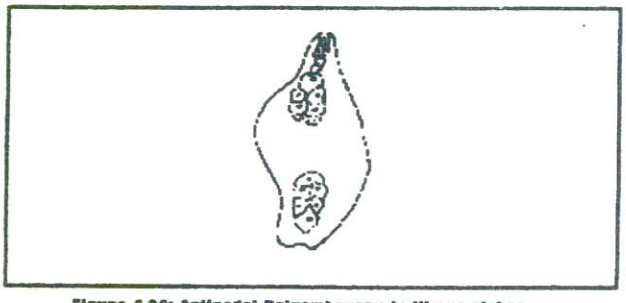


Figure 4.24: Antipodal Polyembryony in Ulmus glabra

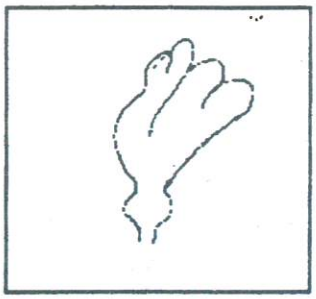


Figure 4.25: Twin embryo

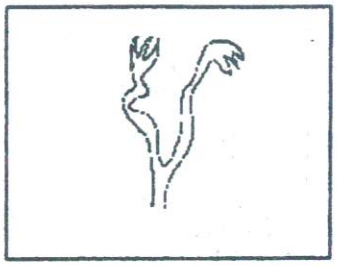


Figure 4.26: Induced polyembryony in Eranthis hiemalis

2. Embryos from Cells of the Embryo Sac Other than the Egg

In this class, the main source of additional embryo is synergids. Embryo either develops from fertilized or unfertilized synergids and embryo may be either diploid or haploid. In Aristolochia bracteata, Poa alpina and Sagittaria graminea, one or both synergids get fertilized at the place of eggs and polar. This process occurs by the entrance of one or more pollen tubes in the embryo -sac or by the presence of additional sperms in the same pollen tube. In this stage, like zygotic, synergids embryos are diploid. In Argemone mexicana, Phaseolus vulgaris, embryos develop from unfertilized synergids. In this case, zygotic embryo can be differentiated from the synergids embryo due to its diploid nature. Rarely, embryos are formed from antipodal. This has been observed in Paspalum scorbiculation, Ulmus americana, U. glabra ets. Sometimes, antipodal cells divide to form polyembryo like structure.

3. More than one embryo sac in the same ovule:

Thus may be many embryosac in an ovule:

(a) Derivatives of the same megaspore mother cell.

(b) Derivative of two or more megaspore mother cell.

(c) Nucellar cells.

One ovule has twin embryosac in *Casurina equisetifolia*, Citrus and Poa pratensis. Commonly embryo sac develops up to 4 cell- stages and multiple embryos develop by the aposporous embryo sac. After fertilization, embryo grows downwards and composite endosperm enters into the ovarian cavity. In Scurrula pulverulenta, 2% seeds show polyembryonate condition. In polyembryonate seeds, among two or more embryos, one is well developed and the other remains undeveloped, non- green. In Struthanthus vulgaris, exceptionally one embryo sac is there, which does not show polyembryony.

4. Activation of some Sporophytic cells of the ovule:

Embryo that develops from the maternal Sporophytic tissues, is known as adventive embryo. Nucellus and integuments are those maternal tissues, which form adventive embryos. Some species of citrus like C. grandis and C. limou shows monoembryonate stage while some species like C. microcarpa and C. reticulata show polyembryonate stage. In polyembryonate species, adventive embryo is formed due to the proliferation of nucellar cells. Sometimes, from the mycropylar half of the nucellus, nucellar embryo develops. In Mangifera, nucellar cells form adventive embryos, which can be differentiated from other nucellar cells by dense cytoplasm and starchy content. At first beginning of nucellar embryos starts outside the embryo sac, but slowly this gets pulled up towards inside the embryo sac cavity, and there it divides and differentiates into mature embryos.

Adventive embryo does not show embryo in many stages of development. Pollination stimulus is necessary for the initiation of nucellar embryos. In Opuntia dillenii, egg apparatus, antipodal and polar and many adventive embryos develop from nucellar cells. Naumova (1981) had shown the relationship between the kind of adventive embryony and ovule and told that nucellar embryony appears in crassinucelate ovules and integument embryony appears in tenuinucellate ovules (e.g. Euonymus). The cells of parietal tissues, not by the cells of nucellus, form Nucellar embryos. In integument embryony, mycropylar and chalazal region of both epidermal cells separate to form embryo.

Causes of Polyembryony

Some theories consider hybridization, necro-hormones and effect of recessive gene as the cause of polyembryony.

(a) Haberlandt (1921, 1922) proposed 'nacrohormone theory' and he proposed that degenerating cells of nucellus as a source stimulates adjacent cells so that

they may divide to form adventive embryo. In Oenothera, Haberlandt induced adventive polyembryony by pricking the ovule and then by carefully squeezing the ovaty and he got 2 embryos from a single ovule.

(b) Levoy (1947) proposed that polyembryony in mango is induced by one or more recessive genes. He told that in Eastern India, monoembryonate form occurs, which has effective genes while in China, Phillippines and Sudan, polyembryony seeds having ineffective genes are found.

(c) Trusato (1957) showed that embryo of citrus seeds are produced by the following factors:

1. Age of the trees; increasing in older trees.
2. Fruit set; being higher in years of higher fruit set.
3. Nutritional status of plant; decreasing with reduced food supply.
4. Orientation of the branch of the tree; being higher on northern or southern branches.

In some species of citrus, monoembryonic state is induced by the synthesis and conduction of volatile and non-volatile embryonic inhibitors from ovule. Ethanol is a volatile inhibitor, which is produced by C. medica. Non-volatile components of inhibitors include IAA, ABA and GA.

Experimental Induction of Polyembryony

In the member- Eranthis hiemalis of Rannculaceae family, in shade, seeds enclosed in undifferentiated embryo, which are pear-shaped, having long suspensor and having radicle like cotyledon and they differentiated after many weeks. These seeds are found in soil. On treating the fresh harvested seeds with 2,4-D, 2,4,5-T or NAA and on 0.1% concentration, abnormalities were induced in them, some seeds developed twin embryo. This treatment destroys plumule and the cells, which produce cotyledons. When older embryo is treated with acidic buffer (pH= 4), then embryonal body gets destroyed but suspensor cells develop into new. Adventive embryo-I destroys and second adventive embryo develops.

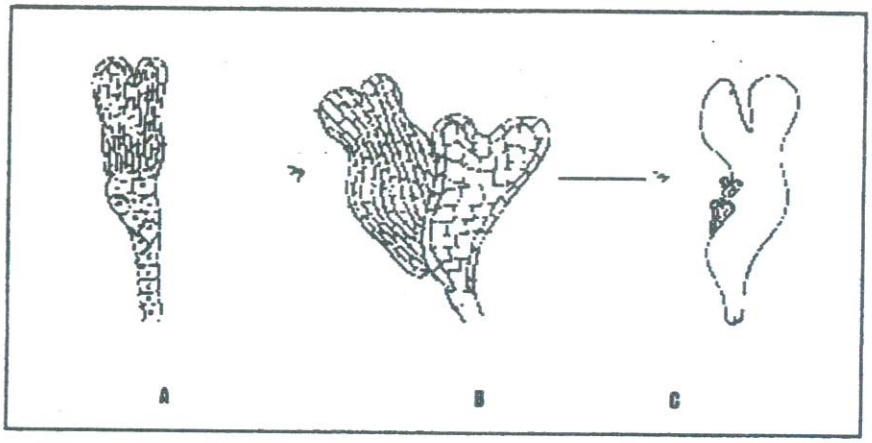


Figure 4.27: Eranthis Hiemalis: Induced Adventative Polyembryony from Suspenser

Special type of physical and chemical environment is needed for the embryo development, which is available in the magic bath inside the embryo sac. If necessary nutritional and environmental condition is made available in culture-vials, cells of plant body stimulate and develop viable embryo. By culture, we can develop accessory embryo, adventive embryo, embryoids, somatic embryos and supernumerary embryo. By culturing zygotic embryo (Dendrophtoe faicata, Solanum melongena) nucellus (monoand polyembryonate species of citrus), root segment (Daccus carota), stem segment (Nicotiana), leaves (Macleaya cordata), fruit tissues etc.,embryoids can be achieved.

Buttercup and carrot plants are specially used for the formation of these both of the plants, embryoids can be obtained.

Proliferated buds of Ranunculus scleratus forms amorphous mass of tissues, which is called as callus. After 6 weeks, many embryoids are formed from callus.

In carrots, for development of embryoids, and processes are used in in vivo state. Each needs separate medium. In 0.5-1mg of 2,4-D, callus initiates, such type of medium is called as proliferation medium (PF medium) and callus gets differentiated into the group of meristmatic cells: -- Embryonic clumps (EC). If PF medium is repeated, then ECs will be multiplying without the presence of mature embryoids. If ECs is transferred into low-level auxin (0.01-0.1 mgl-1) or in medium without 2,4-D then mature embryoids are formed. If medium is called as induction medium, because if in auxin free medium, callus is continuously maintained, then ECs does not develop.

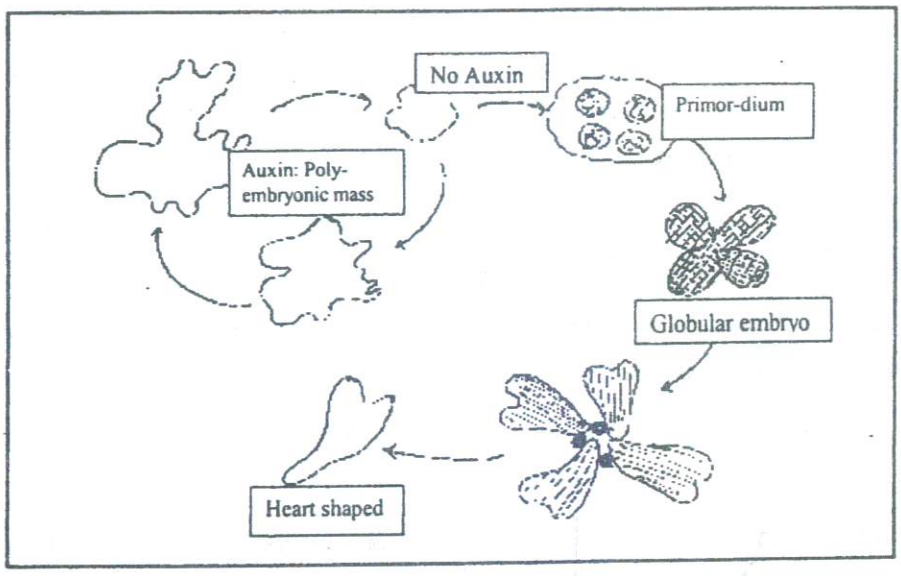


Figure 4.20: Induced Embryogenesis in Suspension Culture

Guha and Maheshwari, first of all obtained embryoids by the culture of pollen grains of Datura. By this technique, large number of haploids in a much less time can be obtained. In this process, pollen grains multiply and divide in a large number and multicellular grains are formed, which burst and form either embryoids or callus. Then, callus differentiates to form embryo.

For embryoids formation, 2 media are considered as factor controlling. These are auxin and source of nitrogen. In citrus sinensis, auxin is needed for somatic Embryogenesis and its nucellar callus needs IAA and kinetin for its growth and embryoid differentiation. Other factors needed for embryoid formation, these are reduced form of nitrogen as: ammonium nitrate, ammonium chloride, glycine, yeast extract etc. In callus, positive correlation is found in between the embryoid formation and concentration of soluble and insoluble organic nitrogen.

Classification of Polyembryony

Polyembryony is of two types:

i. Spontaneous: It includes natural polyembryony.

ii. Induced: It includes practically induced polyembryony Ernst subdivided spontaneous polyembryony into two kinds:

(a) **True polyembryony:** Two or more than two embryo are formed from one embryo sac. Ex: – From zygote and embryo (Eulophia, Vanda), from synergid (Sagittaria), from antipodal cells (Ulmus) or by nucellus/ integuments (Citrus spiranthes) etc.

(b) **False polyembryony:** Embryos develop from more than one embryo sac in an ovule (fragaria) or in a placenta.

Yakovlev (1967) classified polyembryony on the genetic basis. He distinguished spontaneous polyembryony into two:

(I) **Gametophytic:** Production from any gametic cell of embryo sac after fertilization or without fertilization.

(II) **Sporophytic:** Production from initial Sporophytic cells of ovule (nucellus, integument) or proembryo, zygote.

On the basis of origin of additional embryo, Bouman and Boesewinkel (1969) proposed that spontaneous polyembryony is split into four parts:

(i) Supernumerary embryos originate from Sporophytic cells of parental generation.

(ii) Supernumerary embryos originate from the cells of gametophyte.

(iii) Supernumerary embryos originate from new sporophyte (fertilized egg or proembryo).

(iii) Supernumerary embryos originate from male gametophyte.

Practical Value of Polyembryony

Nucellar adventive polyembryony has special importance in horticulture. Adventive embryos provide uniform seedling of parental type, which is obtained by cutting through vegetative propogation. In Comparision to cutting, nucellar seedling of citrus gives better result, because:

(i) Nucellar seedling is having taproot. Hence, as compared to cutting, it develops better root system.

(ii) Restoration of lost vigor occurs through nucellar seedling, which does not occur in the process of cutting.

(iii) Nucellar embryos are free from any disease. Hence, to obtain the virus free polyembryonate clones of citrus, nucellar polyembryony is the only practical

approach. These is no any in vivo method of obtaining virus free clones itself, formation of nucellus and induced embryoids can be done.

By callus culture, plant regeneration can either be obtained by shoot bud differentiation or by somatic Embryogenesis. Shoot bud and somatic embryo can be obtained from the single cell, but by Embryogenesis through tissue culture, solid mutants can be obtained.

There is a special economic importance of haploid in genetic and plant breeding species, because by colchicines treatment, homogenous diploid can be obtained from it. Haploid can also be generated from unfertilized eggs or synergids. Due to this, the frequency of following increases:

(i) Subjection of flowers at high and low temperature.

(ii) To irradiate pollen and foreign pollen by X-rays.

(iii) Delayed pollination.

(iv) Treatment of ovule by many chemicals.

Now-a-days, new methods have also been developed to generate androgenic haploids in a large number by anther culture.

Apomixis

Commonly in sexual reproduction, two methods have been included:

(i) Meiosis,

(ii) Fertilization.

In meiosis, diploid Sporophytic cell transforms into 4 haploid gametophytic cells, while in fertilization, two haploid gametes (a male and other is female) fuse to form diploid sporophyte. Hence, in sexual reproduction, there exists an alternation of generation in between diploid generation (Sporophytic) and haploid generation (gametophytic).

Plants in which sexual reproduction is completely replaced by asexual reproduction, they are called as apomictic and this phenomenon is termed as "apomixis". According to Winkler (1908), Apomixis is that process in which common sexual reproduction is replaced by that reproduction in which meiosis and syngamy do not occur.

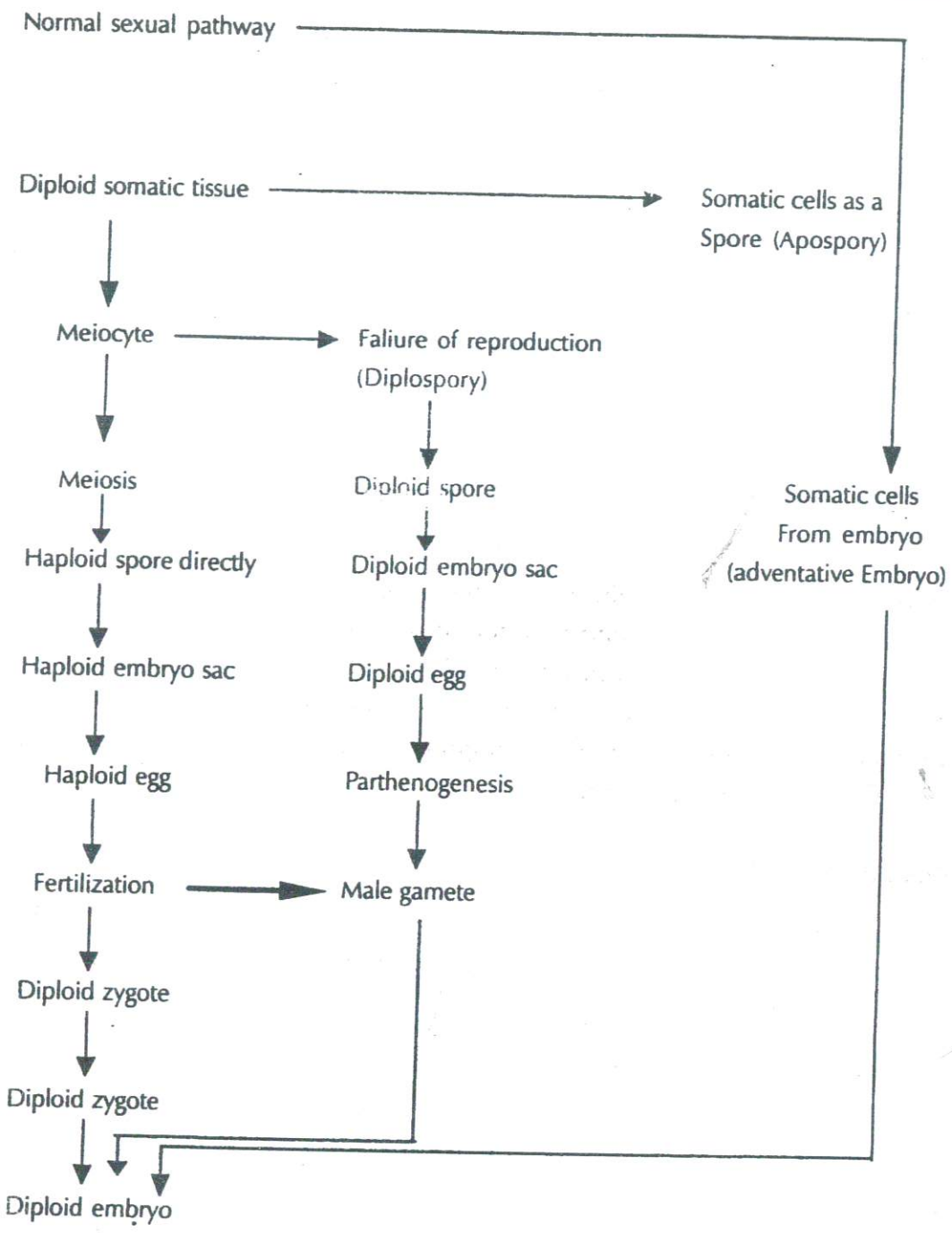


Figure 4.29: Developmental Pathway of Various types of Agamosperny Compared with the Normal Sexual Cycle

Vegetative Reproduction

Gustafsson (1946) differentiated vegetative reproduction by 3 types:

(i) Propagules are formed in plants outside the floral regions and reproduction and seed settings do not occur. E.g.: Agave american and Elodea Canadensis etc.

(ii) Propagules are formed outside the floral region of plants and are sexually sterile. Fritillaria imperialis and Lilium bulbiferum are its examples. These propagate through bulbils and bullbats.

(iii) Propagules are formed on the floral branches of plants. These are formed either along with flowers or at the place of them. This process is found commonly in vivipary.

Vegetative vivipary commonly occurs in grasses (Deschampsia, Festuca, Poa) and in Allium. Actually, it is an adaptation for the multiplication of genotype, which resists the chance of normal pollination under environmental condition. Vegetative vivipary can be induced artificially. By giving light to Poa bulbosa for 16hrs (1week), initiation of inflorescence can be promoted. Normal florets develop under high temperature (21-27°c and long days), but after inflorescence initiation, if low temperature (20°c or below and short days) is given, then bulbils develop in place of normal florets.

Agamospermy

(i) *Adventive embryony:* In such type of agamospermy, gametophytic generation completely disappears. It is close to vegetative propogation, but differs in 2 ways:

1. These posses seed habit.
2. A single diploid Sporophytic cell forms mature embryo by growing in sexual embryo sac.

In adventive polyembryony, formation of more than one embryo in a seed takes place. For e.g.: -- Buxaceae, Cataceae, Euphorbiaceae, Myrtaceae and Orchidaceae etc.

(ii) **Diplospory:** In this process, without regular meiotic division, Megaspore Mother Cell (MMC) forms diploid embryo sac. On the basis of division of MMC, Diplospory can be differentiated:

(a) Type1: In this, MMC divides like meiotic prophase. Pairing of homologous chromosomes also occurs, but dissociation of chromosome also takes place along with the formation of restitution nucleus and dumb-bell-shaped nucleus then rapidly changes into spherical shape. MMC with restitution nucleus directly develops into embryo sac or these divided into 2 cells by mitotic division, among which one degenerates and the other forms embryo sac.

In triploid species, like Ixeris dentate, division of MMC takes place through semi-heterotypic method, in which no any pairing occurs. In anaphase, chromosome spread in spindle. Finally, 3 mitotic division of restitution nucleus forms restitution nucleus,, it forms, 8-nucleated embryo sac.

In diploid species, Taraxacum, sexual reproduction takes place, but in polyploid species, apomictic reproduction occurs. MMC divides by heterotypic division and restitution nucleus gets formed. Along with restitution nucleus, MMC divides by regular mitosis to form dyed. From chalazal cell of dyed, embryo sac is formed and mycropylar cell degenerates.

(b) Type2: In this, nucleus of MMC directly forms unreduced embryo sac by common mitotic division. For ex. —Calamagrotis sp.Eupatorium etc.

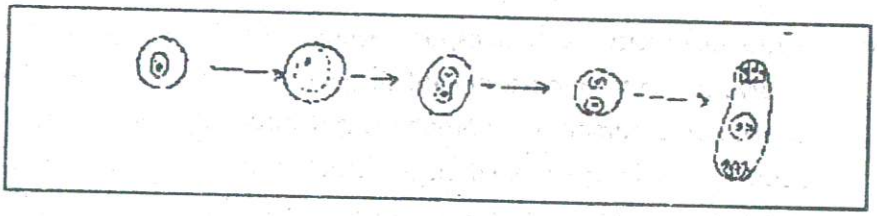


Figure 4.30: Diplospory in Ixeris dentate

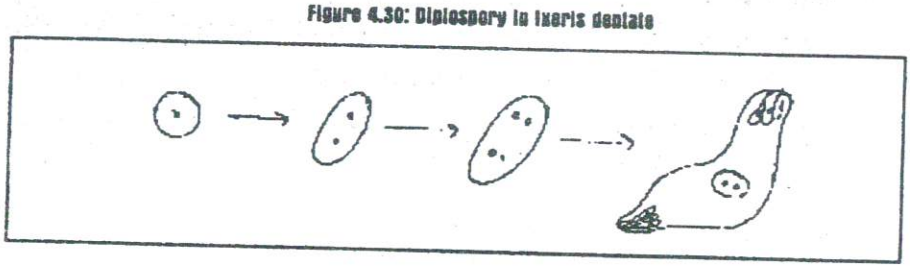


Figure 4.31: Diplospory in Eupatorium glandulosum

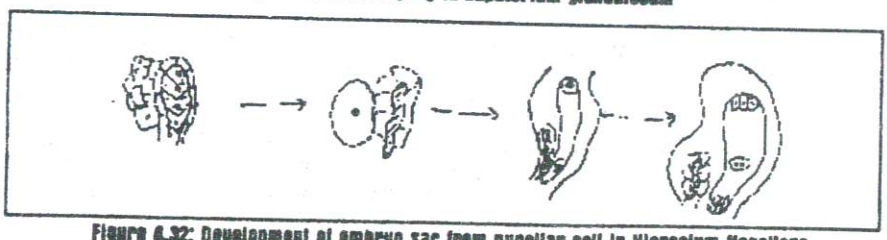


Figure 4.32: Development of embryo sac from nucellar cell in Hieracium flagellare

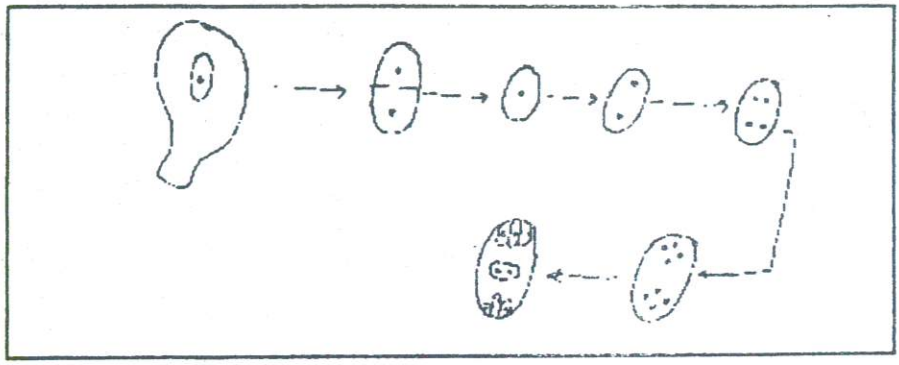


Figure 4.33: Diplospory in Taraxacum officinale

(iii) **Apospory:** First of all, Rosenberg (1907) reported Apospory in Hieracium sp. of angiosperm. In this, MMC, by simple meiotic division, divided to from tetrad. In this process, nucellar cell activates and start to develop. It behaves like mother cell and forms 8-nucleated embryo sac. In grasses, more than one embryo sac develops in a single nucellus and 4-nucleated embryo sac matures. (3-celled egg apparatus and a single polar nucleus).

(a) **Parthenogenesis:** Like Diplospory, Apospory also develops by diploid embryo sac. In the process of parthenogenesis, without any change in the chromosome number of Sporophytic generation and by completion of apomictic cycle, the diploid egg, without male nucleus, develops into embryo, or formation of embryo by unfertilized egg is called as parthenogenesis. In autonomous apomixis, embryo development is independent of pollination stimulus. This process is called as pseudogamy. The most important example of pseudogamy is apomictic grasses. Heslop-Harrison (1972) showed 3 possible roles of pollination in pseudogamy:--

1. To activate growth of ovary and ovule.
2. Supply of male nucleus for endosperm development.
3. To stimulate parthenogenesis.

In Citrus sp. pollination is necessary for initiation and development of adventive embryo. Endosperm develops only after the fusion of male nucleus and polars, hence only after pollination, development of mature apomictic embryo occurs.

(b) Pollen Formation: In diplosporous species, in male, it is a typical irregular meiosis. Therefore pollen formation is common in aposporous types.

Causes of Apomixis

Origin of apomixis is hybrid origin and Polypoidy. Hybrid origin gives the signal that whenever meiosis is present in this species, then it occurs due to failure of synapsis or due to simple contraction of chromosomes. Allium carinatum is indominant apomictic species, in which due to single gene, replacement of flowers occurs by bulbils. Recessive genes control apomixis.

Polyploidy condition is found in parthenium argentatum and experimental study suggests that in it, 3 pairs of genes decide the breeding behavior. In homozygous condition, gene a forms unreduced egg and gene b restricts fertilization. The gene c takes part in development of egg without fertilization. From the plants having the genetic constitution aabbcc, unreduced eggs are formed, but these do not develop into embryo without fertilization. Reduced eggs are formed from the plants having the genetic constitution Aabbcc, but due to the restriction of fertilization, embryo cannot get formed. Plants of AABBcc genotype are having the normal sexual behaviour. Gene C has no effect in the presence of gene A and B. Hence, eggs get reduced and fertilization takes place. Only those plants, which have the genotype aabbcc, they are apomictic. Plants having the mixed genotype show segregation and by complete sexual plants, apomictic offspring develops.

Significance of Apomixis

In apomixis, due to segregation and recombination without any variation, the favorable biotypes can be multiplied.

Somatic Embryogenesis

Somatic embryogenesis is a process in which a single cell or a group of cells initiate a developmental pathway which lead to reproducible regeneration of non-zygotic embryos which can germinate to form complete plants. This is not a natural pathway but occurs frequently in tissue cultures (alternative to organogenesis) & leads to whole plant formation. According to Sharp et al (1982) somatic embryogenuesis is initiated either by "Pre-Embryogenic Determined Cells" (PEDCs) or by Induced Embryogenic Determind Cells (IEDCs).

In PEDCs the embryogenic pathway is predetermined. The presence or removal of inducer activates the cell to start mitotic divisions e.g. callus and embryosac. While in IEDCs the exposure to specific growth the regulator eg. 2,4-D re-letermines the embryonic state of cells. These cells differentiate in anther and callus culture. On reaching the embryogenic state both types of cells proliferate in a similar way i.e. the Embryogenic Determined Cells (EDCs). These cells follow embryogenic pathway and produce plantlets.

Some time cells escape and form embryoids or nodular embryogenic cell, which consists of proembryoids – these are embryo like structure with bipolar unit, which can form full plantlet under suitable conditions.

Embryos formed in culture are called – Accessory embryos, adventive embryos, embryoids & supernumerary embryos classification of embryos (according to Kohlenbach, 1978).

1. Zygotic embryos – those formed by fertilized egg or zygote.
2. Non-zygotic embryos-formed by cells other than zygote.
   (i) Somatic embryos-formed by sporophytic cells (except zygote).
   (ii) Adventives embryos- somatic embryos arising from embryos or organs e.g. stem embryos in carrot.
   (iii) Parthenogenesis embryos –formed by unfertilized cells.
   (iv) Androgenic embryos –formed by male gametophyte

Somatic Embryogenesis in Dicotyledonous Cultures

Explants from embryonic cells or seedling tissues can be used to generate totiopotent embryogenic cells. Explants from other places can also be used. Like from inforescence, scutellum etc. Somatic embryos germinate in situ or when they are excised and cultured on fresh semi-solid medium.

Basic requirement for this are-

(i) **Auxin supply:** The presence of auxins in the medium is essential for somatic embryogenesis.

(ii) **Nitrogen source:** A nitrogen source usually in the reduced form is required for embryo initiation and maturation.

(iii) **Other constituents:** Presence of high concentration of potassium, dissolved oxygen are critical.

(iv) Establishment of suppertime culture: Spinning, stirred cultures or bioreactors can be used.

Somatic Embryogenesis in Monocatyledonous Cultures

Best explants are from embryogenic or meristematic tissues. First an embryogenic suspension is prepared, somatic embryos can be obtained without 2,4-D.

Embryo Maturation and Plantlet Development

Somatic embryos can only germinate when its mature to develop functional shoot and root apices. In presence of high auxin development & growth of shoot meristem can be inhibited. Addition of cytokinin and ABA in low level is beneficial. Various physical factors also effect maturation. For example species, which grow in cold, require chilling for embryo maturation. Somatic embryos germinate on agar medium without growth regulators. After a number of leaves are formed, the small plantlets are transferred to jiffy pots, or vermiculite, for subsequent growth and development.

Loss of Morphogenic Potential in Embryogenic Cultures

The callus or suspension cultures due to aging or long period of subculturing loses morphogenetic ability. This could be to:

1. Genetic – Nuclear changes like polyploidy, aneuploidy etc.
2. Physiological changes : Altered hormonal balance.
3. Competitive growth : Competition between morphogenetic & non-totiopotent cells.

Practical Applications of Embryogenesis

Clone propagation: somatic embryos can be used to proliferate and generate clones. Somatic embryogenesis can be combined with recombinant DNA technology for plant improvement.

1) **Cloning Zygotic embryos for repetitive embryogenesis**

During cloning of zygotic embryos many genotypes undergo auxin-stimulated somatic embryogenesis. They can form somatic embryoids under proper nutritional conditions.

2) **Raising somaclonal variants in tree species**

Embryos from PEDCs produce clonal embryos while embryos from IEDCs generate high frequency of somaclonal variants. Mutations occur during adventive embryogenesis which can generate a new strain of plant. Nucellar embryos like, shoot tips are free of virus and can be used for raising virus free clones, specially from tree species. For colonal propagation of tree species, somatic embryogenesis from nuclear cells is a good process.

3) **Preservation of genotype**

Somatic embryos are convenient organs for cryopreservation and germplasm storage.

4) **Synthesis of Artificial seeds**

Artificial seeds consisting of somatic embryos enclosed in a protective coating are "low-cost-high-volume" propagation system, two types of artificial seeds have been developed- (i) Hydrated (ii) Desiccated Hydrated seeds are formed by mixing somatic embryos with sodium alginate followed by dropping in a solution of calcium chloride to form calcium alginate beads. Coating with synthetic material makes Dessicate seeds; a 5% solution of polyethylene oxide (polyox WSR N-750) is mixed with equal volume of embryo suspension. This water-soluble resin is later dried to form polyembryonic desiccated seeds.

Embryo hardening treatment with 12% sucrose or 10^{-16}M ABA followed by chilling increases survival of encapsulated embryos.

Fluid drilling is also used to obtain transgenic plants from somatic embryos. The embryos are suspended in viscous carrier gel, which extrudes into the soil.

5) **Source of Regenerable Protoplast system**

Embryogenic callus, suspension cultures and somatic embryos are used as source of protoplast isolation. As these cells have regeneration capacity, their protoplasts are capable of forming whole plants.

6) **Genetic Transformation**

By the advent of leaf-disc transformation systems it is possible to engineer species in which tissues are capable of regeneration by somatic embryogenesis. Repetitive embryogenesis from *Agrobacterium* transformed cells has been used to obtain multiple crops of somatic embryos without employing the callus phase.

Transformation technique applied to primary somatic embryo instead of zygotic embryo should give rise to transgenic somatic embryos.

7) **Synthesis of metabolites**

The repetitive embryogenesis system is useful in the synthesis of metabolites e.g. oils of pharmaceuticals. Somatic embryos of Borage by repetitive embryogenesis give continuous supply of g-linolenic acid.

CHAPTER 5

LATENT LIFE-DORMANCY

LEARNING OBJECTIVES

- Importance of Dormancy
- Seed Dormancy
- Bud Dormancy
- Senescence and Programmed
- Cell Death (PCD)
- PCD in the Lifecycle of Plants
- Regulation of Metabolic Activity in Senescing Cells

Importance of Dormancy

Seeds are present in the fruits. When seeds become mature, then these are obtained from fruits. When the seeds are sown, then germination occurs, but in many seeds, it is seen that immediately germination does not occur, but if some resting period is given, then germination takes place. So this resting period, which is needed, for the germination is known as dormancy.

Types of Dormancy

There are mainly two types of dormancy:

The Imposed Dormancy

Germination needs favorable environmental conditions like sufficient water, proper temperature, etc. In absence of such conditions, seed does not germinate or becomes dormant which is sometimes considered as inhibition. This is known as imposed dormancy. Under favorable conditions, this kind of dormancy disappears and the seed germinates immediately.

The Innate Dormancy

Sometimes a seed is unable to germinate due to structural, chemical or physiological properties of the seed itself, this phenomenon is known as *innate dormancy*.

Causes of Dormancy

Some important causes of dormancy are given as below-

1. *Embryonal Dormancy:* It is seen that if seeds are having undeveloped embryos, then they cannot germinate till the maturation of embryo. It needs the resting period for maturation, like – in Rosaceae family. In some cases, embryo may be developed, but it does not show germination. It means such embryos need specific environment for germination.
2. *Hard Seed Coat:* In many seeds, seed coat is very hard and embryo is not having proper space for expansion. So it cannot germinate. These seed coats show mechanical resistance for the growth.
3. *Water Impermeability:* In many plants, seeds are impermeable for water as in legume family. So this impermeability inhibits the seed germination.
4. *Gas Impermeability:* In some seeds, seed coat is permeable for water, but impermeable to dissolved gases, like – oxygen and carbon dioxide and many seeds cannot germinate till the respiratory activity is initiated.
5. *Mechanical Resistance:* In some seeds, seed coat is permeable for water and gases, but is mechanically powerful. So, germinating embryo cannot break it. It is known as mechanical strength.

6. *Poisonous Contents:* In any seeds, seed coat is having poisonous contents, which inhibit the seed germination, like–tannins, organic acids, phenolics, cyanide substances, ammonia releasing substances etc. all these compounds can inhibit seed germination and seedling growth.

7. *Role of Light:* It is observed that far-red light can inhibit the seed germination. The effect of light is controlled through phytochrome, which is a chromophore molecule.

8. *Effect of Hormones:* In many seeds, higher amount of ABA is present, which inhibits the seed germination.

Seed Dormancy

Seed dormancy is a state, in which viable seeds fail to germinate under conditions of moisture, temperature, and oxygen favourable for vegetative growth. It is very peculiar and persistant phenomenon.

Methods to Overcome the Seed Dormancy

Although the seed dormancy is a natural phenomenon and it gives the proper time-period to seed for proper development. But many such methods have been discovered that can break the seed dormancy and show immediate germination. Some important methods are as follows:

1. *Scarification:* In this process, with the help of high pressure, seed coat is broken. So, mechanical resistance gets removed and embryo gets proper space for expansion. Then, it can show germination.

2. *Embryo Culture:* The embryo is if immature, then such seeds are kept in culture medium, then within few days, embryo becomes mature and seeds show germination.

3. *Breaking of Embryo Dormancy:* If embryo is mature, but if does not germinate, then specific conditions are given to embryo, like- seeds are kept at room temperature, but before this, cold treatment is given to them, which is given at 2-5 °C. Then it shows germination.

4. *Impact of Bacteria and Fungi:* In natural conditions, fungi and bacteria are active on the seed coat and hydrolyse the seed coat polysaccharides. So seed coat becomes soft and water can penetrate embryo. Then it shows germination.

5. *Effect of Hormones:* Many hormones can initiate seed germination, as seeds are kept in auxin, gibberrelic acid or cytokinin solution, then these hormones show positive impact on seed germination.
6. *Hot-water Treatment:* When seed are kept in hot water at 60 oC for 30-40 minutes, then toxic compounds present in the seed coat leach out. These compounds inhibit the germination and so exclusion of these compounds initiate seed germination.
7. *Temperature Alteration:* It is observed that if high and low temperature is alternately given to the seed, then seed dormancy breaks down and germination takes place, because temperature fluctuation can change the membrane properties.
8. *Light Treatment:* It has been seen that if the red light treatment is given to the seed, then dormancy breaks down. Actually, in the seeds, phytochromes are present. These phytochromes are changed into Pfr in the presence of red light and Pfr is the signal for the activation of many proteins, which is helpful in the seed germination.
9. *Chemical Treatment:* Many chemicals can affect the seed germination. Like– on giving the treatment of thiourea, germination is initiated.

Bud Dormancy

In the plants, when buds are produced at the floral apex, then these buds take part in the anthesis and show flowering. But in many plants, flowering does not occur after bud formation. It needs some resting period for flowering. This period is known as *dormant period*, and the process, which inhibits flowering, is known as *bud dormancy*. It is common in temperate region. This dormancy occurs before leaf senescence. Buds of many trees stop growth during mid summer and the further growth occurs during next season. It is seen that bud dormancy is related with leaf senescence. Mainly, bud dormancy is controlled by low temperature and day-length, like– if short – day treatment is given to many plants, then these show bud dormancy. The ecotypes show different dormancy responses, like northern race of red maple show winter dormancy, but southern race do not. Mainly, the stem shows response with day-length and temperature, but root does not get affected. Water also affects the dormant period; if the drought conditions occur, then buds show dormancy. Providing moderate temperature and moisture can reverse such dormancy. Morphology is also important for dormancy. The dormant bud is having short internodes

and bud scales. These scales prevent desiccation and restrict movement of oxygen to the Meristem below.

It is seen that ABA can cause bud dormancy, but its exact phenomenon is not known. Although it is seen that dormant tissues have higher amount of ABA.

For overcoming the dormancy, specific temperatures or specific day-lengths or both can be used. If cold treatment is given in the seedling stage, then flowering occurs. It is known as *vernalization*. The mid summer dormancy can be destroyed by long–day treatment. Actually, the bud scales show response against light. It is also observed that if far-red light is given, then dormancy destroys. It is related with phytochrome. When far-red light is given, then the composition of phytochrome is 98% Pr and 2% Pfr. Many chemicals can also break dormancy, like- 2-chloroethanol, which is applied in vapour form. It is also observed that if hot water treatment is given to bud (500-550 °C), then dormancy breaks down. In many deciduous plants, dormancy is broken by gibberellins.

Senescence and Programmed Cell Death (PCD)

Basic Concepts

Senescence is a highly regulated process, during which new metabolic pathways are activated and others are turned off. Senescence and subsequent death are terminal phases in the development of all organs of plant, including leaves, stems, roots and flowers. It remodels the form of plant by disposing of unwanted or inappropriate cells and tissues while simultaneously reclaiming valuable nutrients, especially nitrogen and phosphorus. Senescence generally follows organ maturity and occurs without growth or morphogenesis. It can be influenced by environmental and endogenous (e.g. hormones) perturbations.

In plants, programmed cell death is used to describe the dropping of petals from flowers and leaves from trees. In plants, it has been found that sequentially the following steps occur:

Vegetative growth ⟶ Reproductive growth ⟶ Programmed growth ⟶ PCD

A proposed scheme shows steps in the process of leaf senescence from the initiating signal to cell death is as follows:

1. **Initiation phase**
   - Crossing of metabolic threshold

- Altered redox state
- Signaling cascades

2. **Reorganizing phase**
   - Activation of Salvays pathways
   - Shift from autotrophic to heterotrophic metabolism
   - Detoxification
   - Reversible organelle redifferentiation
3. **Terminal phase**
   - Antibiotic accumulation
   - Release of free radicals
   - Elimination of remaining metabolites
   - Irreversible loss of cell integrity and viability

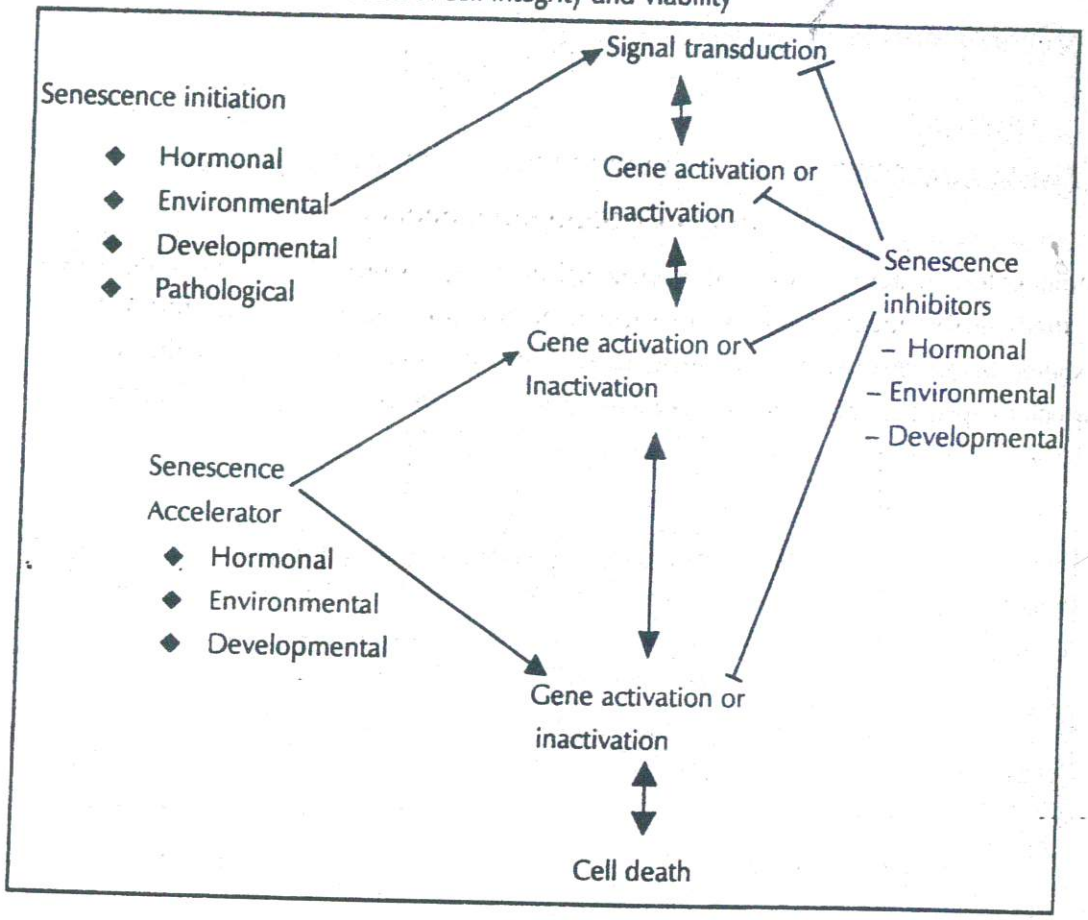


Figure 5.1

Types of Cell Death

Various types of cell death found in plant cells and tissues are as follows:

a. Autophagy

It is a way in which cultured plant cells can degrade their contents. Certain tissues of plants undergo senescence. Cells of the senescing corolla of Japanese morning glory (Ipomoea tricolor) contain autophagosomes, which have been found to fuse with the tonoplast and release their contents into the vacuole, where vacuolar enzymes break them down.

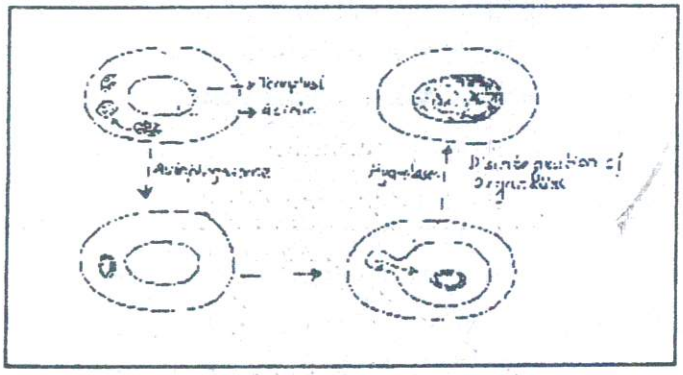


Figure 5.2: Role of Autophagosome in Senescence

Another form of autophagy occurs in the cereal aleurone, in which the number of organelles is dramatically reduced as the central vacuole increases to occupy almost the entire cellular volume before cell death. Some types of PCD may be unique to plants. Another way in which the plant cell protoplast is disposed of has been observed in tracheid differentiation.

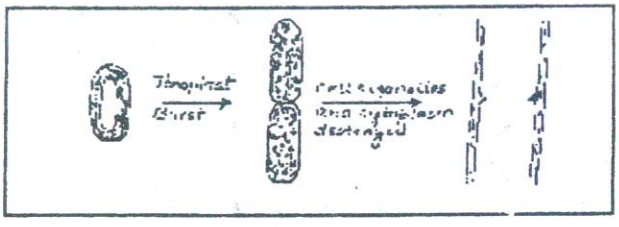


Figure 5.3: Differentiation of xylem elements

The formation of cereal endosperm is an example of specific PCD. Early in grain formation, the cereal endosperm differentiates in to two tissues, the starchy endosperm and the surrounding aleurone layer. In the mature fruit the endosperm cells died away but aleurone layer survived. Endosperms cells mummified and its contents are degraded when germination is initiated in the palnts.

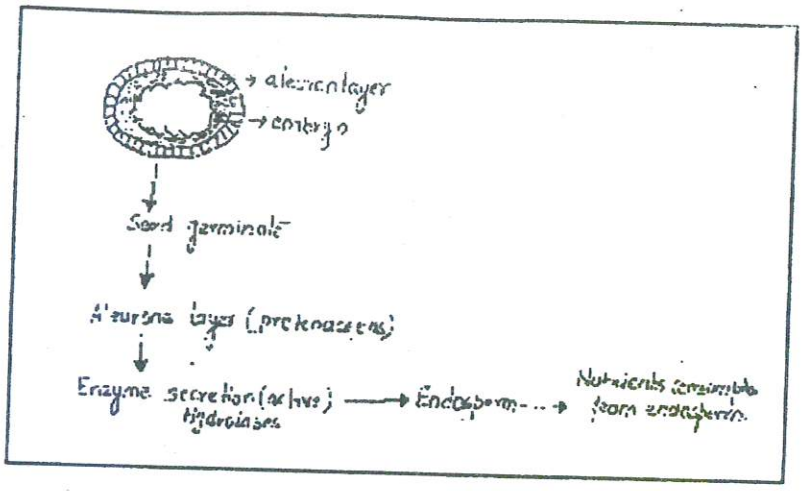


Figure 5.4: PCD in maize grains' endosperm

b. Lysogeny

Various glands on the aerial surfaces of plants arise as a result of cell death. For example, oil glands on the surface of citrus fruit develop because a group of subepidermal cells undergoing PCD formed a cavity that has filled with essential oil. Thus, lysogeny is responsible for the differentiation of secretory ducts, cavities or canals in many species. For example, the mucilaginous canals found in bud scales of the lime tree Tilia cordata arise as a result of lysogenous cell death.

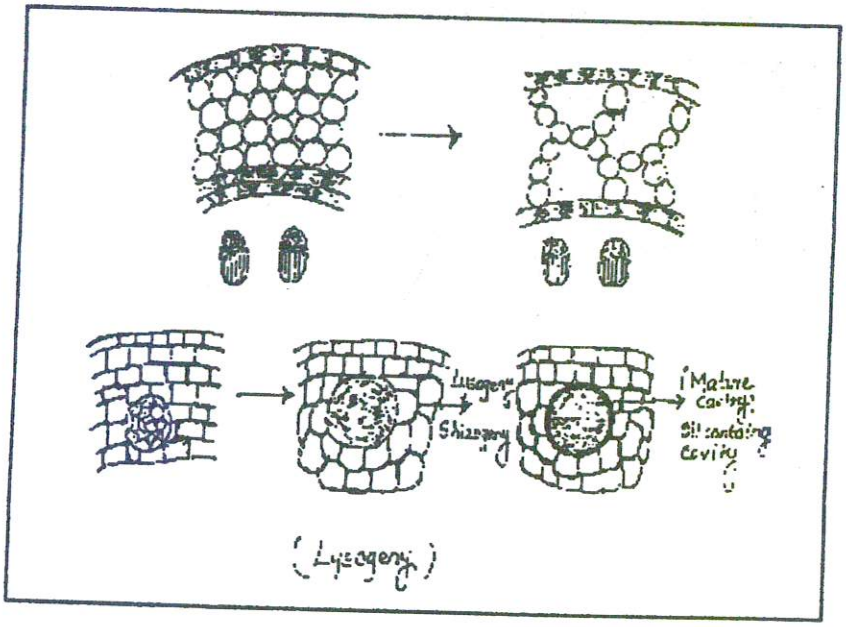


Figure 5.5

PCD in the Lifecycle of Plants

PCD is necessary in the plants. Flower development is radically affected by PCD of selected cells or group of cells. In most plants having unisexual flowers, the developing flower initially contains primordial for both male and female organs. At early stages, these are indistinguishable. During flower formation, either the male or female parts cease growing and are eliminated via a cell death program.

Cell death programs also influence haploid tissues of many plants. During megagametogenesis, in angiosperms, 3 or 4 megaspores undergo PCD, leaving one megaspore to give rise to the egg and other components of the embryo sac. Likewise, PCD plays a role in microsporogenesis, in which the tapetum that surrounds the microsporocytes dies and disintegrates.

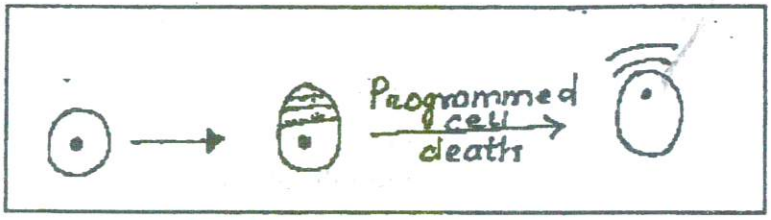


Figure 5.6: PCD during megasporogenesis

After fertilization in most angiosperms, the first mitotic division of zygote gives rise to 2 cells- one produces the embryo, the other the suspensor. The suspensor may undergo a few rounds of mitosis, but eventually suspensor cells undergo PCD.

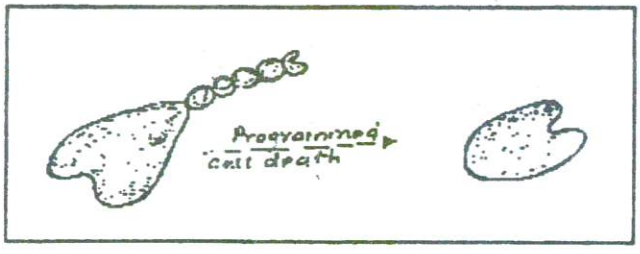


Figure 5.7: PCD in embryonal suspensor

In addition, responses to pathogens and abiotic stresses involve the controlled death of cells.

One example of PCD that causes change in form occurs during development of leaves of Monstera, the swiss cheese plant. In cacti, the green stem functionally replaces leaves and the leaves are reduced to spines.

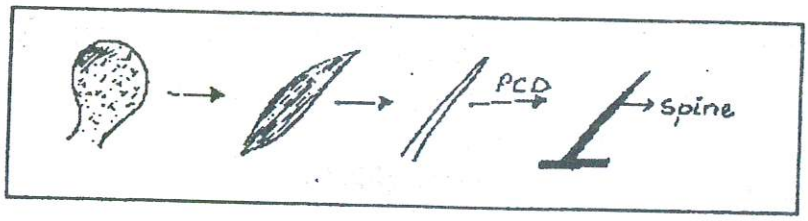


Figure 5.8: Formation of spines through PCD

Cell death occurs in almost all plant cells and tissues. PCD is involved in numerous processes, including:

1. Gamete formation
2. Megaspore formation
3. Degeneration of tissues in seed and fruit
4. Embryo development
5. Senescence
6. Responses to environmental signals and pathogens.

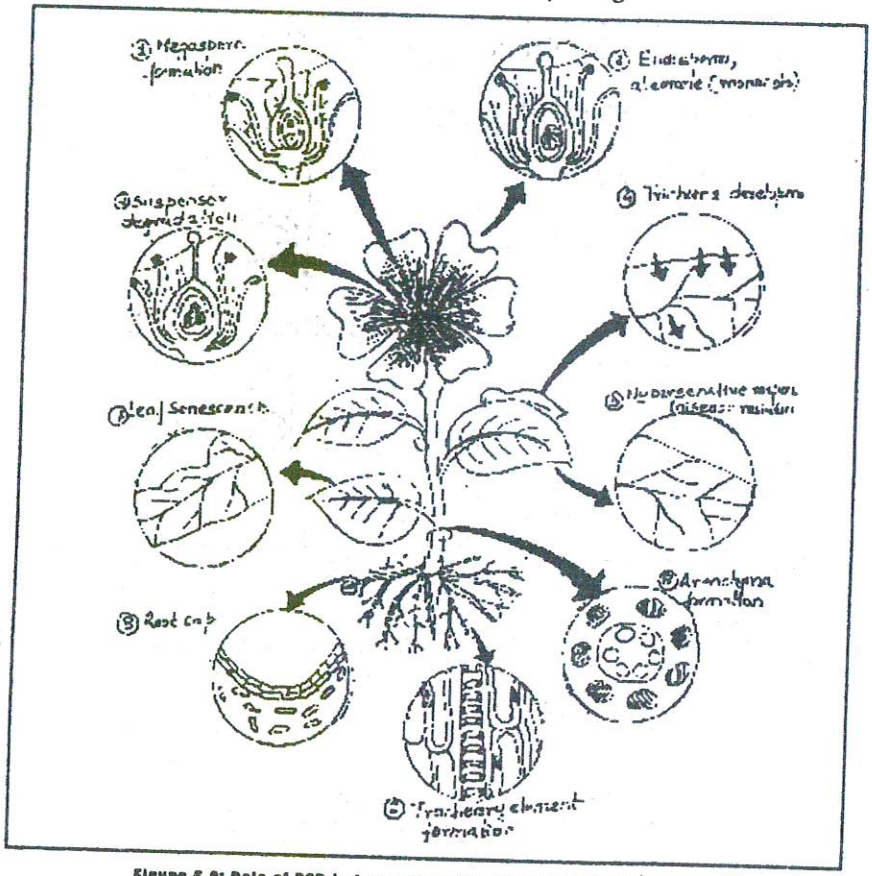


Figure 5.9: Role of PCD in formation and maturation of different organs

Metabolic changes associated with senescence and its regulation

Pigment metabolism during senescence

During senescence, green color of leaves gets lost. In chlorophyll a catabolism, first step is the conversion of chlorophyll to chlorophyllide and yield phytol also along with it. It greatly increases the water solubility of the pigment. An enzyme magnesium dechelatase removes magnesium from chlorophyllide to yield phaeophorbide. Phaeophorbide a oxidase, an enzyme, opens the phaeophorbide ring, introducing one oxygen atom from oxygen and one from water across the methane bridge that links pyrrole groups A & B of phaeophorbide a. The resulting red intermediate, red chlorophyll catabolite (RCC), is converted by RCC reductase to fluoroscent chlorophyll catabolite (FCC), a colorless product that fluoresces blue when excited with UV light. Chlorophyll breakdown ends with accumulation of non-fluorescent chlorophyll catabolites (NCCs)

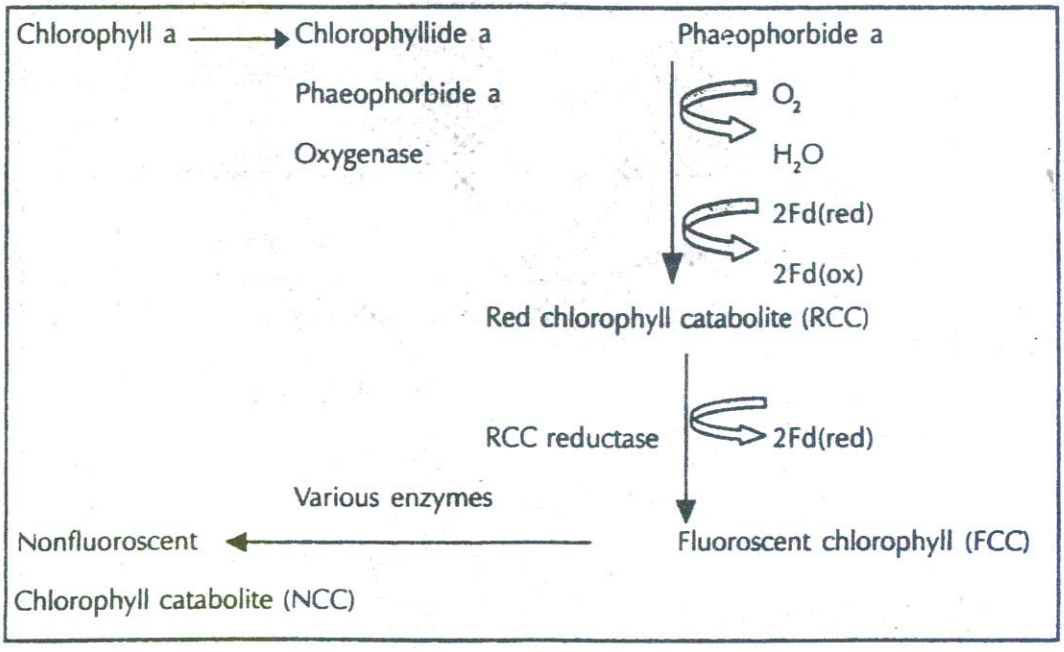


Figure 5.10

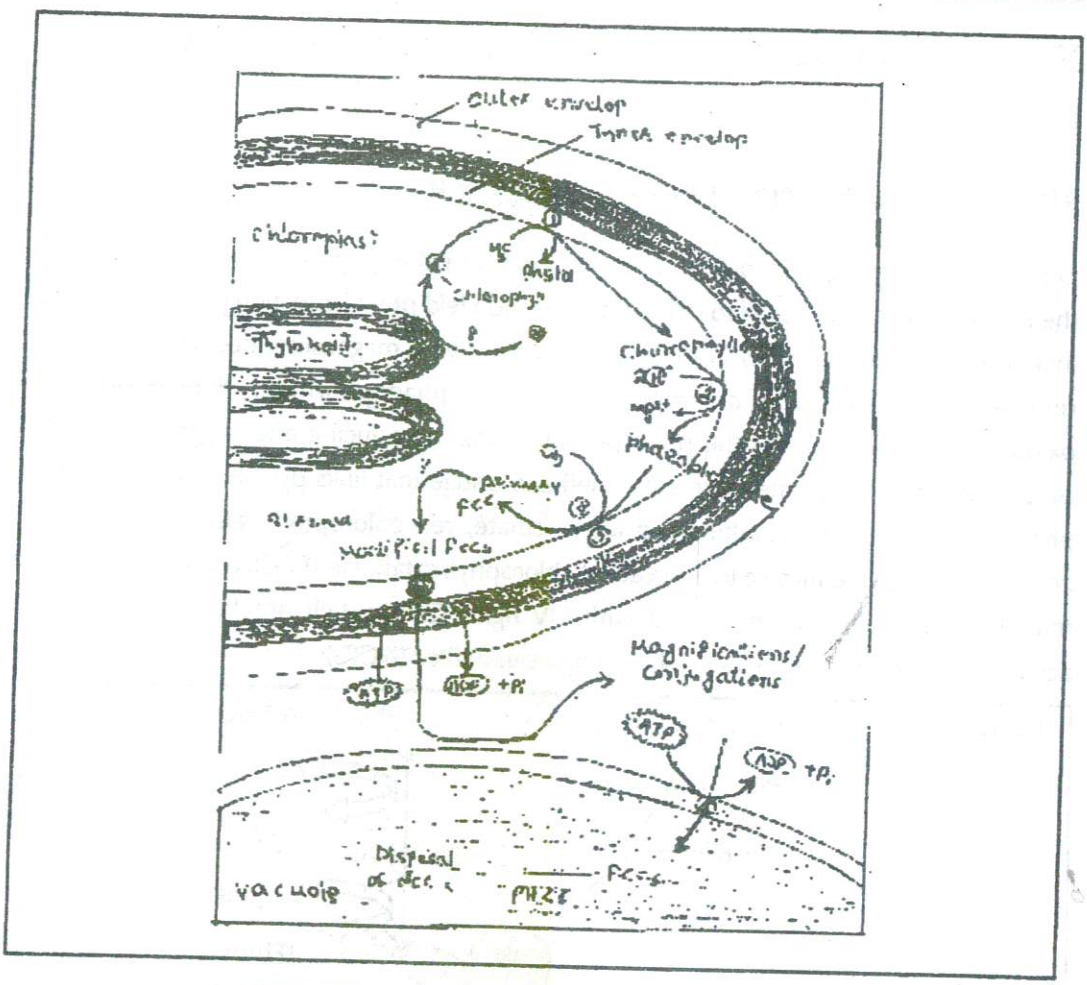


Figure 6.11: Change in metabolism of chloroplast during senescence

1. Chlorophyllase
2. Magnesium dechelatase
3. Phaeophorbide a oxygenase
4. RCC reductase
5. Catabolite translocator
6. ABC transporter

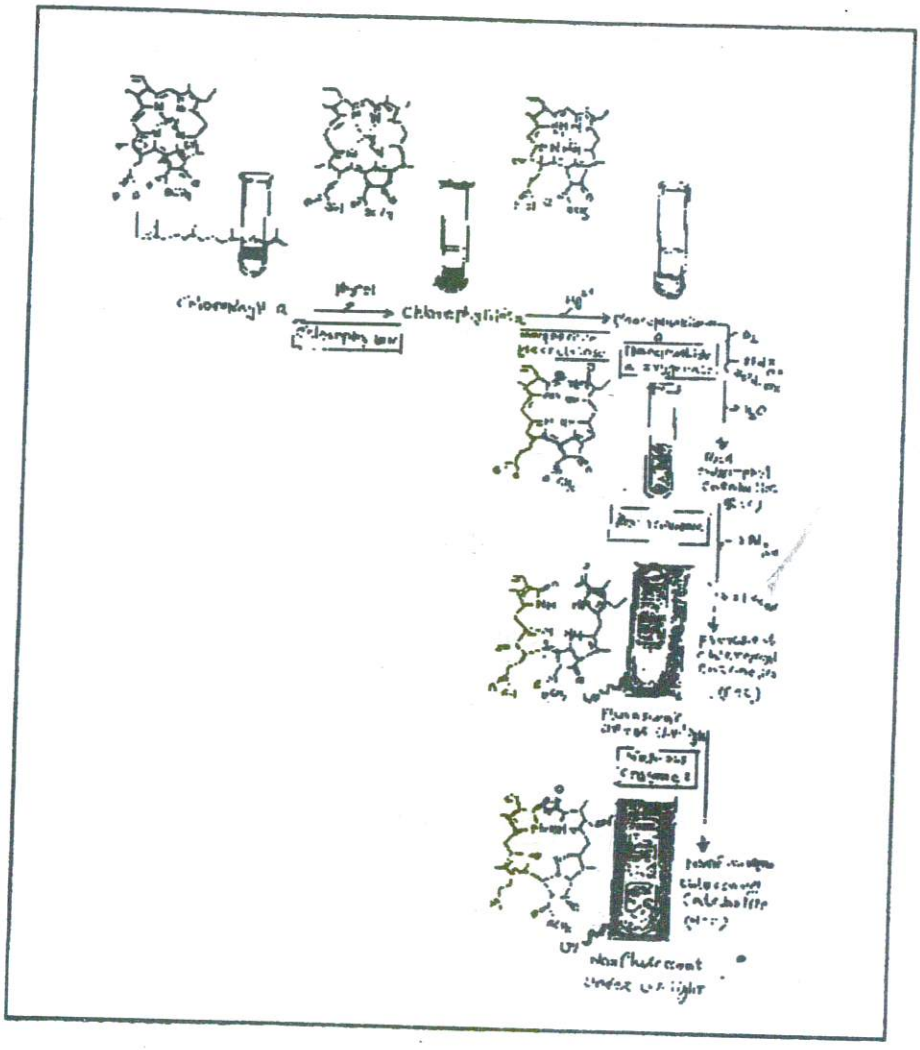


Figure 5.12: Representation of degradation chlorophyll pigment

Phenylpropanoid metabolism also can be altered during senescence. Other pigments and secondary compounds that accumulate in senescing tissues are the products of phenylpropanoid metabolism. This metabolism has been shown in the figure next.

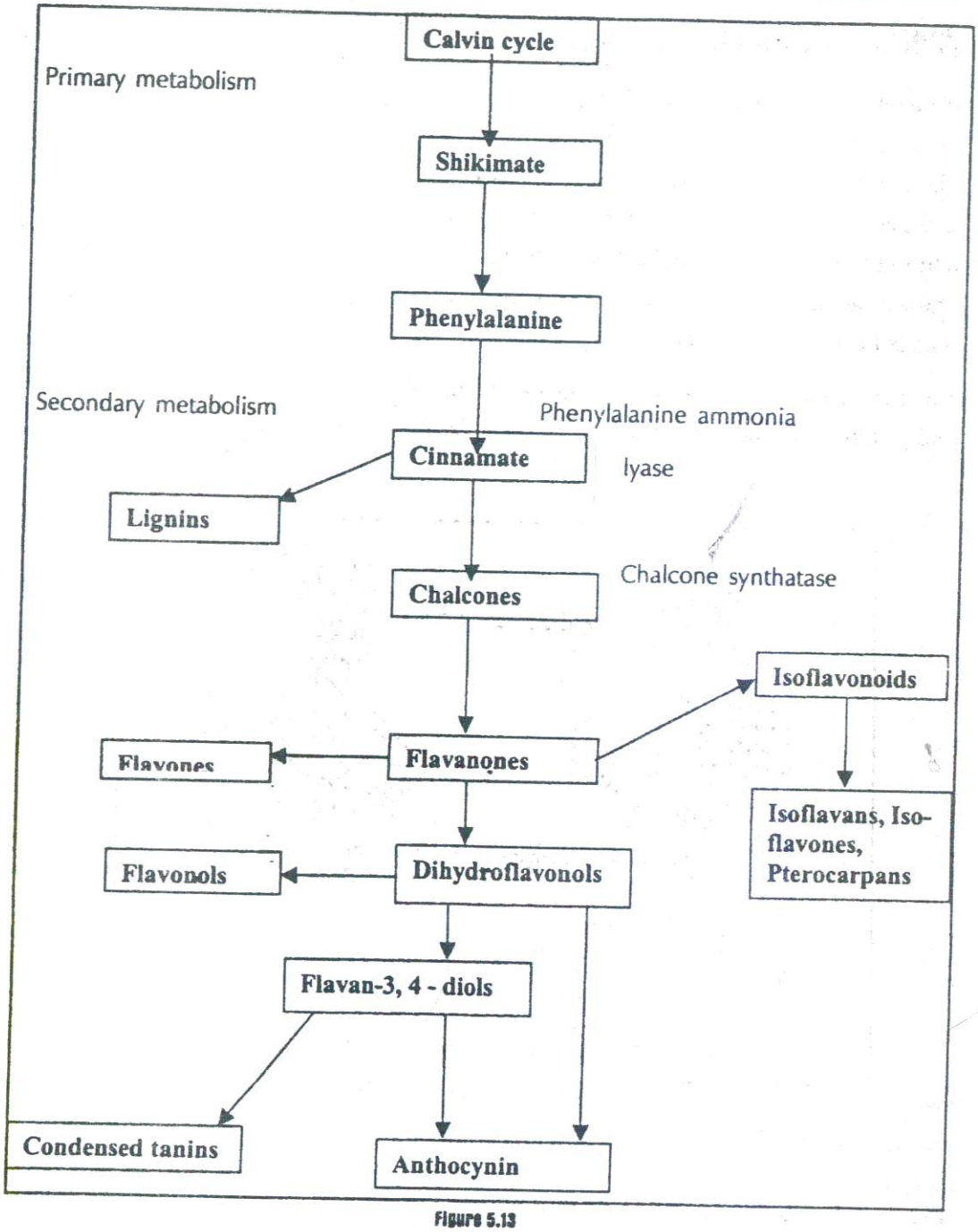


Figure 5.13

Anthocyanin is responsible for some of the vibrant pigments of ripening fruits and senescing leaves.

Protein metabolism during senescence

Pigment breakdown during senescence usually is accompanied by mobilization of chloroplast protein. In green cells, most of the protein is located in chloroplast, so these organelles are the source of most of the organic nitrogen salvaged from senescing tissues. The most abundant of these p[illegible]re rubisco and it is located in the soluble stromal phase of the chloroplast, and the chlorophyll-binding light harvesting proteins (LHCP) of the thylakoid membranes. These proteins are exported to vacuole for degradation. Enzymes cause proteolysis. Protease is called caspases. These cause apoptosis to occur by cascade mechanism.

Senescence may be associated with increasing susceptibility of proteins to breakdown. The two possible mechanisms are illustrated in the figure below:

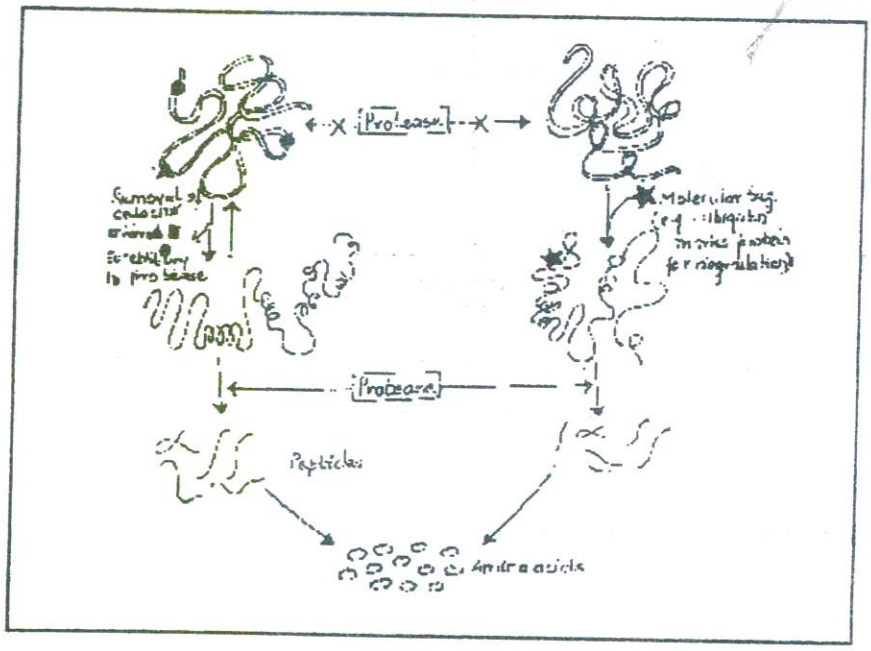


Figure 5.14

Glutamate has a central role in the interconversion undergone by amino acid products of proteolysis.

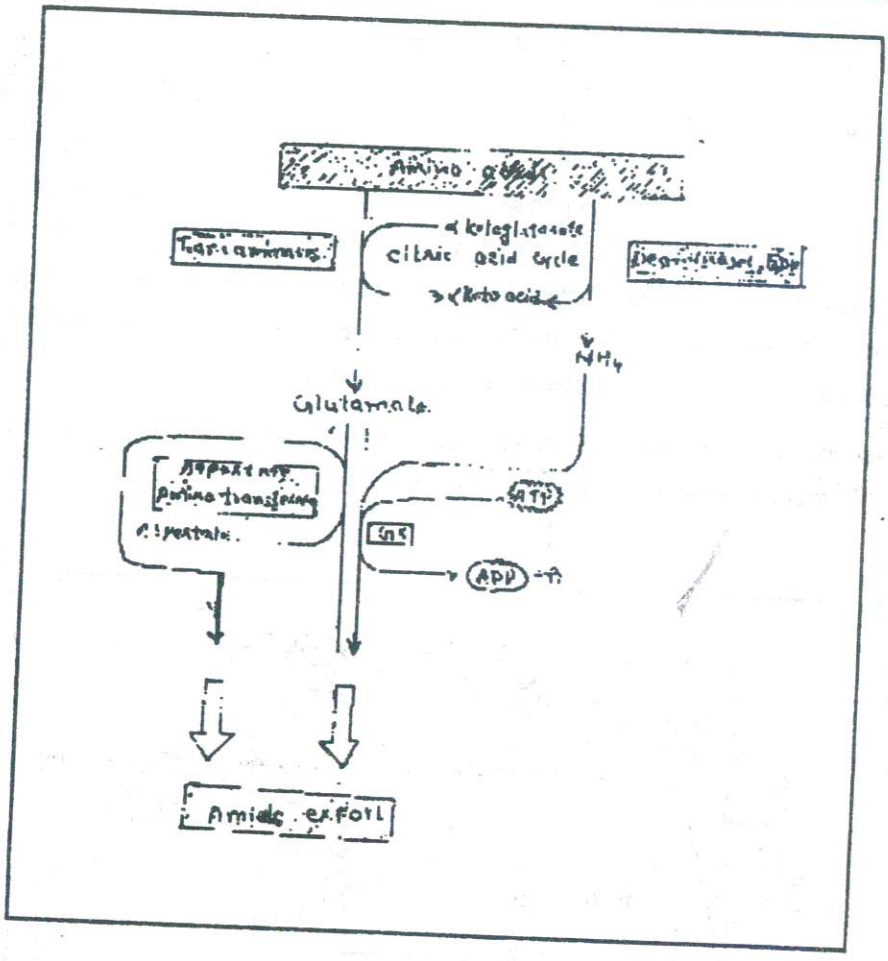


Figure 5.15

3. Metabolism of nucleic acids during senescence

Catabolismof nucleic acid releases inorganic phosphates. Much of the organic phosphorus in the leaves is in the form of nucleic acids. These carry genetic information as well as acts as salvageable reserve substance of high molecular mass. Inorganic phosphate, after releasing from catabolism of nucleic acids, readily translocated through vascular system. The nucleoside products of nuclease and phosphatase are cleaved into sugars, purines and pyrimidines.

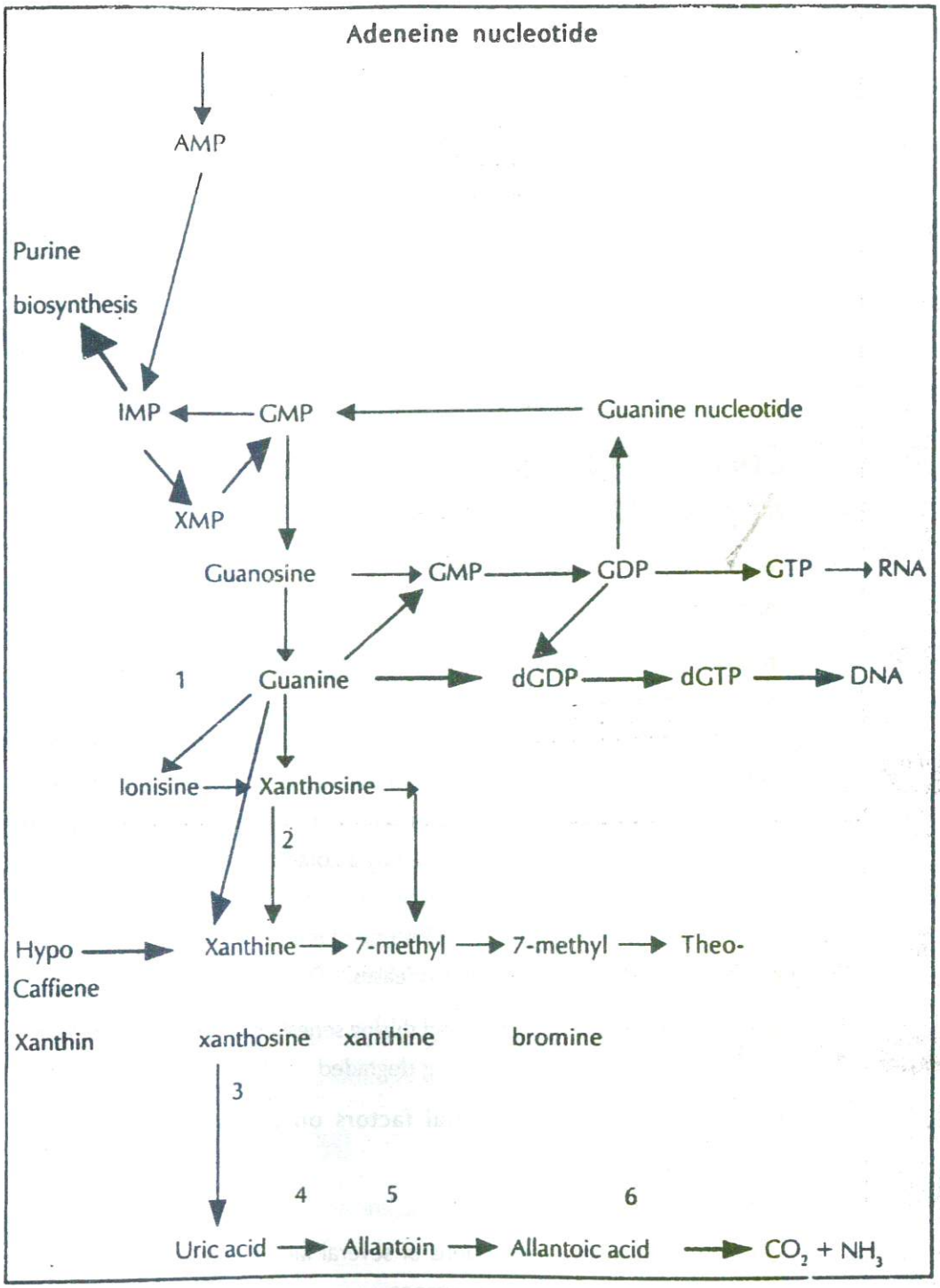


Figure 5.16: Possible Pathway for Guanosine Metabolism in Higher Plants

1. Guanosine deaminase
2. Nucleosidase
3. Xanthine dehydrogenase
4. Uricase
5. Allantoinase
6. Allantoicase

Inter conversion then takes place. GTP is formed. The activity of some RNases increases during senescence.

Regulation of Metabolic Activity in Senescing Cells

1. Differential susceptibility of enzymes to proteolysis can be mediated by several mechanisms.

   Much of the primary active metabolic machinery of senescing cells is present before senescence begins. Senescing cells have a large number of enzymes. If enzymes are more or less equally exposed to degradation, differential activation of gene expression can ensure that some proteins are replenished, where others are removed and not replaced. Another mechanism is compartmentation. The control of degradation might be exercised by a cofactor through its influence on conformational state. If the appropriate balance of metabolic effectors and substrates occupies the catalytic and allosteric sites of an enzyme, the enzyme is comparatively resistant to attack by proteases.

2. Many cytosolic enzymes are unaffected during senescence, while the organellar isoforms of the same protein are being degraded.

Influence of Hormones and Environmental factors on Senescence

Infuence of Hormones

Senescence is mediated by complex interactions of several factors, including hormones. These play a prominent role in regulating senescence.

Influence of Ethylene

Ethylene is typically the strongest promoter of senescence among the known plant hormones. In many plants, treatment with exogenous ethylene can induce senescence in leaves and flowers and ripening of fruit. The concentration of endogenous ethylene correlate with leaf senescence: As leaves get older, they produce more ethylene.

During normal development of climacteric fruit, such as tomato, a developmentally regulated burst of respiration and ethylene production is followed by an increase in ripening-related activities. These result in the changes in fruit color, texture and flavor that characterize ripening. Thus, an increase in ethylene concentration coincides with senescence in many plants. Chemical inhibitors of ethylene biosynthesis inhibit fruit ripening and leaf senescence. The role of ethylene can differ somewhat in different organs, even in the same plant. But ethylene alone is insufficient to trigger senescence.

Influence of Cytokinin

Cytokinin also appears to play a major role in regulating senescence but with an effect opposite to that of ethylene. Cytokinins inhibit the senescence process. The concentration of endogenous cytokinins decreases in most senescing tissues. Exogenous application of cytokinins can cause a delay in senescence in most tissues. The effect of cytokinins also varies with age and type of tissue treated and also from species to species.

Cytokinins probably acts at two levels – at a distance, by promoting differentiation and strong sink activity, and locally in senescing cells themselves, by repressing the senescence program. Thus, it is clear that cytokinin acts as senescence antagonist.

Influence of Abscisic Acid (ABA)

ABA generally acts as a promoter of senescence. ABA most likely affects senescence through its action with other growth regulators. Actually, its impact on senescence is less understood.

Influence of Gibberrelic Acid (GA)

GA usually retards senescence. Its impact on senescence is also less understood.

Influence of Auxin

In most cases, treatment with exogenous auxins delays senescence, but in some species, exogenous auxins promote senescence.

Other growth regulators such as- jasmonates, brassinosteroids and polyamines also may have roles to play in senescence. Polyamine concentration, in plants, is strongly correlated with senescence in many tissues.

Influence of Environment on Senescence

Senescence is as responsive as any other physiological activity to sub-optimal or supra-optimal environmental conditions. Seasonal changes can trigger senescence as a part of an adaptive strategy. Thus, trees prepare for winter by mass leaf senescence in the fall, which is in turn initiated by the declining length of day after mid-summer. Senescence is also a tactic deployed when an unpredictable stress is experienced. Another complex feature of the environmental sensitivities of senescence is the way in which a given factor can determine initiation of the entire senescence syndrome but also often can have a very different effect once senescence is under way. For example, drought invokes prematurefoliar senescence in many species, but water limitation during senescence actually shows the development of yellowing and other symptoms. Frequently, the speed and severity of a developing stress overturn the capacity of the tissue to invoke, co-ordinate, and express the senescence program; as a result, cells are diverted more or less directly to the PCD pathways.

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