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Coniferales: *Pinus*

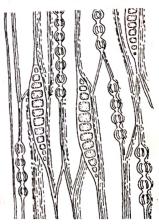
The conifers constitute more than three-fourths of the living gymnosperm flora, and cover vast areas of the temperate regions of both northern and southern hemispheres.

The largest continuous, dense forest in the world is coniferous, and it encircles the earth just below the northern tundra of the Arctic and extends from Canada, Russia, Northern Europe to northern China and Japan. It extended southward along mountain ranges and merged with the deciduous forests of the south. The coniferous trees are ideally suited for the cold and snowy climate of the north.

Coniferales is the largest order of gymnosperms with about 52 genera and 550 species. These plants originated in the Carboniferous period. About 30 genera of the order are confined to the Northern and 14 to the Southern hemisphere and rest of the genera cross the equator on either side. They are usually long, branched and evergreen trees. The branches are usually dimorphic and bear needle-like, linear or lanceolate leaves. The wood is pycnoxylic, characterised by the presence of resin canals. The micro- and megasporophylls form compact cones. The male gametes are non-motile and the fertilization is siphonogamous.

Coulter and Chamberlain (1910) recognised only two families — Pinaceae and Taxaceae — in the order Coniferales, whereas Pilger (1926) divided the order into seven families, Pinaceae, Taxodiaceae, Cupressaceae, Araucariaceae, Podocarpaceae, Cephalotaxaceae and Taxaceae. Chamberlain (1935) recognised only six families as he merged Cephalotaxaceae with Taxaceae. Pulle (1942) considered Taxales as a separate order and included two families Taxaceae and Cephalotaxaceae in it. Florin (1949), Zimmermann et al. (1959) and Sporne (1965) also recognised Taxales as a distinct order. Pant (1957) elevated the order Taxales to the rank of a class Taxopsida. Sporne





(1965) divided Coniferales into nine families: Lebachiaceae, Voltziaceae, Palissyaceae, Pinaceae, Taxodiaceae, Cupressaceae, Podocarpaceae, Cephalotaxaceae, Araucariaceae.

Family Pinaceae

Members of this family are monoecious with spirally arranged, needle-like leaves. Each needle has one or two vascular bundles, enclosed within a common bundle sheath. The pollen grains are mostly winged. The female cone has numerous spirally arranged ovuliferous scales free from the subtending bract scales or adhering only at the base. Each ovuliferous scale bears two ovules on its upper side. There are over 200 species included in the family. All are trees and the family is economically very important.

The life history of *Pinus* is described here as a representative of the order Coniferales.



Systematic Position

Division — Coniferophyta
Class — Coniferopsida
Order — Coniferales
Family — Pinaceae

Pinus, one of the most important taxon of the order Coniferales, is represented by about 105 species, distributed throughout the northern hemisphere. The species of *Pinus* form dense evergreen forests in the north temperate and subalpine regions.

Five species of *Pinus* occur in the Indian subcontinent; of these, four species are confined to the north-east and north-west Himalayan regions (Fig. 1). The distribution of *Pinus* in India is as follows.

[I] Pinus roxburghii

(syn. P. longifolia, Chir pine)

This species occurs in the hilly regions of Kashmir, Punjab, Himachal Pradesh and Uttar

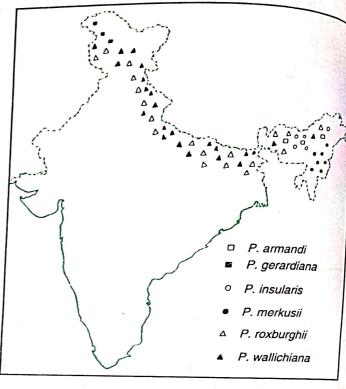


Fig. 1. Distribution of Pinus in India.

Pradesh at an altitude of 450-2,250 meters. The tree attains a height of 35-50 meters. There are three needles in each foliar spur.

[II] Pinus wallichiana

(syn. P. excelsa, Blue pine, Kail)

This species grows luxuriantly in the hills of Kashmir, Himachal Pradesh and Punjab at an altitude of 1,500-3,300 meters. The tree attains a height of about 50 meters. There are five needles in each foliar spur. The female strobili are cylindrical.

[III] Pinus gerardiana (Chilgoza pine)

This species is common in Kashmir and Kinnaur district of Himachal Pradesh at an altitude of 2,100-3,300 meters. The tree is 11-20 meters high with trifoliate spurs. The seeds are 0.5 - 2.5 cm in length and are edible.

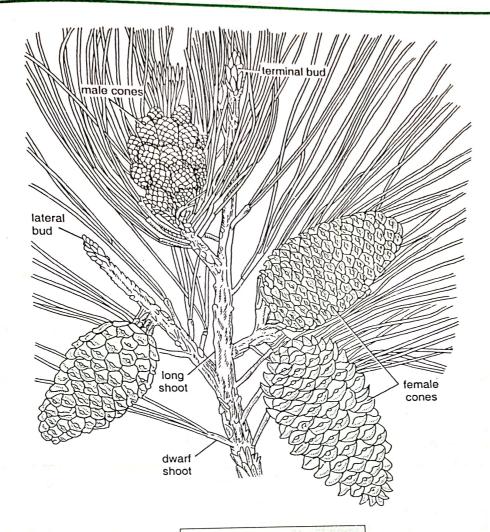


Fig. 2. Pinus: External features.

[IV] Pinus merkusii (Merkus pine)

This species occurs on the hillocks in East Bengal at an altitude of 150-600 meters. The plant is only 3-4 meters high. There are two needles in each foliar spur.

[V] Pinus insularis (syn. P. khasya, Khasi pine) This species is widely distributed in Khasya regions of Assam at an altitude of 700-1,850 meters. The plant attains a height of about 30 meters and the foliar spurs are trifoliate.

Besides several species of *Pinus*, such as *P. armandi*, *P. montana*, *P. laricio*, *P. canariensis*,

P. pinaster, P. radiata and P. sylvestris, are introduced in India.

SPOROPHYTE

External Morphology

The sporophytic plant body of *Pinus* is an evergreen tree. It grows luxuriantly in temperate xerophytic habitats. When young, the plant gives a typical pyramidal appearance. The plant body is differentiated into **root**, **stem** and **leaves**.

[A] Root

The primary root is a typical tap root. It does not penetrate deep into the soil. The lateral roots grow extensively and help the plant to keep firmly in the soil. Root hairs are poorly developed. The roots are covered with fungal hyphae, called mycorrhiza. The fungi present in the ectotrophic mycorrhiza are mainly the species of Amantia, Boletus, Clavaria and Scleroderma of the class Basidiomycetes. They remain in symbiotic association with the roots. It has been reported that in the absence of mycorrhizal association, the death rate of Pinus seedlings is considerably increased. There is a positive correlation between the intensity of infection and increase in the mobilization of soil nitrogen. The internal nutrient status, especially N2, phosphorus and potassium is responsible for determining the intensity of infection.

[B] Stem

The stem of *Pinus* is cylindrical, erect, woody and branched. The branches are monopodial and develop spirally in the axils of scaly leaves present on the stem. Such a branching gives a conical appearance to the plant. But in some species, such as *P. torreyana*, the branches are diffuse or scattered. The stem is dichotomously branched in *P. sabiniana*.

In *Pinus*, the branches are dimorphic. The two types of branches are long shoots or branches of unlimited growth and dwarf shoots or branches of limited growth.

[I] Long shoots or branches of unlimited growth

These branches are present on the main trunk. The apical buds of these branches grow indefinitely, hence these branches are of unlimited growth. They develop in the axils of scaly leaves and spread horizontally. They shorten gradually towards the tip, thus providing a graceful pyramidal appearance to the tree. These branches bear only scaly leaves.

[II] Dwarf shoots or branches of limited growth

These branches do not have apical buds and hence show only limited growth. They are also known

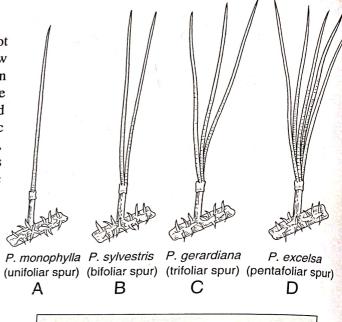


Fig. 3 A-D. Pinus: Various kinds of foliar spur.

as brachyblast. They develop in the axils of scaly leaves and bear both scaly and foliage leaves. Dwarf shoots are shed every two or three years, leaving scars on the stem.

[C] Leaves

The leaves are dimorphic, i.e., there are two types of leaves — scale leaves and foliage leaves.

[I] Scale leaves

The scale leaves are dark brown, membranous, thin and small, and are present on both long and dwarf shoots. They donot help in photosynthesis. Their main function is protection of the young buds. They fall off as the branches mature. The scale leaves on the dwarf shoots have a distinct midrib and they are called **cataphylls**.

[II] Foliage leaves

The foliage leaves are green, acicular and needle-like. They are borne only on the dwarf shoot. A dwarf shoot with a group of needle like foliage leaves is known as **foliar spur**. The number of needles present in a spur is constant for a species and is used for identification of different pines (Fig. 3); for instance, *P. monophylla* is monofoliar with a single needle, *P. sylvestris* and *P. merkusii* are bifoliar with two needles, *P. longifolia* and *P. gerardiana* are trifoliar with three needles,



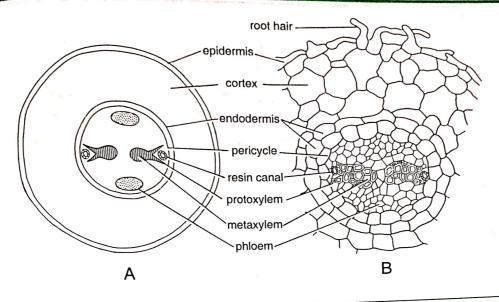


Fig. 4 A-B. Pinus: Young root; A. Diagrammatic representation of transverse section, B. A part cellular.

P. quadrifolia is quadrifoliar with four needles and P. wallichiana and P. armandi are pentafoliar with five needles per spur.

The foliage leaves are photosynthetic and remain persistent for several years. The needlelike nature of the foliage leaves indicates xerophytic adaptation of these plants.

Internal Structure

[A] Root

[I] Primary root

The primary root of Pinus resembles with a typical dicot root. The following regions are discernible in a transection of the young root (Fig. 4 A-B).

- 1. Epidermis. This is the outermost layer which is composed of tightly appressed cells. It gives out unicellular root hairs.
- 2. Cortex. The cortex is composed of 4-5 layers of parenchymatous cells. Some of these cells are filled with resinous substances.
- 3. Endodermis. The innermost layer of the cortex represents endodermis. The cells of this layer have typical thickening bands on their radial walls.

- 4. Pericycle. The endodermis is followed by a multilayered zone of parenchymatous cells which forms pericycle.
- 5. Vascular tissue. The roots are di-to hexarch with radial and exarch vascular bundles. The protoxylem bifurcates to form a 'Y' shaped structure and a resin duct lies in between the two arms of protoxylem. The xylem consists of only tracheids; vessels are, however, absent. The protoxylem consists of mostly scalariform or scalariform-pitted tracheids, the metaxylem of only pitted tracheids.

Phloem strands are present in alternate positions to the xylem strands. The phloem is composed of sieve cells and phloem parenchyma; companion cells are absent.

6. Pith. The pith is poorly developed or altogether absent.

In a mycorrhizal root, the rootlet is enclosed by a fungal sheath, and the fungus is restricted to its cortex in a net of hyphae called Hartig's net. The epidermal layer and the root hairs are replaced and covered completely by a mantle of mycelium.

[II] Secondary growth in root

Secondary growth sets in even before the primary tissues are fully differentiated. The secondary

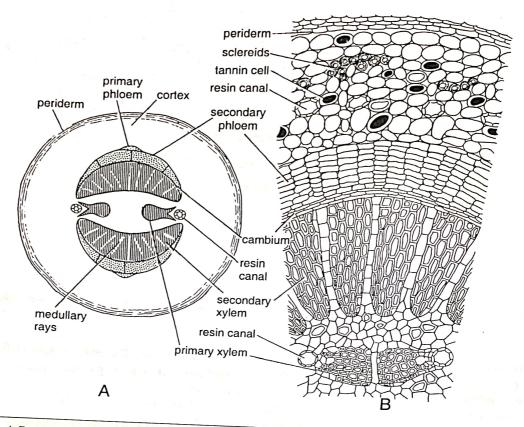


Fig 5 A-B. Pinus: Mature root; A. Diagrammatic representation of transverse section, B. A part cellular.

growth is similar to that of a dicotyledonous root. A few parenchyma cells situated along the inner edges of the phloem groups become meristematic and form crescent shaped strips of cambium. These strips of cambium produce secondary xylem centripetally and secondary phloem centrifugally (Fig. 5 A, B). Consequently, collateral strands of vascular tissue are formed in between the primary xylem bundles. As the activity of these cambial strips proceeds, the cells of the pericycle lying against the protoxylem also become meristematic and thus small strips of cambium are also formed outside the xylem strands. The first formed cambial strips below the phloem strands join with the newly formed strips outside the xylem strands. As a result, a complete but wavy ring of cambium is formed which runs outside the xylem and inside the phloem strands. The first formed cambial strips, located along the inner face of phloem, start functioning earlier than the later formed cambium from the

pericycle. As a result of the formation of secondary xylem opposite the phloem, the cambium is pushed outwards. Thus the wavy cambium ring becomes circular in outline. The cambium ring produces secondary phloem on the outer side and secondary xylem on the inner side. secondary xylem the tracheids comparatively broad and elongated. The primary xylem bundles remain in their original position inside the cylinder of secondary tissue. The primary phloem, endodermis and cortex usually become crushed due to enormous increase in the volume of the secondary tissue.

The cells of the cambium, which arise in the pericycle opposite the primary xylem poles, do not give rise to secondary vascular tissues. They produce primary vascular rays, radiating outwards through the secondary xylem and phloem from the tip of the protoxylem. In addition, short and narrow secondary vascular rays are also formed similarly in the secondary vascular tissue.

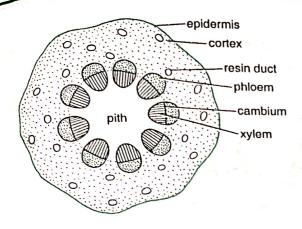


Fig. 6. Pinus: Diagrammatic representation of transverse section of young stem.

The formation of periderm starts soon after the production of secondary vascular tissue has commenced. The phellogen (cork cambium) originates from the outer layers of pericycle. It produces phellem or cork towards the outer side and phelloderm or secondary cortex towards the inner side. The tissue from endodermis to epidermis is sloughed off and consequently the cork becomes outer protective layer. The cork cells may be highly suberized and cutinised, and some of them may be filled with tannin. There are many resin canals in the secondary cortex.

[B] Stem

[I] Primary structure

The structure of *Pinus* stem is similar to that of a dicotyledonous stem. In transection the stem appears wavy or irregular in outline due to the presence of close appressing scale leaves and dwarf shoots. It shows the following regions (Fig. 6).

- 1. Epidermis. It is the outermost layer composed of compactly arranged and heavily cutinised cells.
- 2. Cortex. The outer sclerenchymatous zone of the cortex forms **hypodermis**. It is followed by several layers of parenchymatous cells. Some cells of this zone are filled with tannins. Several resin canals are also distributed irregularly in the cortex. Each resin canal is bounded by a 2-layered envelope of sclerotic cells, followed by a layer of glandular, resin-secreting epithelial cells.

- 3. Endodermis and pericycle. The innermost layer of the cortex represents endodermis, however, it is indistinguishable from the cortical cells. Next to the endodermis is a 2-3- layered pericycle.
- 4. Vascular tissue. The vascular cylinder is composed of 5-9 primary vascular bundles, arranged in a ring. The vascular bundles are conjoint, collateral, open and endarch. As the vascular bundles are closely placed, the medullary rays (parenchymatous zone joining pith and cortex) are narrow. The tracheids are arranged in uniform radial rows. The protoxylem tracheids possess spiral thickenings, whereas those in metaxylem have reticulate thickenings. The phloem consists of sieve tubes and phloem parenchyma. Some albuminous cells are also present in the phloem which are associated with sieve tubes like companion cells. One or two layers of cambium are present between the xylem and phloem.
 - 5. Pith. A parenchymatous pith is present in the centre of the stem. Some pith cells are filled with resinous substances.

[II] Secondary growth in stem

The stem of Pinus grows in thickness by secondary growth. Interfascicular strips of cambium develop in the primary medullary rays in between the vascular bundles (Fig. 7A, B). The strips of with join cambium interfascicular intrafascicular cambium present in the primary vascular bundles and form a complete ring of cambium (Fig. 7 C). This cambial ring cuts off secondary xylem towards the inner side and secondary phloem towards the outer (Fig. 7D). The secondary vascular tissues are thus added continuously by the activity of vascular cambium and the stem increases in girth. The primary phloem is crushed due to the pressure of secondary vascular tissues.

The cambial ring continues to form increments of secondary xylem and secondary phloem every year. The xylem ring formed in a year is differentiated into spring wood and autumn wood. The former is composed of broad tracheids and the latter of narrow tracheids (Fig. 8). This differentiation is due to seasonal activity of the cambium. In autumn, when there is leaf-fall, active translocation of water and nutrients is not required

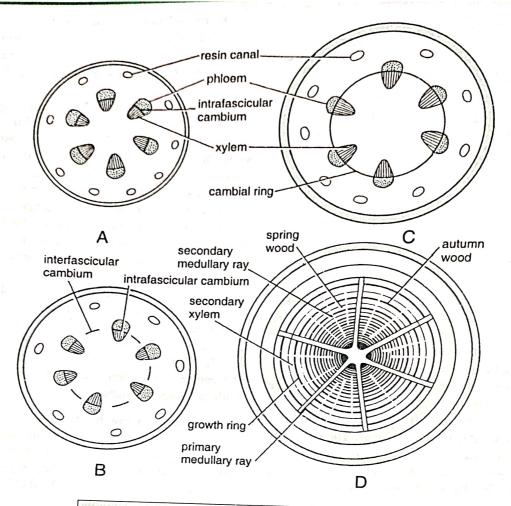


Fig. 7 A-D. Pinus: Stages in secondary growth of a long shoot.

and hence the tracheids formed in this season are narrow. In spring, new leaves and branches are formed and thus there is active translocation of water and nutrients. Hence the tracheids formed in this season are broad.

The increment of a year consists of a ring of spring wood and a ring of autumn wood; the two together constitute an annual ring (Fig. 9). The age of a plant can be calculated with the help of these annual rings.

[III] Secondary xylem

The secondary xylem which constitutes the wood of *Pinus* is pycnoxylic. The tracheids formed in the autumn are narrow and thick-walled with

smaller bordered pits, whereas those formed in the spring are broad and thin-walled with larger bordered pits. The pits are present only on the radial walls. An important characteristic feature of the wood of *Pinus* is the presence of bars of Sanio (Fig. 10). They occur in the form of crescentic bars in between the pits.

These bars are formed by the deposition of cellulose and pectin on tracheid walls. As the wood matures, the bars are separated from the pits, and the bars of the adjacent pits are fused to form rims of Sanio.

The secondary vascular tissues are traversed by parenchymatous secondary medullary rays which extend from the pith to the cortex. They

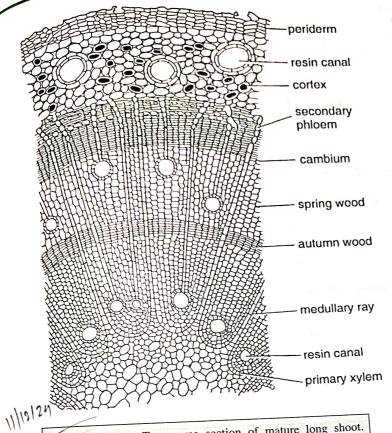


Fig. 8. Pinus: Transverse section of mature long shoot.

are formed by the cambial cells simultaneously with the secondary vascular tissues. The medullary rays are uniseriate and 2-12 cells in height. However, the rays which are associated with the resin canals are multiseriate. In radial longitudinal sections, medullary rays are cut lengthwise and length their revealing (Fig. 11 A). In tangential longitudinal sections, thus they are cut transversely and their height and breadth can thus be noticed (Fig. 11 B). The medullary rays is different in secondary xylem and secondary phloem region. structure of In the secondary xylem, the rays consist of thin walled and starch- filled living cells, and one or more layers of ray tracheids occur on both sides of the medullary rays. The lateral and end walls of the tracheids have bordered pits which help in diffusion of watery substances. medullary rays in the secondary phloem region also consist of living starch-filled parenchymatous cells, but in this region tracheidal cells are

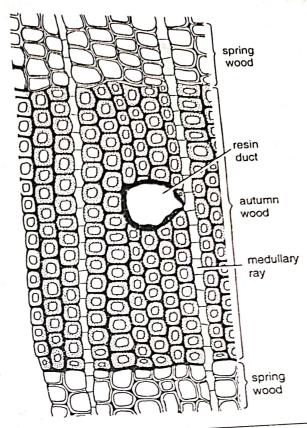


Fig. 9. Pinus: Secondary xylem showing spring and autumn wood.



Fig. 10. Pinus: Bars of Sanio in between bordered pits in tracheids.

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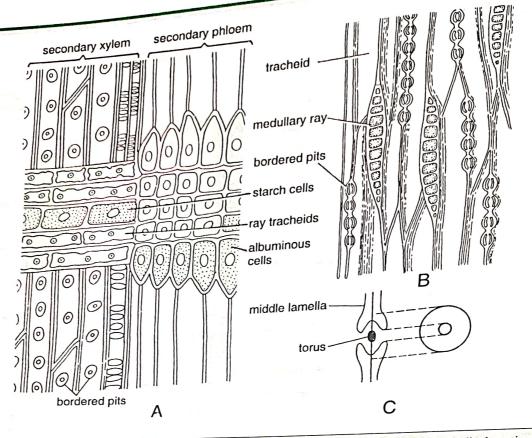


Fig. 11 A-C. *Pinus*: Long shoot; A. Radial longitudinal section, B. Tangential longitudinal section, C. Bordered pit with torus.

replaced by albuminous cells on both sides of the medullary rays. The albuminous cells are small, living, thin-walled cells, which are elongated at right angle to the ray axis.

The bordered pits develop on the walls of tracheids by partial spread of the secondary walls over the pits. The pit possesses a well developed torus (Fig. 11C). The torus is an important structure from functional point of view as it acts as a valve membrane.

[IV] Periderm or cork

Concurrently with the secondary growth in the vascular region, a lateral meristem, known as **cork cambium** (phellogen), develops in the outer region of the cortex. The phellogen divides periclinally to cut **cork cells** (phellem) towards the outer side and **secondary cortex** (phelloderm) towards the inner side. As the stem increases in girth, the epidermis ruptures and the cork cells form a protective covering. The cork cells are impervious

to water and they check transpiration from the stem surface.

[C] Dwarf Shoot

The structure of the dwarf shoot is nearly similar to that of the long shoot (Fig. 12). The internal tissues are differentiated into **epidermis**, **cortex** and **stele**. The epidermis is a single layer of thick-walled cells. It is followed by 4-6- layered parenchymatous cortex. Tannin filled cells and resin canals are also present in the cortex. The vascular cylinder consists of six conjoint, collateral, endarch and open vascular bundles. The medullary rays, which connect the pith and the cortex, traverse between the vascular bundles. The centre of the dwarf shoot is occupied by a parenchymatous pith.

Dwarf shoots show limited amount of secondary growth. The secondary xylem is small and separated by a thin strip of cambium from

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Coniferales : Pinus

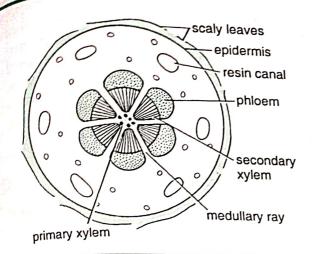


Fig. 12. Pinus: Diagrammatic representation of transverse section of dwarf shoot.

the secondary phloem. Secondary medullary rays are also formed as in long shoots.

The tissues of the dwarf shoot at the base of the spur divide into as many parts as the number of leaves in a spur. For instance, in

P. longifolia, where spur is trifoliate, the tissues divide into three equal parts.

[D] Foliage Leaf

In transection the outline of the foliage leaf varies in different species depending on the number of leaves in a spur. For example, it is circular in *P. monophylla* (with one needle in a spur); semicircular in *P. sylvestris* (with two needles in a spur) and triangular in *P. longifolia* (Fig. 13) and *P. gerardiana* (with three needles in a spur).

- 1. Epidermis. The single layered epidermis consists of heavily cutinised thick-walled cells. It has many deeply sunken haplocheilic stomata. The stomata are arranged in axial rows. Each stoma consists of two guard cells surrounded by 6-9 subsidiary cells. It opens internally into a substomatal cavity.
- 2. Hypodermis. The epidermis is followed by 2-3 layers of thick-walled sclerenchymatous hypodermis. The hypodermis is interrupted by the

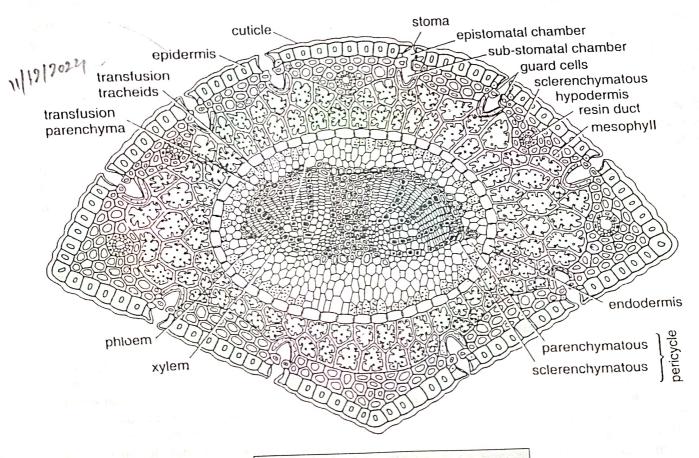


Fig. 13. Pinus: Transverse section of needle.

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presence of sub-stomatal cavities. In *P. longifolia*, the hypodermis is more developed at the corners.

3. Mesophyll. Next to hypodermis there are 3-5 layers of mesophyll tissue, composed of polygonal parenchymatous cells with dense chloroplasts and starch grains. The walls of the mesophyll cells give rise to many peg-like infoldings which increase photosynthetic area of these cells. The structural peculiarity of mesophyll cells compensates the reduced area of the needle leaves.

There are usually two resin canals in the mesophyll, just below the hypodermis. Each resin canal has a layer of secretory epithelial cells which is surrounded on outside by a sclerenchymatous sheath.

- 4. Endodermis. Inner to mesophyll, there is a layer of barrel-shaped cells with prominent casparian thickenings on their radial walls. This layer represents endodermis.
- 5. Pericycle. A multilayered pericycle is present next to endodermis. It consists of the following three types of cells.
- (a) Parenchymatous cells. Most of the pericycle is composed of parenchymatous cells which are densely filled with starch grains.
- (b) Albuminous cells. These cells occur in close contact with the phloem cells. They are also parenchymatous cells which are packed with proteins and starch grains. They do not possess pits and probably help in translocation of nutrients from mesophyll to phloem cells.
- (c) Tracheidal cells. These are tracheid-like cells which occur close to xylem cells. These cells possess pits and help in conduction of water and minerals to mesophyll. They form transfusion tissue.

Besides the above three types of cells, some sclerenchymatous cells also occur in the pericycle which form a T-shaped girdle above the two vascular bundles in *P. roxburghii*.

6. Vascular bundles. The number of vascular bundles varies in different species of *Pinus*. There is only a single vascular bundle in *P. monophylla* and two in *P. roxburghii*. The vascular bundles are conjoint, collateral and open with abaxial phloem and adaxial xylem.

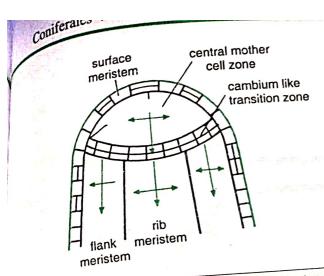
Narrow acicular form, thickly cuticularised epidermis, sunken stomata, sclerenchymatous hypodermis, peg-like infoldings in the mesophyll cells and the presence of sclerenchymatous tissue over the vascular bundles are some of the features of the leaves which indicate xerophytic nature of *Pinus*.

Shoot Apex Organization

The shoot apex of Pinus has four distinct cytohistological zones: (i) the surface meristem (ii) the zone of central mother cells, (iii) the rib meristem, and (iv) the peripheral flank meristem. These zones differ in degree of mitotic activity, directions of growth and in cellular constitution. and show seasonal variations. The surface meristem represents the initiation zone of the entire apex and its cells divide both in anticlinal and periclinal planes. The zone of central mother cells occurs in a median position below the surface layer. Its cells are relatively large, polyhedral, irregularly arranged, and contain numerous vacuoles with large light staining nuclei. Along the sides and the base of the central mother cell zone the other apical regions develop as a result of the diagonal and horizontal divisions of the central mother cells. In this way the peripheral or flank meristem is developed laterally and the rib meristem zone from the base. The cells of the peripheral zone are densely cytoplasmic and from which arise the leaf primordia, cortex and procambium. Later, the procambium differentiates into the vascular tissues that organise into distinct vascular bundles. The cytoplasm of the rib meristem is less dense and contains numerous small vacuoles. Basally, the rib meristem produces the pith (Fig. 14).

Reproduction

The sporophytic plant body forms two types of spores — microspores and megaspores — which develop into male and female gametophytes respectively. The plants are monoecious and male and female strobili are formed on different branches of the same plant. But in some species



organization 14. Pinus : Shoot apex (diagrammatic representation).

(e.g., P. montana, P. maritima, P. roxburghii), bisporangiate strobili also occur occasionally (Fig. 15). Both male and female strobili develop on the branches of the current year.

[A] Male Cone

Male cones develop in the axils of scale leaves on the branches of unlimited growth. As dwarf shoots are normally formed in the axils of scale leaves, the male cones are considered as modified dwarf shoots. The male cones develop in groups just behind the apical bud on the branches of unlimited growth (Fig. 16 A). The main shoot thus continues to grow further. The number of cones in a cluster varies from 15 (P. wallichiana) to 140 (P. roxburghii). The male cone is a small oval structure, about 2- 4 cm long and 5-6 mm in diameter (Fig. 16 B). The development of male cones starts before the female cones.

The male cone consists of a centrally located cone axis surrounded by numerous spirally arranged microsporophylls (Fig. 16 C). The number of microsporophylls in a cone varies

The microsporophyll is a membranous, stalked from 60-150. and roughly triangular structure (Fig. 16 D). It can be compared with the stamen of an angiosperm flower. The terminal expanded sterile part of the microsporophyll is known apophysis. It bears two microsporangia at the

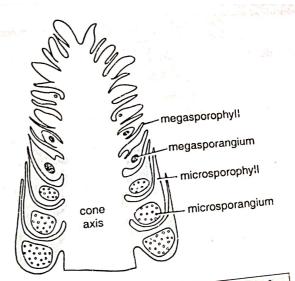


Fig. 15. Pinus maritima: Longitudinal section of a bisporangiate strobilus.

base of its abaxial side. Each microsporangium has numerous microspores.

Microsporophylls are arranged at right angle to the cone axis in such a manner that the apophysis of the upper microsporophyll is overlapped by the apophysis of the lower microsporophyll (Fig. 16 C). Except for a few basal sporophylls, all sporophylls in a male cone are fertile.

[I] Development of microsporangium microsporangium Development develops from 3-7 hypodermal cells of the eusporangiate microsporophyll. The microsporangial initial cells divide by a periclinal wall into outer primary wall cells and inner primary sporogenous cells (Fig. 17 A). The primary wall cells undergo several periclinal and anticlinal divisions and form a 2-4-layered wall (Fig. 17 B- D). The innermost layer of the sporangium wall differentiates into a nutritive layer, known as tapetum (Fig. 17 E).

The developemnt of tapetum and sporogenous cells takes place simultaneously. The differentiation and maturation of the sporogenous tissue starts in the centre of the sporangium and proceeds centrifugally.

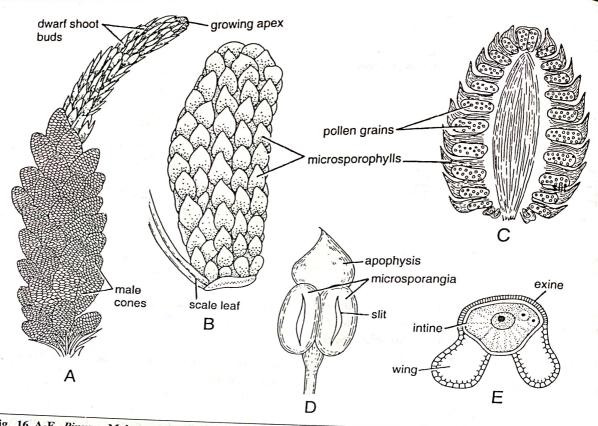


Fig. 16 A-E. Pinus: Male cone; A. A group of male cones, B. A single male cone, C. Longitudinal section of male cone, D. Microsporophyll, E. Pollen grain.

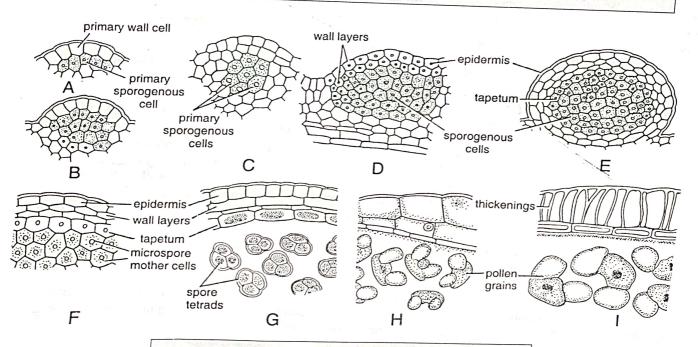


Fig. 17 A-I. Pinus: Development of microsporangium and microspores.

The tapetum is of the secretory type. Young tapetal cells are richly cytoplasmic and multinucleate. They become very conspicuous during meiosis in the microspore mother cells, and degenerate soon after the spores are released from the tetrads. The tapetum is involved in the nourishing of the sporogenous cells / young microspores, and in the formation of exine on the spores.

The cells of the sporogenous tissue separate from each other and function as microspore mother cells. Each microspore mother cell divides meiotically to form four microspores (Fig. 17 F-I). Following meiosis, wall formation is simultaneous, separating the four nuclei, and tetrahedral and isobilateral tetrads are formed. There are no plasmodesmatal connections either between the dividing mother cells or the microspores in a tetrad.

The microsporangium is a sac-like sessile structure present on the ventral surface of the microsporophyll. There are a large number of microspores (pollens) in each sporangium. The microspore has an outer thick exine and an inner thin intine. The exine expands on sides in the form of two wings (Fig. 16 E).

[II] Dehiscence of microsporangium

A longitudinal line of dehiscence is present on the dorsal surface of the microsporangium (Fig. 16 D). At maturity, the sporangial wall bursts along this line and pale yellow microspores are released through the slit. In India, dehiscence of microsporangia takes place in warm and dry environmental conditions. The microspores are released in such a large quantity that pine forests appear yellow at the time of dehiscence of microsporangium. This is called shower of sulphur.

[B] Female Cone

Female cones are formed in groups of 1-4 in the axils of the scale leaves towards the tips of long shoots of the current year (Fig. 2). They replace the branches of indeterminate growth. The female cone takes about three years to mature and the cones of the successive years may be seen below each year's growth of the long shoot on the terminal branches. The first year cones are compact (1-2 cm in length) and red-green with closely arranged sporophylls (Fig. 18 A). The second year cones are large and woody with sporophylls still compactly arranged (Fig. 18 B).

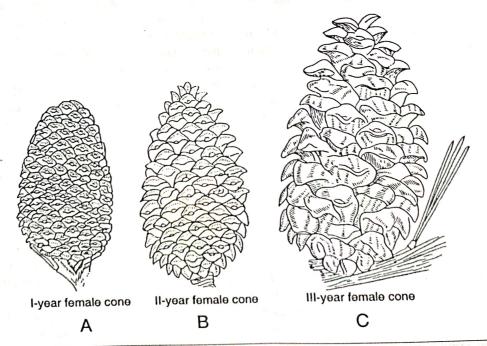


Fig. 18 A-C. Pinus: Female cone; A. 1st year cone, B. 2nd year cone, C. 3rd year cone.

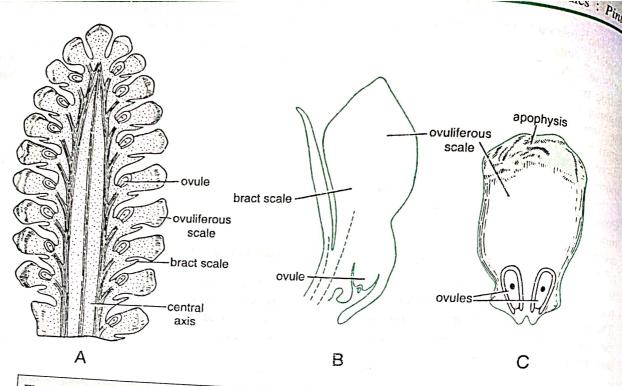


Fig. 19 A-C. Pinus: Female cone; A. Longitudinal section, B. Megasporophyll, C. Ovuliferous scale.

The third year cones become loose and the sporophylls separate from one another due to the elongation of the cone axis.

The female cones are much larger than the male cones. They are usually 15-20 cm long, but in *P* coultre they are 25-35 cm long and in *P*. tambertiana they attain a length of about 60 cm.

The female cone is much complicated in structure. It has a central axis around which many megasporophylls are arranged spirally (Fig. 19 A). Some basal megasporophylls are small and sterile and the rest are fertile. The megasporophyll is a compound structure, consisting of two types of scales (i) bract scales or cone scales, and (ii) ovuliferous scales or seminiferous scales (Fig. 19 B, C).

1. Bract scale. It is a small membranous structure which is directly attached to the cone axis just below the ovuliferous scale. At the maturity of the cone, the bract scales curve inwards so that seeds are dispersed easily. Each bract scale has a single vascular bundle with its xvlem pointing upwards. The bract scale is

sometimes also known as carpellary scale or cover scale.

2. Ovuliferous scale. It is a thick, large, woody and brownish structure, attached to the dorsal surface of the bract scale. It is roughly triangular in shape and its upper broad and thick part is known as apophysis (Fig. 19 C). In surface view, the apophysis is known as umbo. Two ovules are present at the base of the ovuliferous scale on its dorsal surface. The micropyle of each ovule is directed towards the cone axis.

Morphological Nature of Ovuliferous Scale

There is a great deal of controversy regarding the morphological nature of the ovuliferous scale as it is present in the axil of a bract scale. Several theories have been advanced to explain its morphological nature. Some important ones are briefly discussed below.

(1) According to excrescence or ligular theory of Sachs (1882) and Eichler (1889), the female cone is equivalent to a simple flower.

The central cone axis is equivalent to receptacle or thalamus and the bract scales to open carpels. They considered the ovuliferous scale as an outgrowth of the bract scale, similar to the ligule of *Selaginella* or the placenta of angiosperms.

- (2) Robert Brown (1827) considered the ovuliferous scale as an open foliar carpel which is present in the axil of bract scale and bearing naked ovules.
- (3) Schleiden (1839) considered ovuliferous scale as an axillary placenta, present in the axil of an axillary leaf (bract scale).
- (4) According to Alexander Brown (1842), the ovuliferous scale represents the first two leaves of an axillary shoot which had fused. Growth of the axillary shoot is, however, stopped after the formation of first two leaves.
- (5) Van Tieghem (1869) considered the bract as a leaf and the ovuliferous scale as a single leaf branch in the axil of the bract.
- (6) Bessey (1902) regarded the ovuliferous scale as a chalazal development of two ovules. According to the foliar theory of Delpino (1889) the ovuliferous scale was formed by inward turning and fusion of two lateral lobes of the bract scale. He considered the bract as a tripartite structure with a median sterile and two lateral fertile lobes which were fused together.
 - (7) Hirmer derived the ovuliferous scale and bract scale from a single structure which forked vertically like the sporangiophore of Sphenophyllales.
 - (8) Brachyblast theory of Braun suggests that the female cone is equivalent to an inflorescence rather than a flower. The ovuliferous scale was considered as a determinate axillary shoot bearing 2-3 fertile leaves. Each fertile leaf bears an ovule on the dorsal side.
 - (9) On the basis of his extensive studies of fossil conifers, Florin (1951) arrived to the conclusion that the female cone of *Pinus* is comparable to the inflorescence of angiosperms. The cone axis represents peduncle, the bract scale is a true bract

and the ovuliferous scale developed in its axil represents a rudimentary female flower (modified reproductive shoot). The female cone of *Pinus* is thus a compound strobilus. He introduced a combined term 'seed-scale-complex' for the bract scale and ovuliferous scale. According to Florin, the flattened seed-scale-complex usually has a rudimentary axis, two basal megasporophylls and two or three sterile distal scales, the latter fused to form the so-called ovuliferous scale.

Describing the morphology of the seed-scale-complex in *Pinus pinaster*, Guedes and Dupuy (1971, 1974) established that only two prophylls make up the scale. On the basis of epidermal features and the developmental order of the various zones of the scale, Lemoine-Sebastian (1973, 1975) visualised that the complex seed scale is composed of an axial portion (which represents the body) possessing the ovules and a foliar part (which corresponds to the apophysis).

From the foregoing discussion it appears that morphologically the ovuliferous scale is a reduced shoot and the female cone is an inflorescence.

Development of Megasporangium or Ovule

The development of the megasporangium (ovule) is of eusporangiate type, i.e., the megasporangium develops from more than one superficial initial cells. It appears as a small protuberance on the dorsal surface of the ovuliferous scale. The protuberance develops to form nucellus. The neighbouring cells at the base of the nucellus become meristematic, they grow upwards and form integument. The integument surrounds the nucellus, except at the top, where a small pore, known as micropyle, is left. The micropyle is directed towards the base of the ovuliferous scale and opens by a narrow oblique canal at the apex of the nucellus. In early stages the integument remains free from the nucellus but later on due to increased growth of the nucellus towards the chalazal region the basal part of the integument unites with the nucellus. However, the integument

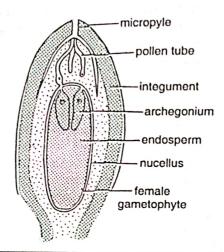


Fig. 20. Pinus: Longitudinal section of ovule.

remains free from the nucellus at the micropylar end (Fig. 20).

At the apex of the nucellus (towards the micropyle), a hypodermal cell is differentiated from the other cells by its larger size. This cell is known as archesporial cell. The archesporial cell becomes distinguishable while the female cone is still covered by the scale leaves. It divides by a periclinal wall to form an upper parietal cell and a lower primary sporogenous cell. The former undergoes both vertical and transverse divisions, so that the sporogenous cell is pushed deep into the nucellus, and later functions as the megaspore mother cell. The megaspore mother cell undergoes reduction division and forms four haploid megaspores, arranged in a linear tetrad. The three megaspores towards the micropylar end degenerate and the remaining one is the functional megaspore.

Three to five layers of cells round the functional megaspore become densely cytoplasmic with prominent nuclei. This is the **spongy** or **nutritive tissue**. The spongy tissue comprises a definite zone of physiologically active cells which are concerned in the nutrition of the young gametophyte.

Structure of Ovule or Megasporangium

The ovule of *Pinus* is orthotropous as in other gymnosperms. The following parts can be seen in a longitudinal section of the ovule (Fig. 20).

[I] Nucellus

It is the massive parenchymatous region of the ovule.

[II] Integument

It is the thick protective covering around the nucellus. It has a very narrow aperture at the apex of the nucellus. The integument is differentiated into three layers; the outer and inner layers are fleshy and the middle layer is stony.

In the upper part of the ovule a space is formed in between the integument and nucellus. This space is known as **pollen chamber**. After pollination, the pollen grains are collected in the pollen chamber and further development of the male gametophyte takes place inside the pollen chamber.

[III] Micropyle

It is a narrow aperture at the apex of the ovule, formed by the integument.

[IV] Vascular system

The vascular system of the ovule is not very well-developed. It is represented by some tracheidal elements, present at the base of the ovule.

GAMETOPHYTE

Pinus is heterosporous. The microspores and megaspores are formed in the male and female cones respectively on different branches of the same plant. Both micro- and megaspores are haploid and represent the first cell of the gametophytic phase. The microspore and megaspore develop into male and female gametophytes respectively.

Development of Male Gametophyte

The microspore is a uninucleate structure with an outer cuticularised **exine** and an inner thin **intine**. The exine expands on the lateral sides to form two balloon-like structures, known as wings. The outer wall of the wing has reticulate sculpturing (Fig. 21A).

The germination of microspores starts in situ, i.e., they germinate while still inside the microsporangium. The microspores are released from the microsporangium after partial

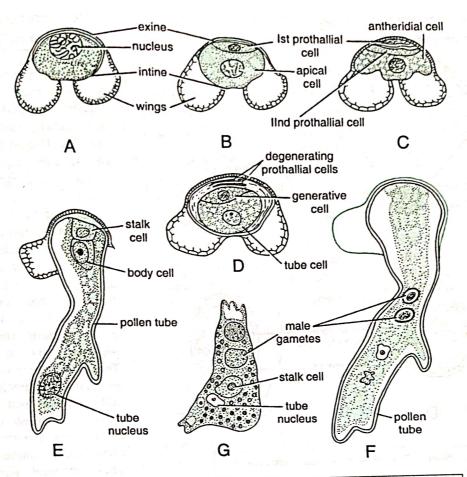


Fig. 21 A-G. Pinus: Development of male gametophyte; A-D. Stages before pollination; E-G. Stages after pollination.

development of the male gametophyte. Further development takes place in the pollen chamber of the ovule after pollination.

[A] Development of Male Gametophyte within the Microsporangium before Pollination

The microspore divides asymmetrically into a small lenticular cell and a larger cell (Fig. 21 A-B). The former, known as first prothallial cell, does not divide further. The larger cell, known as apical cell, divides asymmetrically and forms a lenticular second prothallial cell and a large antheridial cell (Fig. 21 C). The second prothallial cell does not divide further and remains attached to the first prothallial cell. The antheridial cell divides to form a generative cell (adjacent

to the second prothallial cell) and a large tube cell (Fig. 21 D). At this stage, the male gametophyte has four cells-first and second prothallial cells, a generative cell and a tube cell. The microspores are released from the microsporangium at 4- celled stage. They are disseminated by wind.

[B] Development of Male Gametophyte after Pollination

After pollination the 4-celled male gametophyte reaches the pollen chamber and there it remains inactive for about 11 months. Further development of the male gametophyte starts in the next spring. The exine bursts and the intine comes out in the form of a pollen tube (Fig. 21 E). The pollen tube gradually progresses towards the archegonium,

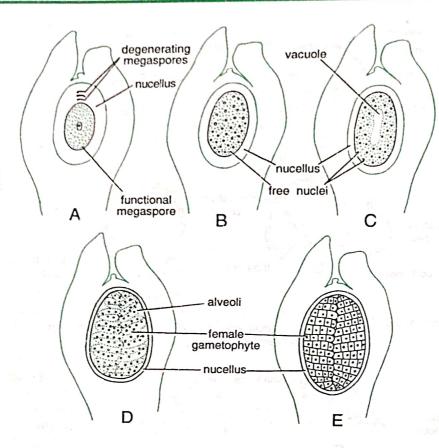


Fig. 22 A-E. Pinus: Development of female gametophyte.

penetrating the nucellar tissue. It is rich in starch grains and it may be branched or unbranched. It acts only as a sperm carrier.

generative cell of the 4-celled gametophyte divides to form a body cell and a stalk cell. The stalk cell is larger and the body cell remains attached at its free end (Fig. 21 E). The nucleus of the body cell divides to form two nuclei just before fertilization. These two daughter nuclei are known as male nuclei and they swim in the cytoplasm of the body cell (Fig. 21 F). The wall of the body cell gradually disappears and both male nuclei pass into the cytoplasm of the pollen tube. At maturity, four nuclei (a tube nucleus, a stalk nucleus and two male nuclei) are present at the tip of the pollen tube (Fig. 21 G). The male nuclei function as sperms, which are non-flagellate microscopic, and ephemeral structures.

Development of Female Gametophyte

The megaspore is the mother cell of the female gametophyte. It remains embedded deep inside the parenchymatous tissue of the nucellus. During the development female gametophyte, functional megaspore enlarges in size (Fig. 22 A) and its haploid nucleus divides repeatedly to form 2,000-2,500 daughter nuclei (Fig. 22 B). A vacuole develops in the centre of the megaspore and the multinucleate cytoplasm forms a thin layer near the periphery (Fig. 22 C). Thereafter, wall formation starts from the periphery towards the centre. Thus, numerous radially elongated multinucleate tube-like cells are formed, which are known as alveoli (Fig. 22 D). Later, each alveolus divides to form many uninucleate cells. They represent endosperm or female prothallus (Fig. 22 E).

As in other gymnosperms, the development of endosperm in *Pinus* occurs before fertilization and hence it is a haploid structure (in angiosperms endosperm is formed by triple fusion and as such it is a triploid (3x) structure. The nucellar cells surrounding the multinucleate endosperm form a 2-3-layered nutritive layer. This layer is also known as spongy layer or endosperm jacket and is equivalent to the tapetal layer.

[A] Development of Archegonium

All superficial cells at the micropylar end of the female gametophyte have the capacity to develop into archegonia, but only a few cells differentiate into archegonial initials. The number of archegonial initials varies in different species; for instance, there are 3 archegonial initials in *P. strobus*, *P. rigida* and *P. resinosa*, 5- 6 in

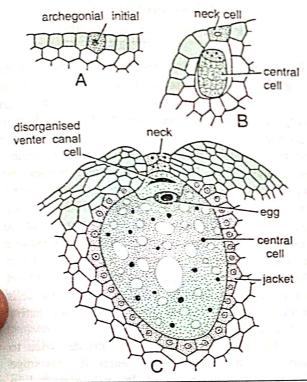


Fig. 23 A-C. Pinus: Archegonium; A-B. Stages in the development of archegonium, C. Mature archegonium.

P. laricio and up to 9 in P. montana. The archegonial initials remain separated from each other due to the presence of vegetative cells in between.

The archegonial initial can be distinguished from other cells by its larger size (Fig. 23 A). The nucleus of the archegonial initial moves towards the periphery and then divides by a transverse wall into an upper smaller primary neck cell and a lower larger central cell (Fig. 23 B). The primary neck cell divides by two anticlinal walls at right angle to each other to form four cells. These cells divide transversely to form eight cells which are arranged in two tiers of four each. Thus a 8- celled neck is formed. However, in *P. roxburghii* and *P. wallichiana* the neck remains only 4-celled.

The central cell increases in size considerably and the cytoplasm becomes vacuolated. It divides asymmetrically into an upper smaller venter canal cell and a larger egg (Fig. 23 C). This division takes place just before fertilization, approximately at the same time when the body cell of the male

gametophyte divides to form male nuclei. The nucleus of the venter canal cell degenerates just before fertilization.

A nutritive layer is differentiated around the central cell and this layer is known as archegonial jacket. The jacket helps in conduction of nutrients from the endosperm to the archegonium.

Pollination

Pinus is wind-pollinated. At the time of pollination a secretion oozes out from the micropyle of the ovule which entangles pollen grains disseminated by wind. This secretion is generally given out at night and remains at the tip of the micropyle till morning. Then it is sucked inside alongwith the pollen grains. The pollen grains are thus collected in the pollen chamber at the tip of the nucellus. Thereafter, the mouth of the micropyle is closed.

At the time of pollination, the female cone assumes vertical position and its bract scales are curved inwards. In this position the pollen grains can easily reach the ovules. In *Pinus* the pollen grains are formed in enormous quantity but only a few are utilized for fertilization and the rest go waste.

Fertilization

Fertilization takes place after about an year on pollination. The pollen tube, containing four nuclei (tube nucleus, stalk nucleus and two male nuclei), elongates and reaches near the tip of the archegonium, it penetrates neck cells, and when it comes in contact with the egg it releases all its contents in the vicinity of the egg. Out of the two male nuclei only one fuses with the egg and the other male nucleus and the tube and stalk nuclei degenerate.

The functional male gamete is devoid of the cytoplasmic sheath as it moves down the archegonium to fuse with the female gamete. The mitochondria and plastids brought by the pollen tube are morphologically different from those of the female gamete. They remain grouped in the upper part of the egg cytoplasm during the division of the zygote. The functional male gamete

is lodged in a depression on the female gamete. Soon, at the point of contact, junctions between the nuclear membranes of the two gametic nuclei are established at several points. The junction areas between the nucleoplasm gradually enlarge to form islets of cytoplasm surrounded by nuclear membrane. The latter slowly breaks down at the region of contact between the two nuclei and the two groups of chromosomes (of male gamete and egg) converge and lie on a common spindle. Finally the chromosomes of the two gametes lose their identity.

The diploid zygote thus formed represents the first cell of the sporophytic phase. More than one archegonia in an ovule may be fertilized but the nutrients present in the ovule are sufficient only for the development of one zygote. The other zygotes, if formed, degenerate.

EMBRYO DEVELOPMENT

Development of embryo can be studied in the following two steps.

- (1) Development of proembryo from zygote.
- (2) Development of embryo from proembryo.

Development of Proembryo from Zygote

Following fertilization, the diploid nucleus of the zygote migrates towards its base and then divides by two mitotic divisions to form four nuclei (Fig. 24 A-C). All the four nuclei thus formed get themselves arranged in one plane at the base of the zygote. Thus, only two nuclei are visible in lateral view. These nuclei divide further and form eight nuclei which are arranged in two tiers of four each. Thereafter, wall formation takes place in such a way that four complete cells are formed in the lower tier (i.e., these cells possess a wall all around) and four incomplete cells in the upper tier (i.e., these cells have walls only on the lower side; Fig. 24 D). The incomplete cells belonging to the upper tier do not take active part in embryo formation.

The cells of the lower tier divide twice to form three tiers of four cells each. At this stage the proembryo consists of 16 cells arranged in four tiers (Fig. 24 E-G). Each tier has a specific function: (i) the lowermost tier is known as the embryonal tier; its cells divide to form embryo,

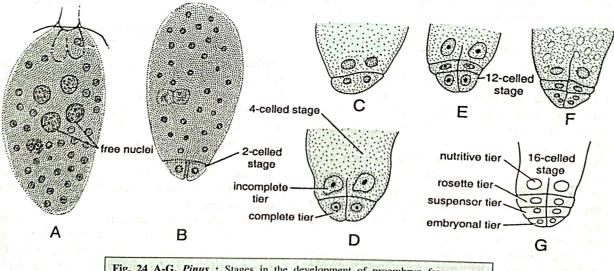


Fig. 24 A-G. Pinus: Stages in the development of proembryo from zygote.

(ii) the cells of the next tier (the suspensor tier) elongate to form suspensor, (iii) the cells of the third tier, known as the rosette tier, conduct nourishment to the embryo, and (iv) the uppermost tier consisting of incomplete cells is known as nutritive tier and it provides nutrition.

Development of Embryo from Proembryo

All the four cells of the suspensor tier elongate considerably and as a result the embryonal cells present at their base are embedded in the endosperm (Fig. 25 A). Due to the limitation of space in the endosperm, the primary suspensor cells become coiled to each other (Fig. 25 B). At this stage, the cells of the embryonal tier divide by transverse wall to form another tier of cells, known as embryonal tubes or secondary suspensor (Fig. 25 C).

The cells of the embryonal tier separate from each other and form four independent embryos. The phenomenon of the formation of more than one embryo from a zygote is known as polyembryony. In *Pinus*, as the polyembryony occurs by the splitting of a zygote, it is known as cleavage polyembryony. Sometimes cells of the rosette tier may develop into embryo and then it is called rosette polyembryony. But due to limited nutrients available, only a single proembryo develops into embyro and the rest degenerate.

The proembryo first divides by a transverse wall to form two cells. These cells undergo repeated divisions and form embryo. The embryo is differentiated into: (i) cotyledons, usually more than two in number, (ii) plumule or shoot apex, present between the cotyledons in the opposite direction of the micropyle, (iii) radicle, directed towards the micropyle, and (iv) hypocotyl, present in between the plumule and radicle (Fig. 25 D).

Polyembryony

Polyembryony is common in *Pinus*. Additional embryos originate by simple polyembryony, i.e., from multiple zygotes, as well as due to cleavage polyembryony, i.e., a single zygote gives rise to

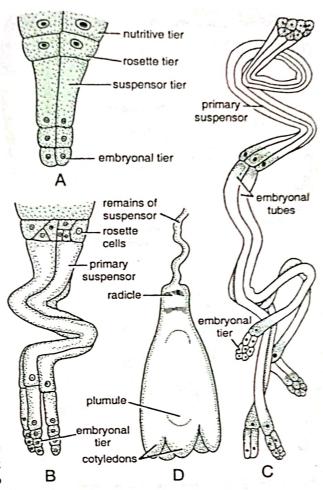


Fig. 25 A-D. Pinus: Stages in the development of embryo from proembryo.

multiple embryos by cleavage or splitting of the embryonal tier into several embryonal units. These units remain unchanged during the earlier stages of development, but later they divide and form a multicellular mass. Generally, the deep-seated terminal embryo succeeds and develops further. The remaining embryos become arrested at different stages of development.

SEED

Concurrently with embryo development, various parts of the ovule, outside the female gametophyte,

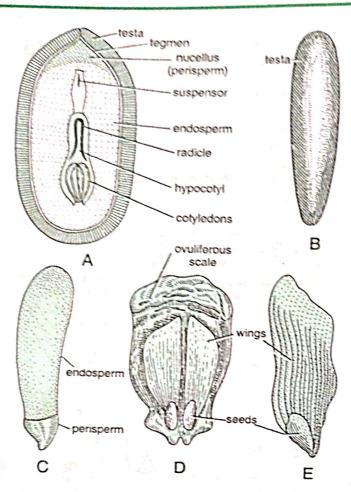


Fig. 26 A-E. Pinus: Seed structure; A. Longitudinal section of seed; B. Seed with testa, C. Seed without testa, D.E. Winged seeds.

undergo several changes by which the ovule is transformed into seed.

- The outer fleshy layer of the ovule wall disappears and the inner fleshy layer remains in the form of a thin membrane. The middle stony layer, however, develops into seed coat (Fig. 26 A-C).
- (2) Due to the growth of endosperm, the nucellus persists in the form of a thin brownish layer, known as perisperm.
- (3) The most part of the seed is occupied by endosperm which becomes massive by absorbing nutrients from the nucellus. The embryo remains embedded within the massive endosperm.

- (4) The micropyle persists as a small aperture at the apex of the seed.
- (5) A thin layer of the ventral surface of the ovuliferous scale is attached to the seed and thus the seed becomes winged (Fig. 26 D-E). This helps in the dispersal of the seed.
- (6) At maturity of seeds, cone axis elongates and as such the space between the megasporophylls is increased. Thus seeds are easily dispersed.

SEED GERMINATION

Under favourable conditions seeds of *Pinus* germinate soon after their dissemination. They donot require any resting period before germination. But they can remain dormant for several years under unfavourable conditions. The germination is epigeal, i.e., cotyledons come outside the soil surface by elongation of the hypocotyl. The seed coat bursts by absorbing water and radicle emerges out through the micropylar aperture and enters the soil. The radicle develops into primary tap root (Fig. 27 A- C). Thereafter plumule emerges, which forms the shoot system

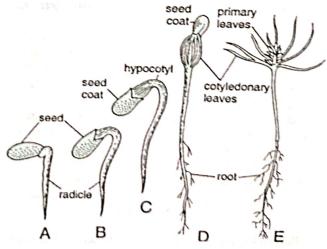


Fig. 27 A-E. Pinus: Stages in seed germination.

(Fig. 27 D). The spirally arranged leaves at the apex of the plumule are known as primary or juvenile leaves (Fig. 27 E). As the shoot grows, juvenile leaves are reduced to scaly leaves and the branches of limited growth develop in their axils.

Karyotype

The karyotype analysis shows that Pinus has uniformly 2n = 24 chromosomes (Mehra, 1988). The chromosomes are numbered I-XII according to their decreasing size. Chromosome pair XII is heterobrachial in P. gerardiana and P. roxburghii, whereas in P. kesiya, chromosome XI is also heterobrachial. In P. gerardiana, there is a secondary constriction in the proximal arm of the heterobrachial pair. However, in P. kesiya, each of the two isobrachial pairs have secondary constrictions.

Economic Importance of Pinus

The species of Pinus are of considerable economic important as the source of food, timber, oil, resins, etc.

- (1) The wood of P. roxburghii (Chir) and P. wallichiana (Kail) is an important timber. It is suitable for making railway sleeper, packing cases, furniture, etc. Besides, it is also used for making match sticks.
 - Pinus roxburghii, P. wallichiana, P. insularis and P. merkusii are the chief sources of turpentine in India. Rosins, obtained as a residue after the distillation of pine resin, are used in paper sizing, varnish making, enamels and in the preparation of plasters and ointments.
- The roasted seeds of P. gerardiana (Chilgoza pine), P. edulis and P. monophylla are edible.
- Several species of Pinus are grown as ornamentals in parks, gardens and in landscaping.
- (5) Young plants of P. merkusii are used in the manufacture of craft paper.
- The oleo-resin obtained from P. roxburghii is diuretic.
- The fossilized resin, obtained from a fossil species of Pinus (e.g., P. succinifera), is known as amber. It is widely used in ornaments and decoration work.
- (8) The wood and female cones of Pinus are used as fuel.

Important Ouestions

>>Long answer questions

- 1. Write a note on the distribution of Pinus in India and also comment on its economic importance.
- 2. With the help of suitable diagrams describe the anatomy of Pinus stem. Also comment upon the important characters of the wood.
- 3. Give an illustrated account of the internal structure of the Pinus needle and point out its xeromorphic features.
- Describe the structure and morphological nature of the ovuliferous scale of Pinus.
- Give a comparative account of the male gametophytes of Cycas and Pinus.
- Describe the post fertilization changes occurring in the ovule of Pinus.
- 7. Describe the development of female gametophyte of Pinus. How does it differ from that of angiosperms?
- 8. Why is the female cone of Pinus considered to be comparable to an inflorescence rather than a flower?
- 9. Draw labelled diagrams of R.L.S. and T.L.S. of Pinus stem.
- 10. Describe the internal structure of Pinus needle and compare it with the leaflet of Cycas,

>> Short answer questions

- 1. What is polyembryony? Explain it with reference to Pinus.
- Write a note on the seeds of Pinus.
- 3. Describe the structure of male cone of Pini s.
- 4. What are bars of Sanio?
- 5. Write a note on the economic importance of Pinus.
- 6. Describe the events occurring in the pollen grain before pollination.
- 7. What conclusions were drawn by Florin about the female cone of Pinus?
- Describe briefly the development of microsporangium in Pinus.

(GYMNOSPERMS)