1. Details of Module and its Structure

Subject Name	Gymnosperms
Paper Name	Systematics II- Pteridophytes and Gymnosperms Section B: Gymnosperms
Module Name/Title	Gnetopsida
Module Id	Bot/SystII/ Gnetopsida óII: Reproductive biology 31
Pre-requisites	Gymnosperms, basic knowledge of cell and structural biology
Objectives	To provide reproductive biology knowledge about Gnetopsida
Keywords	Gnetopsida, *Ephedra, Gnetum, Welwitschia*

Structure of Module / Syllabus of a module (Define Topic / Sub-topic of module)

Ephedra 1. Male strobilus and gametophyte	1.1 Male strobilus 1.2. Microsporogenesis 1.3. Development of male gametophyte
2. Female strobilus and gametophyte	2.1. Female strobilus 2.2. Megasporogenesis 2.3. Development of female gametophyte
3. Pollination and Fertilisation	3.1. Pollination 3.2. Fertilisation
4. Embryogeny	4.1. Seed
Gnetum 5. Male strobilus and gametophyte	5.1. Male strobilus 5.2. Microsporogenesis and development of male gametophyte
6. Female strobilus and gametophyte	6.1. Female strobilus 6.2. Megasporogenesis and development of female gametophyte
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Welwitschia 9. Male strobilus and gametophyte	9.1. Male strobilus 9.2. Microsporogenesis and development of male gametophyte
10. Female strobilus and gametophyte	10.1. Female strobilus 10.2. Megasporogenesis and development of female gametophyte
11. Pollination and Fertilisation	
12. Embryogeny and seed development	12.1. Seed and seedling
13. Summary 14. References	

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e-Text

The content write should send the elaborate information about the topic and sub-topic in textual format. e-Text is the descriptive textual documents with graphics/animations and video to elaborate the concept. The content writer should provide the information as per the e-text template. Textual document consisting of at least 8-10 pages with detailed write-up on the topic of module.

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Ephedra L.

Ephedra

Ephedra is typically dioecious. The male and female strobili are borne on separate individuals in the axils of leaves of determinate shoots (shoots of limited growth) and also in whorls on the nodes of older branches (Fig. 1A. a). The cone may be solitary but mostly arranged in dichasial cymes (Bhatnagar and Moitra 1996).

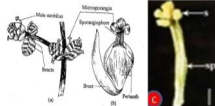## 1. Male Strobilus and Gametophyte

1.1 Male Strobilus

Fig. 1A: *Ephedra foliata* a) A part of *twig* with a cluster of male strobili. b) A male fertile shoot with three bilocular microsporangia at the tip (After Tiagi, 1966). c. *E. gerardiana* close up synangiophore with synangia

It consists of a central axis on which a few (10 in *E. foliata*), opposite and decussately arranged bracts occur. In the axil of each bract a fertile shoot or a microsporangiate 'flower' arises. Each 'flower' consists of a pair of basally fused perianth leaves surrounding a sporangiophore, which bears 2 to 6 bilobed, sessile, microsporangia at its tip (Fig. 1A. b, c).
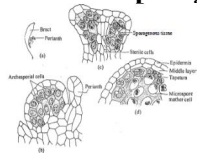
1.2 Microsporogenesis

Fig. 1B: *Ephedra* sp. microsporogenesis, a) L.S. young male flower with subtending bract. Note the perianth and the initiating sporangium. b) Same, at an older stage showing perianth and hypodermal archesporial cells. c) Still later stage depicting the development of a band of sterile cells. Also seen are two groups of sporogenous cells. d) A sector of sporangium showing microspore mother cells undergoing meiosis and multinucleate tapetal cells (after Singh & Maheshwari 1962).

A group of archesporial cells gets differentiated in the hypodermal region of the young sporangium (Fig. 1B. a, b). Subsequently, differentiation of a band of sterile cells separates sporogenous cells into two chambers (Fig. 1B. c). Surface view of the sporangium also indicates lobbing. A primary parietal layer and a primary sporogenous layer are cut off by the periclinal division in the outermost layer of the archesporial cells. The former undergoes both anti- and periclinal divisions giving rise to wall layers, the innermost of which differentiates into tapetum (Fig. 1B. d). The primary sporogenous cells, in turn, give rise to sporogenous tissue, whose cells eventually become the microspore mother cells (Fig. 1B. d). The microspore mother cells undergo meiosis to give rise to tetrads of microspores embedded in a massive layer of callose. The microspores are set free after the dissolution of callose (Singh & Maheshwari 1962).

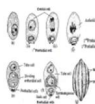### 1.3 Development of Male Gametophyte

Fig. 1C : (a-g): *Ephedra* sp., developmental stages of male gametophyte . a) A microspore, b-e) Stages in the development of male gametophyte. f) A five-nucleate pollen grain. g) The surface view of a mature pollen grain showing ridges and furrows. (after Singh & Maheshwari, 1962).
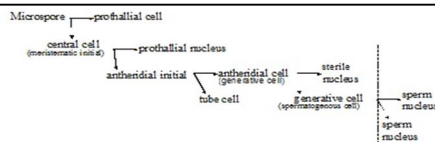
Fig. 1D. Male gametophyte development in *Ephedra* (Fernando et al. 2010).

The microspore nucleus moves to one end (Fig. 1C. a) and divides to form two cells: a small, ephemeral, lens-shaped prothallial cell (designated as first prothallial cell) and a large central cell (Fig. 1C. b). The central cell divides again in the same manner, forming a second prothallial cell and the large antheridial initial (Fig. 1C. c, d). The antheridial initial divides and gives rise to a tube cell and an antheridial cell. The antheridial cell on division forms the spermatogenous cell and stalk cell (Fig. 1C. e, f). The shedding of pollen thus takes place at 5-celled stage, having two prothallial cells, one tube cell, a stalk cell and a spermatogenous cell (Fig. 1D). Pollen grains are spherical in shape. The pollen wall is laid down concurrent

to the release of microspores from the tetrads. It consists of two layers - an outer exine and an inner intine. The exine is coated with sporopollenin, which is autofluorescent, and resistant to acetolysis. The intine is cellulosic in nature. The exine is ribbed, i.e., it consists of ridges and furrows (Fig. 1C**. g).** About 16 such ridges run longitudinally in *E. foliata* whereas in *E. gerardiana* only eleven such ridges are present (Singh & Maheshwari, 1962).

2. Female Strobilus and Gametophyte
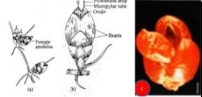
2.1 Female Strobilus –

Fig. 2A. *Ephedra foliata.* a) A part of the twig bearing female strobili. b) A female strobilus in an enlarged view. It consists of two large ovules and six pairs of bracts. Each ovule shows a micropylar tube with a distinct pollination drop (after Tiagi, 1966). c. Close up female strobilus showing fleshy red bracts and single ovule.

A female strobilus consists of four to seven pairs of opposite and decussate green bracts, fused at the base forming a cup-like structure. In the axil of each of the uppermost pair of bracts there is an ovule (Fig. 2A. a, b, c). Each ovule consists of two envelopes. The inner envelope, which is called integument, is thin and fused with the nucellus except in the upper region and protrudes to form a long micropylar tube (Fig.2A. b). The outer envelope is known as perianth, which is thicker and completely free from the integument. The apical meristem transforms into an ovule. The initiation of ovule is marked by periclinal divisions in the outermost layer of the lateral shoot meristem. The outer envelope (perianth) is formed by anticlinal and periclinal divisions in the epidermis. Immediately above the outer envelope, the integument arises as a protuberance. After initiation the integument becomes annular, but later shows one-sided growth or becomes asymmetric on the dorsal side. The integument remains asymmetric throughout and this is also reflected in the micropyle. The integument grows at a much higher level than the outer envelope. The outer envelope grows by an independent meristem. In most species, a massive nucellus arises as a result of periclinal divisions in the epidermis forming a nucellar cap; a parietal tissue is thus absent. However, in some species the nucellus is of dual origin having been contributed by the nucellar epidermis and parietal tissue (Tiagi, 1966; Singh and Maheshwari 1962).
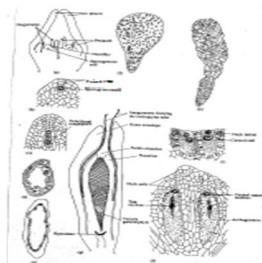
2.2 Megasporogenesis –

Fig, 2B. *Ephedra* spp. a) Longitudinal section of a female cone showing two young ovules and the subtending bracts. b) A part of apical portion of the nucellus showing sporogenous cell and parietal cells. c) Same, with a triad of megaspores. Note, the lowermost cell is the functional megaspore. d, e) A young, free nuclear gametophyte in longitudinal section. I) Whole mount of the top-shaped female gametophyte at free nuclar stage. g) Longitudinal section of ovule at the cellular gametophyte stage; mark the hypostase, The integument forms the micropylar tube. h) Cellular gametophyte formed through alveolation, enlarged from figure g. i) L.S. female gametophyte showing two young archegonia, each with a neck initial and a central cell. j) Same, at a later: stage of development. Each archegonium shows an egg nucleus, a ventral canal nucleus and a long neck whose cells merge with the surrounding gametophytic cells. (g, j) redrawn from Maheshwari, 1935; b, c, i) redrawn from Narang, 1956; d-T, h) after Singh & Maheshwari,1962).

A single hypodermal archesporial cell differentiates in the nucellus, and then divides periclinally to produce a primary parietal cell and a sporogenous cell (Megasopore mother cell). The primary parietal cell gives rise to several layers of parietal cells (Fig. 2B. a, b). The megaspore mother cell remains deep in the massive nucellus. The megaspore mother cell enlarges and divides meiotically to form a linear tetrad of haploid megaspores, of which the lowermost megaspore is functional and upper three degenerate. Such a development where only one of the megaspores takes part in the formation of female gametophyte is termed as monosporic (prevalent in conifers). This is in contrast to tetrasporic development in *Gnetum* and *Welwitschia* where all four megaspores are functional and form the female gametophyte, is called tetrasporic. Sometime the upper cell of the dyad fails to divide resulting in a triad of cells (Fig. 2B. c).

2.3 Development of Female Gametophyte –

The functional megaspore enlarges considerably and a central vacuole appears in its cytoplasm. Its nucleus divides mitotically to give rise to as many as 256 or 512 free nuclei. The nuclei arrange themselves in a thin layer of cytoplasm around the central vacuole (Fig. 2B. d-f). The wall formation takes place through alveolation. The cellular gametophyte (Fig. 2B. g) can be demarcated into two zones: a broad micropylar zone of radially elongated thin-walled cells with sparse cytoplasm; and a narrow chalazal zone of compact,

small and polygonal cells with dense cytoplasm (Fig. 2B. h). The chalazal part has an upper region of actively dividing cells and a lower region of large, densely cytoplasmic cells with prominent nuclei. So the mature gametophyte is distinguished into three zones: an upper fertile zone that bears archegonia, middle storage zone and a lower haustorial zone. Three to five cells at the micropylar end of the female gametophyte become distinguished by their large size, dense cytoplasm and prominent nuclei, and function as archegonial initials. They divide periclinally to form an outer, small primary neck initial and an inner, large central cell (Fig. 2B. i). A long column of about 30 - 40 neck cells for the archegonial neck (Fig. 2B. j), which looks similar to the transmitting tissue in the style of angiosperms. The central cell divides to form to form a ventral canal nucleus and an egg nucleus (Fig. 2B. j). The ventral canal nucleus appears to degenerate soon after, but in some species it persists and remains intact near the upper part of the archegonium. The egg nucleus enlarges and comes to lie in the centre of archegonial cytoplasm (Maheshwari, 1935; Narang, 1956; Singh & Maheshwari,1962).

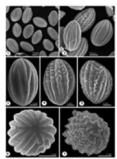## 3. Pollination and Fertilization
3.1 Pollination

Fig. 3 A. *Ephedra trifurca*. 1. Overview showing the morphology of typical pollen. 2. Normal grain with straight and branched valleys of ectexine and variant grain showing ectexine folding (upper middle). 3. Normal grain showing branched valleys and detail of normal ectexine. 4. Variant grain showing details of ectexine folding. 5. Variant grain that is apparently the result of a non-disjunction during microsporogenesis (note two apical poles). 6. Polar view of normal grain showing number of ridges. 7. Polar view of variant grain showing details of highly folded ectexine.
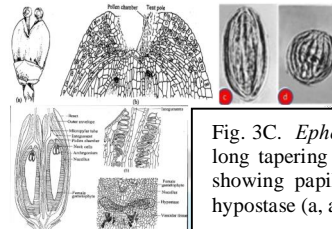

Fig. 3B. *Ephedra* spp. a) A mature female strobilus with ovules showing prominent pollination drops. b) A portion of ovule cut in longitudinal section showing well developed pollen chamber and tent pole (a, Courtesy Singh, M.N. : b, after Narang, 1956). c-d. Diamorphic pollen grains.

Fig. 3C. *Ephedra* spp. a) LS female cone showing two devdoped ovules. each showing two envelopes, a pollen chamber, long tapering female gametophyte bearing two archegonia with long prominent necks, b) L.S. micropylar part of ovule showing papillate projections from inner lining of outer envelope. c) A portion of nucellus from chalazal end with hypostase (a, after Narang, 1956; b & c. after Singh & Maheshwari, 1962).

The pollen grains of *Ephedra* are semicircular and wingless. In *Ephedra torreyana*, *E. trifurca* and *E. funerea*, the pollen grains show a marked pollen dimorphism when examined using scanning electron microscopy. Typical pollen grains in all of these taxa have straight ridges, but the variant forms exhibit a highly folded ectexine (Fig. 3A; Ickert-bond et. al. 2003, Fig. 3B. c, d). Wind blown pollen grains are caught in the pollination drop (Fig. 3B. a) at the micropylar tip and which subsequently sucked in and lie in the pollen chamber tip of the nucellus. The pollination drop is rich in amino acids, peptides, malic acid, citric acid, inorganic phosphates and sugars. The concentration of sucrose is as high as 25%. Although wind-pollination is the common mode of pollen delivery, but entomophily is prevalent in some species such as *E.aphylla* and *E. campylopoda*. Some ants and other insects feed on the sugar rich nectar in both the male and the female strobili and aid in the transfer of pollen grains that stick to their bodies to the ovules. The tip of the female gametophyte protrudes into the pollen chamber. Such a projection is called a 'tent-pole' (Fig. 3B. b; Narang, 1956). The pollen chamber extends to the top of the female gametophyte (Fig. 3B. b, 3C. a). The pollen grains swell in the pollen chamber which cause rupturing of exine and extension of intine as tube. The tube nucleus and spermatogenous cell move into the growing pollen tube. The Spermatogenous cell divides to give rise to two male nuclei. Several pollen tubes grow from a number of pollen grains. One of the pollen tubes makes its entry the mature archegonium and releases the two male gametes. On fertilization of an achegonium, the growth of other pollen tubes cease. In post-pollination stages, the cells of outer envelope shows papillate projections, which later elongate and become thick-walled (Fig. 3C. b). This results in closing the space between the two envelopes. There is a well-developed hypostase and the lower tapering end of the gametophyte lies very close to it. The cells of the hypostase are small, thin-walled and colourless. Some spiral and pitted tracheids are present below the hypostase (Fig. 3C. c; Singh & Maheshwari, 1962).
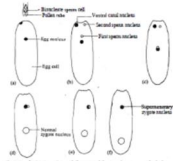
3.2 Fertilization

Fig. 3D. a-f) : *Ephedra* sp. Schematic diagrams depicting the various stages culminating in double fertilisation. a) Diagrammatic representation of a pollen tube with binucleate sperm cell approaching egg cell. b-d) Stages of fertilization of egg nucleus by the first sperm nucleus forming a normal zygote nucleus. d-f) The zygote nucleus eventually migrates to the brw of the former egg cell. The stages of fusion of the ventral canal nucleus and the second sperm nucleus are depicted in the figure b-f (see the upper region of the former egg cell) (Redrawn from Friedman, 1990).

One of two sperm nuclei of a pollen tube fuses with the egg nucleus and forms zygote. In *E. nevadensis* a regular occurrence of double fertilization is reported in which one male nucleus fuses with the egg nucleus and form ÷normal zygote nucleusø with dense cytoplasm (Fig. 3D. a-d). The second sperm nucleus fuses with ventral canal nucleus and form ÷supernumerary zygote nucleusø (Fig. 3D. b-f), Thus both sperm nuclei of a pollen tube are functional. Similar reports of double fertilization have also been made on some other species of *Ephedra* (Land 1907, Herzfeld 1922, Khan 1943, Moussel 1978). One could hypothesize that double fertilization arose in a common ancestor of both *Ephedra* and angiosperms (Friedman 1994). Phylogeographic Evidence for a link of species divergence of *Ephedra* through morpho-molecular variations suggests that a wide sampling of species is helpful to investigate the origin of observed haplotypes and make reliable phylogeographical inference (Ickert-bond et. al. 2009; Rydin et. al. 2010; Ickert-bond and Rydin 2011; Yang and Wang 2013; Qin et. al. 2013).
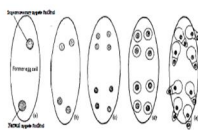
4. Embryogeny

Fig. 4A. *Ephedra sp.* Outline diagrams depicting stages of early embryogeny. a) The two products of double fertilisation. b, c) The two fertilization products undergo mitotic divisions to form two groups of nuclei, each having four nuclei. d) Diagrammatic depiction of the cellularisation of free nuclei, consequently each nucleus acquires a cell wall. Thus two sets of unicellular/uninucleate proembryos are formed. e) Further, each proembryo begins a cellular pattern of development with filamentous growth (After Friedman, 1994).

Fig. 4B. *Ephedra sp.* Further stages in embryogeny, a) One embryonal unit at a later stage showing elongated embryonal suspensor and an embryonal cell. b-d) Later stages in embryo development. (Redrawn from: a, d, Khan, 1943; b, c, Lehmmn-Baerts, 1967).

The synchronous mitotic divisions in the two zygote nuclei (Fig. 4A. a) lead to the formation of two sets of daughter nuclei. A total of eight free nuclei are formed, which are typically arranged in two groups within the former egg cytoplasm: the basal four from the 'normal zygote nucleus' and the apical four arising from the 'supernumerary zygote nucleus' (Fig, 4A.b, c). Subsequently cell walls are deposited around individual nuclei (Fig. 4A. d). Thus two sets of proembryonal cells or units are formed (Fig. 4A. d). A tubular outgrowth appears on one side in each of the eight cells, which grow towards the archegonial base. The nucleus in each of the eight cells move in the tube and divides by a transverse wall to form an embryonal cell and a suspensor cell (Figs 4A. e; and 4B. a). Suspensor cells push the developing embryos deep into the female gametophyte (Friedman 1994). All the eight proembryos in an archegonium are potentially alike, but those present at the micropylar end, gradually degenerate. One of the deep-seated, centrally placed, chalazal embryos finally develops into a mature embryo (Fig. 4B. b-d). The shoot apex is organized at the tip towards the chalazal end and the cotyledons arise as two small protuberances just below the shoot apex. Root apex differentiates near secondary suspensor cells (Khan, 1943; Lehmmn-Baerts, 1967).

4.1 Seed
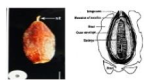
Fig. 4C. a. Seed with a straight micropyle b. *Ephedra* sp. Outline diagram of a seed cut longitudinally (Redrawn from Khan, 1943).

The two bracts of the megasporangiate strobilus in a mature seed (Fig. 4C. a) form the outer fleshy envelope (Fig. 4C. b). The outer envelop is vascularized and it forms the seed coat. The integument (inner envelop) remains papery. In the ovule at the pre-pollination stage, the nucellar cells around the pollen chamber become binucleate and distort after fertilization. In a mature seed, only the compressed remains of the nucellus persist. It is followed by a massive starch rich female gametophyte (=endosperm). The embryo is dicotyledonous. The germination of the seed is epigeal. The cotyledons develop chlorophyll and represent

the first two cotyledonary leaves (Khan 1943). Seed dispersal appears to be facilitated by the ovulate bracts, which in some species are bright red and fleshy and indicative of endozoochory. Bird dispersal has also been observed by Hollander et al. (2010). In some species, dry wing-bracted strobili are adapted for anemochory. The seeds of *E. aspera*, *E. californica*, *E. funerea*, *E. nevadensis*, and *E. viridis* are not fleshy and their ovulate bracts are not winged. Seeds of these õintermediate bractedö taxa often fall at the stem base, and seed-caching rodents have been observed as dispersers (Ickert-Bond et. al. 2009).
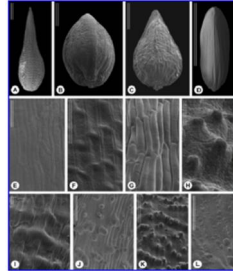

Fig. 4D. Seed shapes in longitudinal outline (AóD) and surface patterns of the seed envelope (EóL). Scale bars: AóD, 2 mm; EóG, IóL, 100 mm; H, 20 mm. A, Seed lanceolate in *Ephedra torreyana* (Franklin 3368 [NY]); B, seed elliptic in *Ephedra foeminea* (1526 [PE seed bank]); C, seed ovate in *Ephedra multiflora* (Ickert-Bond 1211 [ASU]); D, seed oblong in *Ephedra transitoria* (Collenette 9095B [E], at pollination stage of development, i.e., smaller than the others); E, outer periclinal cell walls flat in *Ephedra sarcocarpa* (Freitag 13.988 [KAS]); F, outer periclinal cell walls depressed in *Ephedra regeliana* (K.C. Kuan 1067 [PE]); G, outer periclinal cell walls convex in *Ephedra trifurca* (Ickert-Bond 577 [ASU]); H, outer periclinal cell walls papillate in *Ephedra equisetina* (Yang 2004003 [PE]); I, ÷wavyø transverse ridges in *E. multiflora* (Ickert-Bond 1231[ASU]); J, ÷papillate lamellaeø in *E. torreyana* (Porter & Porter 8998 [S]); K, ÷ridge-like papillaeø in *E. equisetina* (Yang 2004003 [PE]); L, ÷wartlike projectionsø in *Ephedra pachyclada* (Regel s.n. [S]).

Variation in micromorphological pattern of the seed envelope of *Ephedra* is a useful taxonomic tool for species determination in the subclade delineation (Fig. 4D; Ickert-bond and Rydin 2011).

Gnetum.

5. Male Strobilus and Gametophyte

5.1 Male Strobilus

Fig. 5A *Gnetum* sp. a) Reproductive branch. b) male strobilus.

Gnetum is dioecious and male and female strobili develop on separate plants. Fig. 5A shows the morphology of the male branches.
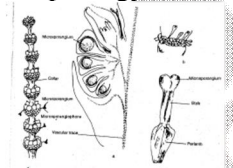

Fig. 5B. a-c) *Gnetum sp.* : a) Branch bearing a panicle of male cones at dehiscence. At each collar, just above male flowers, there is a ring of imperfect female flowers or abortive ovules. b) Portion from an enlarged; male flowers are dehiscing. c) Elongation of stalk and emergence of anthers from perianth. d) L.S. part of male cone to show the position of sporangia and ovule and vasculature to collar, male flowers and ovule (a-c, after vasil, 1959; d-g, after Sanwal, 1962).

The cone branch (inflorescence) is a solitary or fascicled panicle. Both male and female megastrobili terminal or lateral, sometimes arranged in dense, cauliflorous clusters on old stems. Each male and female strobili consists of a straight axis above a basal pair of opposite, connate bracts. The axis bears usually three to six superposed cupules one above other in whorls, each of which contains several to many male or female strobili (Fig. 5B. a). A young strobilus appears compact with very short internodes and the collars appear to be continuous but as the axis elongates the collars get separated. The upper two or three collars in *G. gnemon* are much reduced and sterile. Each collar bears three to six rings of 12-15 male flowers encircling the axis and above them a single ring of 7-12 imperfect (non functional) female flowers or abortive ovules (Fig. 5B. a, b). Each male flower has two unilocular anthers on a stalk (antherophore) enclosed in a sheath of bracts or perianth (Fig. 5B. d). The stalk elongates at maturity and the anthers come out of the perianth through a slit (Fig. 5B. c). During the development of the male strobilus, the collars are formed in an acropetal succession (Vasil 1959; Sanwal 1962).

5.2 Microsporogenesis and development of male gametophyte
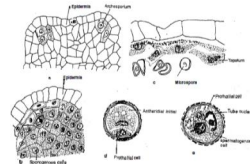
Fig. 5C : *Gnetum sp.* Microsporogenesis and male gametophyte. a) L.S. very young male flower to show few hypodermal archesporial cells. b) Same, at a later stage; primary parietal layer has been cut off above sporogenous tissue. c) Part of sporangium showing degenerating tapital cells and a few microspores. d) Two-nucleate pollen grain; prothallial cell has been cut off, e) Mature, three-celled (prothallial cell, tube cell and spermatogenous cell) pollen grain at shedding stage (after Sanwal, 1962).
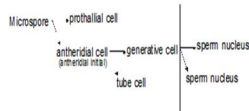
Fig. 5D. Male gametophyte development *Gnetum* and *Welwitschia* after Fernando et. al. 2010

A ring of meristematic cells differentiates at the base (axil) of each collar. The microsporangium is formed by a hump of tissue which becomes notched to form two anthers with a stalk. Two groups of hypodermal archesporial cells, by repeated divisions, give rise to multicellular archesporium (Fig. 5C. a). The outermost layer of the archesporial cells divides to form the primary parietal layer and the sporogenous cells (Fig. 5C. b). The primary parietal layer, by periclinal division, gives rise to a wall layer towards outside and tapetum towards inside. The tapetal cells become densely cytoplasmic and are normally binucleate; the nuclei may fuse and become polyploid. The tapetal cells start degenerating after meiosis in the spore mother cells and their remnants are discernible at uninucleate stage of microspores (Fig. 5C. c). Ubisch granules or orbicules are seen on the wall of the tapetal cells. The epidermis remains persistent in the mature sporangium which dehisces by a median longtitudinal slit. The sporogenous cells divide and increase in number, the last cell generation of which differentiates into microspore mother cells. Broad cytoplasmic channels interconnect microspore mother cells (meiocytes) forming a syncytium. As the mother cell enters meiosis, it is surrounded by a thick layer of callose. The meiosis results in the formation of tetrahedral or isobilateral tetrads embedded in the callose cover, which is soon absorbed and releases individual haploid microspores (Fig. 5C. c). The microspore nucleus divides to form a small lens-shaped prothallial cell and a large antheridial initial (Fig. 5C. d). The prothallial cell rounds up and does not undergo any further division and degenerates as such. The antheridial initial divides forming an antheridial cell and a tube cell and former directly functions as the spermatogenous cell as ther is no stalk cell. Pollen grains are shed at the 3-celled stage (Fig. 5C. e; Fig. 5D, Fernando et al. 2010). Pollen dyads are also observed in some species probably arise due to the non-separation of two or more cells of a tetrad. The pollen is sticky in nature (Sanwal 1962).

6. Female Strobilus and Gametophyte
6.1 Female strobilus

Fig. 6 A: *Gnetum* sp. a) female strobilus. b) seed bearing branch (after Kubitzki, 1990).

The female strobilus resembles the male strobilus at young stage, but become distinct at maturity. In a female strobilus, a ring of four to ten female flowers (ovules) encircling the axis is present above each collar (Fig. 6A. a, b; Kubitzki, 1990).
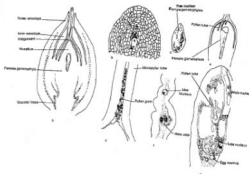

Fig. 6B: *Gnetum sp.* a) L.S. ovule to show outer and inner envelopes, integument and well developed nucellus; the integument forms the micropylar tube. The female gametophyte is at the free nuclear stage. Dotted lines represent the vascular supply to various parts. b) A two nucleate megaspore mother cell. c) Free nuclear female gametophytes, d) L.S. part of micropyle showing entrapped pollen grains, e) L.S. ovule to show three pollen tubes in nucellar tissue. One of the pollen tubes is about to enter the lower female gametophyte. f) Portion of pollen tube showing tube nucleus and two equal male cells. g) L.S. upper part of female gametophyte with several pollen tubes. Two large nuclei with radiatlng cytoplasm near the lower pollen tube will function as egg nuclei, (c-e after Vasil, 1959; a-b after Madhulata, 1960; f-g after Sanwal, 1962).

All the ovules look alike initially but, later, only a few grow to maturity. The upper few collars usually lack ovules. Nearly four to ten ovular primordia differentiate from an annular meristem below each collar of the female cone. The three envelopes; outer, middle and inner (integument) arise in a centripetal manner. The ovule is stalked or subsessile. The outer envelop is called perianth, which gets thickened and somewhat succulent at maturity (Fig. 6B. a). The middle envelope is known as outer integument, which forms a tapering ring-shaped rim at its apical part. Stomata differentiate in the outer epidermis of this envelop and it also the laticifers at the time of pollination. The major part of the stony layer of the seed coat is formed by the fibrous elements and sclereids in this envelope. The innermost envelope or inner integument is fused with the nucellus in the lower part. It elongates considerably to form a long micropylar tube or so-called "style" projecting beyond the apical cleft of the outer envelope. The exposed part is nearly one-third the length of the mature ovule. This part starts degenerating before or at the time of pollination. The apical lobes of this envelope become prominent and irregularly twisted and their number varies from 7 to 11(Fig. 6B. a). The

inner integument lack stomata and sclereids. Massive nucellus develops in the middle and its epidermis divides to form a nucellar cap. Prior to meiosis in the megaspore mother cells, some nucellar cells divide to form a pavement tissue tissue in where cells are arranged in radiating rows. As the female gametophyte grows in the chalaza1 region, the pavement tissue gets absorbed. This tissue is considered to have nutritive in function (Madhulata 1960).

6.2 Megasporogenesis and development of Female Gametophyte -

Some hypodermal archesporial cells differentiate deep in the young nucellus (Fig. 6B. b). These cells form outer primary parietal cells and inner primary sporogenous cells. The parietal cells together with nucellar epidermis produce a massive nucellus. The primary sporogenous cells divide and form 8-16 linearly arranged as megaspore mother cells. These cells divide meiotically and no walls are laid down after meiosis I and II (Fig. 6B. d), thus many tetranucleate coenomegaspores are produced. The development of the female gametophyte is thus, tetrasporic. Although several coenomegaspores develop in the same nucellus but generally only 2 or 3 grow beyond 16-nucleate stage. The four nuclei of a coenomegaspors divide and form female gametophyte with a large vacuole in the centre and the nuclei in the peripheral cytoplasm that undergo repeated divisions (Fig. 6B. c). Lower part the gametophyte accumulates most cytoplasm and free nucli (Fig. 6B. e). Unlike that of conifers, the archegonia are absent in the female gametophyte of *Gnetum*, and this feature is shared with *Welwitschia* (Vasil 1959). When the pollen tube makes contact with the female gametophyte, one or more nuclei in the middle region of female gametophyte are distinguished by their large size and dense cytoplasm, which function as egg nuclei. The differentiation of the egg is stimulated by the presence of the pollen tube in the vicinity of the female gametophyte (Fig. 6B. g; Sanwal, 1962). All the differentiated egg nuclei do not mature simultaneously nor do all the pollen tubes reach the gametophyte at the same time. The female gametophyte, which for most of its part is free nuclear, now starts becoming cellular in its upper portion soon after the fertilization of one of the eggs. Eventually the upper part of the gametophyte becomes almost cellular. From a structural perspective, the most divergent female gametophytes among all seed plants are found in *Gnetum*, a clade within Gnetales. Coenocytic organization at sexual maturity, absence of defined egg cells (free nuclei are fertilized), lack of centripetal cellularization, and post-fertilization development of embryo-nourishing tissues are features of the female gametophytes of *Gnetum* unparalleled among seed plants (Friedman and Carmichael 1998).
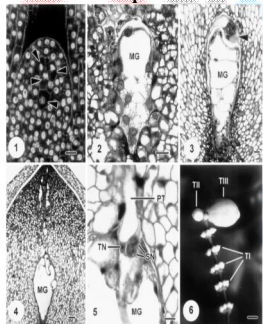

Fig. 6C: 166. Development of megagametophytes and ovules on bisexual cones of *Gnetum gnemon.* 1. DAPI-stained section showing four megasporocytes (arrowheads). Bar - 20 µm. 2. Longitudinal section through a young coenocytic megagametophyte. Bar - 20 µm. 3. Coenocytic megagametophyte at the time of pollination. Note the numerous vacuoles and degenerate megagametophyte (arrowhead). Bar - 20 µm. 4. Longitudinal section through a pollinated ovule. Pollen tubes are found growing through the nucellus toward the megagametophyte. Bar - 50 µm. 5. High-magnification view showing a pollen tube with sperm nuclei entering the apex of a coenocytic megagametophyte. Sperm remained within pollen tubes and fertilization did not occur. Bar - 20 µm. 6. Bisexual cone ;3 mo after pollination. All of the microsporangia have abscised by this point. The majority of ovules did not develop after pollination (Type I). Some ovules enlarged, but only slightly (Type II). Other ovules developed into large seed-like structures and turned red in color (Type III). Bar - 1 cm. *Figure Abbreviations:* MG, megagametophyte; N, nucellus; RFU, relative fluorescence units; SN, sperm nucleus; TN, tube nucleus; TI, type one ovule; TII, type two ovule; TIII, type three ovule.

A recent study demonstrates three different developmental patterns of ovules formed on bisexual cones of *Gnetum gnemon.* Type I ovules did not develop at all after pollination and represented the majority of ovules on each cone. Type II ovules enlarged slightly after pollination due to the enlargement of nucellar tissue. Type III ovules were typically found on the terminal whorl and developed into seed-like structures (Fig. 6C; Haycraft and Carmichael 2001).

7. Pollination and Fertilization

Fig. 7. Diagram illustrating double fertilization in *Gnetum gnemon.* Binucleate sperm cells are produced within pollen tubes that enter a coenocytic female gametophyte. Shortly after pollen tubes enter, free nuclei within the female gametophyte migrate around the tips of pollen tubes, None of the female nuclei differentiate as eggs and all represent potential gametes. Pollen tubes discharge both sperm nuclei into surrounding female cytoplasm and each sperm nucleus fuses with a separate female nucleus. Double fertilization results In the formation of two viable zygotes. Although unfertilized female nuclei may become cellular (arrowheads), they ore clearly distinguishable from diploid zygotes. Concurrent with fertilization, the chalazal region of the female gametophyte becomes cellular, will eventually enlarge and serve to nourish developing embryos. As many as six pollen tubes may fertilize a single female gametophyte, (after Friedman 1996).
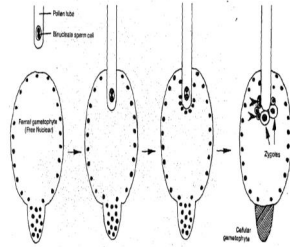
In the evening time both male and female strobili emit a strong, putrid odour; pollination droplets are also secreted from the ovules. Evaporation of the exposed pollen droplet is very slow at night in the tropical rain forest. This nocturnal entomophily is attained by replacing showy petals with a strong odour. The pollination takes place when the female gametophyte shows nearly 250 free nuclei in *G. ula* and 32 or 64 nuclei in *G. gnemon*. Pollination may be carried out by wind or insects. The tip of the micropylar tube becomes flared and lacerated. Pollination drop which is formed by the degenerated cells of the nucellar tip The sugar rich pollination drop is exuded at the tip and collects pollen. As the fluid dries, the pollen grains are sucked into the micropylar canal and reach to the pollen chamber (Fig. 6B. d) (Vasil 1959). A pollen chamber develops at the apical portion of the nucellus. When megasporogenesis is complete, the cells in the upper region start degenerating. The degeneration of the cytoplasm is followed by the breakdown of nuclei and cell walls. A shallow pollen chamber is formed into which pollen grains reach. The cells between the pollen chamber and female gametophyte are gorged with starch grains. The growing endosperm (female gametophyte) nearly consumes the nucellus. The pollen grains are devoid of germ pores. A pollen tube emerges as extension of the intine, which grows and traverses the nucellus through intercellular spaces. Meanwhile the prothallial cell degenerates in situ and spermatogenous cell divide and forms two male cells in the pollen tube (Fig. 6B. f). The male gametes come to lie near the tip of the pollen tube (Fig. 6B. g; Sanwal, 1962). A closing tissue is formed by the proliferation of inner epidermis of integument at the level of the flange. This results in the closure of the micropylar due to the elongation and interlocking of the inner epidermal cells. The plugging tissue has been called "obturator" as it fits in the pollen chamber. During post-pollination stages, a ring shaped swelling appears just above the insertion of inner envelope. Another proliferation develops in the apical part below the pollen chamber. At the time of pollination, a circular rim or an umbrella-shaped structure, called "flange" develops from the integument. Double fertilization is reported to occur regularly in *G. gnemon,* and is possible because two nuclei per pollen tube have access to at least two potential female nuclei in the gametophyte. In *Gnetum gnemon* a binucleate sperm cell is formed in the pollen tube. In the free nuclear female garnetophyte, undifferentiated female nuclei function as eggs. Both male nuclei are released in the surrounding female cytoplasm and each fuses with a separate female nucleus thus causing **double fertiiizalion**, resulting in two diploid zygotes. After fertilization each zygote nucleus develops into an embryo, but usually only one attains maturity in the seed (Fig, 7; Friedman 1996).
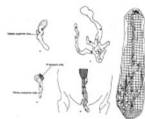
8. Embryogeny and seed development

Fig. 8 A Stages in development of embryo of *Gnetum gnemon*. a) Zygote giving out primary suspensor tube, b) Two-celled zygote; some endosprrrn cells are also seen. c) Branched primary suspensor tube. d) L.S. ovule showing numerous primary suspensor tubes penetrating the female gametophyte. e) Lower portion of ovule showing the primary suspensor tubes growing beyond endosperm in the nucellar tissues.

Fig. 8B *Gnetum sp.* (et, embryonal tubes). a) Tip portion of primary suspensor tube. b, c) Same showing 2 and 8 celled stage; nucleus of primary suspensor tube Is persistent. d, e) Upper cells of the cellular mass have enlarged and undergone division to give rise to secondary suspensor (embryonal tubes) l) Young embryo showing primary suspcnsor tube with long, coiled multicellular secondary suspensor. A few cells of secondary suspensor have elongated to produce long tubes growing on the sides of primary suspensor tube (after Vasil 1959).

Fig. 8C. *Gnetum gnemon* a) L.S. mature embryo showing a well developed feeder which is much longer than the embryonal axis. The dotted lines represent vnsculature. b) Polyembryony; note the development of additional embryos from prlmary suspensor tube (after Sanwal, 1962).

Fig. 8D. a-e) Germination of seed and seedling formation in *Gnetum* (after Maheshwari & Vasil, 1961).

The zygote may give rise to a small protuberance into which the nucleus moves (Fig. 8A. a). It may even divide into two cells and both or one of the cells may form a tube like structure (Fig. 8A. b) called primary suspensor tubes. The tubes become septate and much elongated and coiled, and penetrate the female gametophyte or endosperm (Fig. 8A, c). These primary suspensor tubes always move towards chalaza1 end (Fig. 8A. d, e). Embryo development starts at the tips of some of these primary suspensor tubes. The nucleus of the primary suspensor tube migrates towards the tip and divides into two unequal nuclei (Fig. 8B.a, b). The smaller one divides twice producing four cells. Subsequent divisions are irregular forming a mass of cells, upper cells of which give rise to the secondary suspensor (embryonal tubes) (Fig. 8B. c, d). Cells at the tip of the secondary suspensor are compact and form the embryo proper (Fig. 8B. e). The secondary suspensor becomes quite massive. Some cells elongate producing long tubes grow on the sides of the primary suspensor tube (Fig. 8B. f ; Vasil 1959).

Embryo Development: Although several primary suspensor tubes grow initially, but only a few continue to develop and the remaining embryos along with their primary suspensor system degenerate. The growing embryo exhibits an elongated mass of cells with distinct shoot apex at the tip. On the two sides of the embryonal mass tip, the cells divide actively to form two cotyledons covering the central zone of the shoot apex. The root tip is differentiated at the opposite end. The root cap cells are confluent with the massive secondary suspensor. A small protrusion so-called "feeder", appears in the region between the root and shoot apices. In the mature embryo the feeder is very prominent and is generally longer than the hypocotyl (Fig. 8C. a). Polyembryony is frequently recorded in *Gnetum* (Fig. 8C. b). Both, simple and cleavage polyembryony are observed. The cleavage polyembryony may occur due to the fragmentation in embryonal mass of the primary or the secondary suspensor tubes. Sometimes cells of the secondary suspensor may become meristematic and produce many embryos, at the tip.

In Most of the gymnosperms a cellular endosperm (female gametophyte) is formed before fertilization, but in *Gnetum* although the cellularization begins before fertilization, a part of the gametophyte remains free nuclear. Wall formation results in rnultinucleate compartments. The nuclei in each cell eventually fuse forming a single polyploid nucleus. Chalazal cells of female gametophyte undergo transverse divisions producing a distinct area called axial tissue. The upper part is gradually consumed by downgrowing suspensors, and later becomes compressed and crushed. The endosperm is rich in starch and oil droplets, and provides nutrition to the undifferentiated embryo which continues to grow even after the seed dispersal (Sanwal 1962).

8.1 Seed :

The seed in most species of *Gnetum* is oval and green to red coloured. Seeds are shed at a stage when the embryo is not fully formed. The nucellus represents a thin strip at the apex; the endosperm is massive, surrounded by a three-layered seed coat. There is always a time lag between seed shedding and seed germination. In *G. gnemon* seeds are shed in April and germinate in September, whereas in *G. ula* they take one year to germinate. The gemination is epigeal (Fig. 8D; Maheshwari and Vasil 1961).

Welwitschia

9. Male strobilus and gametophyte

9.1 Male strobilus

Fig.9A *Welwitschia mirabilis*: one complete strobilus and parts of two others: X5.2.ô After Church 1914 .

The male strobilus consists of dichotomously (2-4 times) branched stalks. The cones are covered by the opposite decussate bracts (Fig. 9A). The flowers push out beyond their subtending bracts at maturity. Six stamens are present on the rim of the staminate tube. A funnel-shaped tip of the integument of non functional ovule is present in the center of group of stamens. Thus the male flower is bisporangiate, having both male

and female parts, the latter one is non functional. Two opposite bracts alternate with two opposite bracts at right angles to them, throughout the entire cone, and in the axil of each bract, there is a single flower. The opposite decussate arrangement of bracts in the cone is continual in the four parts of the perianth of the flower. There are six trilocular stamens, one opposite each of the smaller bracts of the perianth, and two opposite each of the larger ones. The stamens are borne on a tube, and below each stamen is a nectary like protuberance. In the center is an ovule, with a well-developed nucellus, but never lack any sporogenous tissue. There is an inner integument, which elongates considerably, although it never reaches the extreme length of the inner integument of the ovulate flower. However, the integument of this sterile ovule has an expanded funnel-shaped tip, which has caused some to write about stigmas. Outside the inner integument is the perianth, consisting of four bracts: two at the ends, small and sharply angled at the midrib, and two at the sides, parallel with the large bract in the axil of which the flower stands. Thus, each flower is a strobilus in the axil of a bract, and the whole cone is a compound strobilus. The parts of the flower are in two's or four's. Each anther has three fused locules.

9.2 Microsporogenesis and development of Male gametophyte

Fig. 9 B . *Welwitschia mirabilis*: A, three-nucleate stage of pollen grain, X940; B, shedding stage, X600: ex, exine; in, intine; n^1, n^2 and n^3 interpreted respectively as prothallial, generative, and tube nuclei. ô .After Pearson 1929.

The microspore (male gametophyte) consists of two spore coats, exine and intine, which are of about equal thickness and usually become more or less split apart (Fig. 9B). It is evident that the first division forms a prothallial nucleus and an antheridium initial and the latter divides to produces a tube cell and a generative cell. Pollen grains are shed at this three-nucleate stage.

10. Female strobilus and gametophyte

10.1 Female strobilus

Fig. 10 A . *Welwitschia mirabilis*: A, flower stalk with ovulate strobili of various ages, one-half natural size.

The ovulate strobili look similar to staminate strobili are borne in considerable numbers on branching stalks (Fig. 10 A). The young strobili are green, but at maturity, thy turn to reddish brown. Strobili are covered with the symmetrically arranged bracts. A few of the lower bracts have no flowers in their axils, and the flowers in the axils of a few of the upper ones do not mature. Each strobilus produces from fifty to seventy flowers, but comparatively less numbers of seeds are able to germinate. Each consists of a central nucellus surrounded by two interments and then to lateral bracts. The inner integument (true integument) is prolonged to form a long micropyle. The outer integument, also called perianth, is two-lobed structure and it functions as wing and help in the seed dispersal.

10.2 Megasporogenesis and development of female gametophyte

Fig. 10 B *Welwitschia mirabilis*: A, late free-nuclear stage of female gametophyte: H, cell of nucellus; nt, megaspore membrane; B, early cellular stage, with most of the cells still multinuclear; at f, nuclear fusions have taken place until the cells are uninuclear; f, at bottom, shows that cells may divide after the fusions; a, in the middle cell at the left, shows that nuclei may divide before fusion; C, four of the cells, f, have become uninuclear and nuclear fusions are advancing in the rest.

Megaspore mother cells differentiate in the nucellus and one of them divides meiotically to form four nucleate megrapore. All four haploid nuclei take part in the formation of female gametophyte thus it is known as tetrasporic development. This megaspore enlarges and its four nuclei divide by free nuclear division and form nearly 1000-1300 nuclei. The nuclei are equally distributed throughout the gametophyte (Fig. 10 B) and there is no central vacuole. The wall formation occurs irregularly, often inclosing a multiple nuclei (10-12) in each cell especially at the lower region. Nuclei in each of these cells fuse together and form polyploid cells of the female gametophyte (Fig. 10 B). Archegonia do not develop in *Welwitschia*. Some cells of the female gametophyte at micropylar end, which are regarded as archegonial initials, elongate and grow up as tube like structures into the nucellus. They are called 'prothallial tubesø They grow about half-way through the nucellus, where they meet the pollen tubes coming down. The cells of female gametophyte keep dividing mitotically. This behavior stops before fertilization, but is resumed as soon as fertilization has taken place, a feature quite characteristic of angiosperms.

11. Pollination and Fertiliation

The pollination drop, characteristic of gymnosperms, ooze out at the tip of the projecting micropylar tube. Hooker presumed the insect pollination in *Welwitschia.* Baines found a parasitic insect, *Odontopus expunctidatus*, on plants. Pearson found the insect with pollen on its legs and abdomen on both male and female plants that might be effecting pollination. Pollen are shed at three-nucleate stage, which are caught in pollination drop and drawn down through the long micropyle to the nucellus. The pollen tube may begin to develop, even in the micropyle. The exine splits longitudinally throughout the entire length. In the young pollen tube the generative cell and the tube nucleus enlarge, and the prothallial nucleus can no longer be distinguished. There is no stalk cell. The nucleus of the generative cell soon divides, forming the two male nuclei, which lie in a common mass of protoplasm, with no wall between them.

Fig. 11 ô *Welwitschia mirabilis*: upper part of nucellus with prothallial tubes going up to meet the pollen tubes which will be coming down: t, prothallial tubes; s, sterile cells of upper region of female gametophyte; n, nucellus; m, megaspore membrane; X140.ô After Pearson 1929.

The pollen tubes grow downward into the nucellus and the prothallial tubes grow upward into the nucellus (Fig. 11). A pollen tube and a prothallial tube come into contact, their walls are fused and a swollen portion is formed, which is called fertilization bulb. The male nuclei of a pollen tube and nucleus of the prothallial tube move into the fertilization bulb, where one of the male nuclei fuses with female nucleus of the prothallial tube. The cytoplasm from the prothallial tube surrounds the zygote. The zygote nucleus moves down to the female gametophyte.

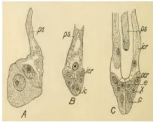## 12. Embryogeny and seed development

Fig. 12A *Welwitschia mirabilis*: early embryogeny: A, first division of zygote; ps,primary suspensor cell and, below it, the cell from which the embryo is to develop; B, later stage, with inner cortical ring (icr) and terminal initial cells (ic) ; C, still later stage; ps, primary suspensor; icr, cells of inner cortical ring; ocr, cells of outer cortical ring; the embryonic plate (e), ring (x), and cap (c), are each represented in section by two cells; A, X700; B and C, X305.ô After Pearson 1929.

Tho zygote elongates and its nucleus divides. The wall begins to form at the periphery and closes in toward the center. The wall is complete before another mitosis takes place, so there is no free nuclear stage (Fig. 12A). The upper cell elongates and form the primary suspensor. The embryo is derived from the lower cell. The primary suspensor cell elongates but does not divide. The inner cortical ring cells elongate immensely (icr in Fig. B and C of Fig. 12A), surrounding the primary suspensor cell. The outer cortical ring cells then behave like the inner cortical ring cells, elongating and surrounding the inner cortical ring cells, so that a transverse section shows the primary suspensor in the middle, surrounded by two cortical rings, the inner consisting of eight cells and the outer of sixteen. Later, third and fourth rings of cortical cells (x in Fig. 12A, C) are cut off and added to the suspensor. The cells (C) undergo division, forming a cap, which is cast off later. The stem, leaves, cotyledons and root are formed from the four cells of the plate (c, in C of Fig. 12A). As the embryo grows down into the endosperm, the suspensor becomes coiled and twisted in typical gymnosperm fashion and, after the cap has been cast off, the outer cells become dermatogen, and the meristems of the root and shoot are organized.

12.1 Seed and seedling

Fig. 12 B . *Welwitschia mirabilis*: seedling in greenhouse at the University of Chicago

Fig. 12C. *Welwitschia mirabilis*: same seedling as that in fig. 380, two years later

The seedling has two cotyledons and two leaves (Fig. 12B). The embryo has elongated hypocotyls and two cotyledons. At the lower part of the hypocotyl, a lateral outgrowth, called the feeder, acts as a haustorium until the food supply of the seed is exhausted. The cotyledons persist for two or three years and then fall off: the two leaves, at right angles to the cotyledons, persist throughout the life of the plant. The cotyledons and leaves are erect initially, but they begin to droop and the leaves become horizontal after one year (Fig. 12 C). In the axil of each cotyledon a bud appears and becomes flatter and broader until the two buds meet, forming a continuous plate, which covers the stem apex. Meanwhile, the tissues above and below the base of the leaves grow so that the leaf bases become inserted in a deepening groove.

13. Summary

Ephedra, Gnetum and *Welwitschia* are dioecious. In Ephedra a flower consists of a central axis on which a few bracts occur. In the axil of each bract a micro or megasporangiate flower arises. In *Gnetum,* the inflorescence is either solitary or a fascicled panicle. In *Welwitschia* the strobilus consists of dichotomously (2-4 times) branched stalks and the cones have flowers, which push out beyond their subtending bracts at maturity. In *Gnetum* and *Welwitschia* the male strobili have male (stamens) as well as female (ovules) parts, though the latter one in non functional. In *Welwitschia* the integument form long micropylar canal and its flared tip fom a stigma like structure. The pollen grain is a 5-celled in *Ephedra* at the time of shedding, but in *Gnetum* and *Welwitschia* they shed at the three-celled stage, comprising a prothallial cell, a tube nucleus and the spermatogenous cell. Its exine of pollen has ridges and furrows in *Ephedra*, with their number varying with species. The ovule in female flowers of three general is surrounded of two envelops (outer perianth and inner integument). The development of female gametophyte is monosporic in Ephedra, however, it is tetrasporic in *Gnetum* and *Welwitschia*. In *Ephedra* the mature archegonium contains a long neck an egg nucleus and a ventral canal nucleus, however in *Gnetum* and *Welwitschia,* the archegonia are absent and some free nuclei (in *Gnetum*) or some cells (in *Welwitschia*) of the female gametophyte function as acrhegonia. Pollination takes place through the agency of wind or insects. The pollen grains are caught in the pollination drop that later gets sucked in and the pollen grains come to lie on the gametophyte tip. Of the two male nuclei, one fuses with the egg nucleus forming the normal zygote nucleus. The second male nucleus fuses with the ventral canal nucleus (in *Ephedra*) or one of the other free nuclei (in *Gnetum*) of female gametopphyte to form the supernumerary zygote nucleus, thus causing double fertilization, resulting in two zygotes. Only one or two zygotes reach maturity, whereas the rest degenerate In *Ephedra* zygote nucleus divide to form eight proembryos in an archegonium, but only one, which is centrally placed develops to maturity. Both simple and cleavage polyembryony are recorded.The embryo is dicotyledonous. Seed germination is epigeal.

Some of the characters which these three genera share are a) the male and the female reproductive parts borne on fertile shoots resembling flowers, arranged in compound strobili, b) better protection of the ovules as compared to other gymnosperms, and c) formation of a micropylar tube by the prolongation of the integument.. The development of the female gametophyte is monosporic in *Ephedra,* whereas it is tetrasporic in *Gnetum.* The female gametophyte in *Ephedra* becomes cellular before fertilization takes place, whereas it is partly free nuclear in *Gnetum* at that stage. A "tent pole" is present in many species of *Ephedra* but is absent in *Gnetum,* except for *a* rudimentary structure in *G. africanum.* Archegonia are formed in *Ephedra,* whereas they are absent in *Gnetum.* The formation of archegonium is completely suppressed in both groups. Some free nuclei of female gametophyte act as eggs in *Gnetum.* vi) In *Gnetum,* though the cellularization starts prior to fertilization, it is complete only after fertilization and is haploid. vii) The zygote does not undergo any free nuclear division. Based on the above apparent resemblances it is regarded that angiosperms passed through some stage during the evolution which is now presently shown by *Gnetum.* Pollination appears to be partly, if not entirely, due to insects. The division of the spore-mother-cell is of the "simultaneous" type. No pollen-chamber is formed. The development of the microsporangium proceeds along very similar lines in the three genera *Ephedra*, *Gnetum*, and *Welwitschia*. In the germination of the microspore *Ephedra* reveals its gymnospermous affinity, while *Gnetum* and *Welwitschia* show a greatly reduced prothallus. While in the unicellular archesporium of the macrosporangium *Welwitschia* agrees with *Ephedra*, in the later stages of the embryo- sac and in the early condition of the prothallus closer affinity between *Gnetum gnemon* and *Welwitschia* is indicated. But in the septation of the micropylar end of the sac and in the production of the prothallial tubes, *Welwitschia* possesses characters which are quite distinct from anything known in *Gnetum*.