LIFE-HISTORY OF CYCAS CIRCINALIS L.

Part V. Seedling Anatomy

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ABSTRACT

The paper deals with the germination of the seeds of Cycas circinalis L., the growth and structure of the different parts of the seedling in the stem, root, cotyledons and plumule. The anatomy of four different types of roots, is given. The anatomy of the stem, cotyledonary plate, procambial strands and their orientation in the stem and root are described. The origin and development of stomata found on the hypocotyledonary region of the embryo are given in detail. The cotyledons, their vasculature and final fate are described.

MATERIALS AND METHODS

Cycas seeds were germinated in the garden and their various parts were collected and fixed in different stages of development. The material was subsequently embedded in wax and microtomed. Sections were cut in the transverse, longitudinal tangential, longitudinal radial, and paradermal planes. They were stained in Iron-Alum Haematoxylin.

OBSERVATIONS

As the ripe seeds of Cycas are shed by the mother plant their fleshy coat gradually deteriorates and exposes the hard shell.

During germination, the coleorhiza splits the shell and peeps out through the rupture (Figs. 30 and 11). The emergence of coleorhiza and its further growth result in the breaking and deterioration of the attached suspensor (Fig. 41). The coleorhiza now becomes free and occupies the terminal end of the radical pole of the embryo which is straight and not bent as reported by Sloover (1964). The root-tip develops from behind the coleorhiza and gradually digests and destroys it (Fig. 39). Thus the coleorhiza is only a temporary structure preparing the way for the formation of the root-tip.
which is at first protected by the coleorhiza. Ultimately the root-tip enters the soil and the elongated radicle turns into the tap root.

Well developed stomata in the enclosed embryo of *Cycas revoluta*, *Macrozamia* and *Stangeria* have been described by Pant and Nautiyal (1962) and they appear to be confined to the hypocotyledonary region, *i.e.*, towards the base of the cotyledons, and lie coinciding with the length of the embryo. They have shown the cuticle, stomatal outline, their distribution and orientation out of macerated embryo of *Cycas*. In *C. circinalis* microtome sections shows that the hypocotyl is short and well developed stomata are found on all exposed parts of the embryo, *i.e.*, the coleorhiza and the root and very few on the cotyledons.

The stomatal development in the seedling is just like that of the foliar stomata of *C. circinalis*. (Pant and Mehra, 1964). An examination of the epidermis of the coleorhiza shows that the spherical stomatal mother cells divide to give rise to two daughter cells which become the guard cells (Figs. 1–5). The stomatal aperture appears later when the guard cells assume their final shape (Figs. 1–5 and 27 a). The stomata are haplocheilic (Florin, 1931).

Median, submedian and polar transverse sections of the stomata present different shapes of the guard cells (See Figs. 6–9 and 24 a and b). The guard cells are situated at the bottom of a pit (Figs. 7–9) which appears squarish in a surface view. The surrounding subsidiary cells may have another ring of encircling cells around them.

At the time when the root grows into soil the seedling shows well developed cotyledons, hypocotyl and plumule. The cotyledons are embedded in the endosperm but their petiolar portion are projecting out of the shell. Between the petioles can be seen the plume, the stem apex and the young leaves (Figs. 11 and 30). The stem apex is broad and circular and new leaves are formed all round its margin. The top portions of the cotyledons are fused (Figs. 13–18). Dorety (1909) states that *Microcycas* is peculiar in the extensive fusion of its cotyledons. This is true of *C. circinalis* also. The fusion is so complete that the abutting epidermal layers disappear and do not show even the "seam" which is characteristic of such fusion (Fig. 24 c).

At the junction of the base of the cotyledons, and the hypocotyl which is very small, can be seen a number of lenticels forming a ring all round the hypocotyl (Fig. 30 a). Above this ring of lenticels are the two opposite bases of the cotyledons, and these persist even after the cotyledons have disappeared.
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on the depletion of their reserves. The next pair of leaf-bases is formed at right angles to the cotyledonary bases. The lamina of these leaves is not developed and they are covered by ramenta. Then follows the bulbous swelling of the seedling due to subsequently formed leaf bases developed in spiral manner inside the first pair. The leaf bases are thick with rich storage of starch and cover the central xylem cylinder (Figs. 15 and 35 b).

Seedling anatomy of several cycads has been worked out by many workers like Thiessen (1908), Dorety (1909), Hill and De Fraine (1909), Chamberlain (1917). Thiessen (1908) found that the vascular bundle of the stem in Dioon edule is very short and he prefers to call it vascular plate instead of a vascular cylinder. It is squarish with protoxylem groups at each corner from which a strand extends downwards forming the protoxylem of the tetrarch root. Four strands extend in the opposite direction to the cotyledons. According to Angell (1909) the embryo vascular plate from its first appearance, is an endarch siphonostele, probably the only siphonostele in the early stages of a cycad seedling. Hill and De Fraine (1909) conclude that the number of vascular bundles at the base of each cotyledon ranges from 2 to 8 and they are mesarch and become exarch in the root. The transition phenomenon occurs so rapidly that most of the hypocotyl shows root structure. However, the following points have been observed and recorded in C. circinalis seedling anatomy.

When the root-pole of a seedling is bending towards the earth, section from the junction of its epicotyl and hypocotyl regions shows all the cells at the center to be pro-meristematic and constituting the future cotyledonary plate. It appears to be protostelic to begin with, which later on at a higher level becomes siphonostelic (Figs. 26 and 28). As the hypocotyl elongates, the pro-meristematic tissue organises a young cotyledonary plate consisting of partly meristematic and partly parenchymatous cells the latter cells occupying the center (Figs. 26 and 28). This is the beginning of the siphonostelic condition of the future stele. The oval cotyledonary plate with the meristematic cells organises a few (8-10 or fewer) pro-cambial groups all along its margin which later on grow out as pro-cambial strands (Figs. 10 and 28). Some of them divide on their way up the cotyledons and become the cotyledonary strands (Fig. 24 a). They appear to diverge in all directions (Figs. 32 and 12). First they take a downward bend and then take an outward and upward direction and enter the cotyledons which are free at this level of the embryo (Figs. 15-17 and 39). During their growth the pro-cambial strands are transformed into xylem and phloem which take up their relative position in the future vascular bundle (Fig. 19). Usually there
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Figs. 13–23
are seven vascular bundles in each cotyledon. The differentiation of the procambial strands starts from the centre of the cotyledonary plate towards its periphery. It is an endarch vascular bundle that starts from the cotyledonary plate which gradually becomes mesench as can be seen in vascular bundle of the cotyledons (Figs. 19 and 29).

At the protostelic stage of the cotyledonary plate (Figs. 25 and 34), i.e., a stage previous to the siphonostelic stage the oval cotyledonary plate give rise to two pro-cambial strands one at each pole towards the apex of the root, which become the vascular traces of the root-tip (Figs. 39, 41 and 12). The proto-xylem of this trace is exarch to begin with, while that of the cotyledon is endarch. The change from endarch to exarch position takes place in the short length of the hypocotyle (Figs. 20-23). Thus the root gets its diarch vascular supply and becomes the tap root of the old seedling (Figs. 39 and 35). The first pair of lateral roots developed on the tap root at the level of the soil or just below it, become corollloid roots and are apo-geotropic. The tap root at this stage is much bigger and stouter than the stem which is very inconspicuous. It is diarch and develops secondary thickening giving the young root the characteristic appearance when seen in a transverse section (Fig. 36).

The first and second leaves which appear as conical elevations from the centre of the stem apex are supplied from the upper side of the vascular plate and the traces are sent up which go to the first and subsequently formed leaves. First, second and third leaves are supplied by three vascular traces which subsequently divide into a number of leaf traces (Figs. 15-17 and 41). All the above leaf and cotyledonary traces are derived from the vascular plate and directly pass on to the respective organs without making a loop or girdle as has been otherwise described in other cycads specially Dioon (Chamberlain, 1917). At this stage of investigation of Cycas circinalis it is only possible to state that the vascular supply to the first few leaves is direct and does not take a loop. The diagrammatic figure of the vascular supply of the seedling with the first and second leaves clearly illustrates the above point (Fig. 12). There is no girdling of the leaf traces in younger stages, the leaf traces being vertical but with radial increase of enclosed leaves and stem tip, the girdling becomes apparent.

No extensive study of the cycad root has been undertaken (Chamberlain, 1919). There appears to be a tap-root which tapers gradually and extends down to a great depth, so that small amount of water is brought up even when the soil surface is very dry. Lateral branches are usually small and weak,
In *Stangeria* there is a strong main root with weak branches. In *Cycas circinalis* the tap root is strong but the lateral roots are much branched and weak. The tap root in some cases before giving rise to first pair of lateral apo-geotropic and corolloid roots, gives rise to a number of swollen adventitious roots. They arise above the apo-geotropic roots and below the ring of tentacles at the hypocotyledonary region (Fig. 31). They vary in number from one to six and are endogenous. Further, these adventitious roots become swollen with storage of water and other soluble food materials and hence are translucent or pearly white in colour. They look like radish (*Raphanus sativus*) without the vertical row of root-lets. They taper to a point and grow down deep into the soil. They bear lateral rootlets very sparingly. The rootlets are not arranged in vertical rows whereas the taproot displays the vertical rows. Such swollen lateral roots have not been observed so far in *Cycas circinalis*. However, Bryan (1936) has described fascicled roots in *C. revoluta*. From the description one could make out that the swollen lateral, roots of *C. circinalis* differ from the fascicled roots of *C. revoluta*, in size, place of origin, number per tap-root and anatomy as well (Rao, 1971). Anatomically the swollen roots of *C. circinalis* as seen in four year old plants have a thick cortex and a central cylinder which is one fifth the diameter of the cross-section. The cortex is made up of hexagonal, slightly elongated, thin walled cells with intercellular spaces. The central cylinder has a pith and pentarch xylem with protoxylem being exarch. Thus, the swollen roots differ in anatomical features from the diarch tap-root.

Rao and Murthy (1937) state that the diarch or tetrarch condition of the roots of *Cycas* is not dependent upon the algal infection but upon the level of the root in the soil, the roots at a deeper level showing the diarch condition and those near the surface showing tetrarch condition. This feature is not confined to the primary root alone but even to the all secondary roots.

The study of the roots of *Cycas circinalis* reveals that there are four different types of roots in a *Cycas* plant specially at seedling stage. (1) Main root or primary root or the tap root, (2) Lateral roots or the secondary roots, (3) Corolloid roots, and (4) Swollen roots or fleshy roots. The tap root shows the diarch vascular cylinder throughout its length, and its diameter decreases from base to the tip (Figs. 30, 31 and 12). The thin and wire-like lateral roots are also diarch at the time of their emergence from the tap-root and they maintain the same feature upto the tip. They arise on the tap-root roughly in two vertical rows and branch very sparingly. Corolloid roots take their origin on the tap-root as a single unbranched root just below the hypocotyl and are situated on either side of it (Fig. 35 c). The unbranched
root before it dichotomously branches, shows diarch stele while after branching the vascular cylinder shows tri- or tetra and even pentarch condition. The question of depth of the soil does not arise at all in this case, since the corolloid roots are found just below the soil level. In the case of tap root and the lateral roots, though they penetrate deep into the soil, the vascular cylinder remains only of diarch nature. The algal infection does not take place at all. In the ultimate fine tips of the lateral roots, however, the vascular elements are reduced in number and are found scattered due to permeation of parenchymatic cells (Figs. 36 and 37). Even from embryogeny, it is noticed that only two pro-cambial strands proceed downwards from the vascular plate to the root apex, which finally become the vascular cylinder of the root with exarch bundle, while the stem apex gets many pro-cambial strands. The fourth type of roots are the swollen or fleshy roots and they are not found in every seedling. Their anatomy is peculiar in that they start from the diarch stele of the tap root and from the very beginning they are polyarch and finally end in tri- or tetra-arch. The polyarch nature is perhaps partly due to the special function of the roots, viz., storage and partly due to their swollen condition. The anatomical features of the swollen roots are diffuse secondary growth of parenchyma in the pith and in the secondary xylem and the consequent splitting up of the central cylinder into many parts leading to polyarch condition. This appears to have been brought about by a proliferation of the parenchyma among the vascular elements and a massive development of parenchyma, some of its cells being differentiated into vascular elements. Generally the roots contain more parenchyma than the stems.

The adventitious swollen roots are not found on seedlings of one or two years of age. They are generally found on seedlings which are 3 to 5 years old, grown in soil under natural conditions of Bangalore. Further the seeds of Cycas germinate on the ground and send roots down the soil. The germination is thus epigeal. The stem and the epicotyl develop more above the soil than in the sub-soil. This above ground position may not be congenial for the growth of the plant and its water requirements in a dry soil where Cycas generally grows. Further, a careful examination of the swollen roots not fully saturated with water, i.e., under dry conditions, shows wrinkles or furrows on the surface running horizontally. Median longitudinal sections of roots with furrows were examined and surprisingly enough the structure revealed horizontal bands of contracted cells alternating with bands of normal cells. This function of contracting roots generally helps the tuberous stem to get itself embedded well in the soil which is essential at the vulnerable stage in the plant's life-history.
Dorety (1908) states that when roots of Coratozemia pierces the soil, the starch is transferred to it from the endosperm and the root thickens into a tap-root. By its further penetration into the soil, it often draws the upper portion further down embedding into the soil and possibly giving the first series of lateral roots their initial upward slant. The lateral roots almost always appear in groups of three whether the root is tetrarch or triarch. The swollen roots of *Cycas circinalis* having special contractile tissue pulls down the tuberous stem to the soil more efficiently than the tap-root.

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**REFERENCES**


EXPLANATION OF FIGURES

Figs. 1–12. Fig. 1. Spherical stomatal mother cell with two nuclei. Fig. 2. The same with cell partition formed. Fig. 3. The same with cell partition being laid down. Fig. 4. Stoma with 2 guard cells formed. Fig. 5. Stoma with 2 guard cells, aperture fully developed with a ring of accessory cells. Figs. 6–9. Sotmatal sections taken at different planes. Fig. 10. Cotyledonal plate showing vascular elements being organised and vascular bundles going out to the cotyledons. A = Vascular bundle with xylem—phloem (Dotted), B = Vascular bundle going out of the cotyledonal plate, C = Pith, D = Cortex with mucilage canal. Fig. 11. An enlarged and diagrammatic view of seed of Cycas circinalis showing the seed coats. A. Disorganised nucellar beak, B. Endosperm, C. Embryo, D. With the suspensor still attached to coleorhiza, E. Archegonial cavity F. Cotyledons and plumule seen through the split H. Fig. 12. An enlarged diagrammatic sketch of the embryo showing radicle and portion of the cotyledons. Cotyledonal plate A. I and II leaves B and C Root-traces at C. Vascular bundles to the cotyledons going out from the cotyledonary plate D (Nearer ones in black and distant ones in dotted lines). Split coleorhiza seen F and root-tip meristem G. Mucilage ducts at H. Pith I.

Figs. 13–23. Figs. 13–17. Transverse section of the embryo passing at different levels starting from below the cotyledonal plate. Fig. 13. Two cotyledons are fused. The fused margins are seen at Am. Precambial strands are formed and found diverging out as vascular bundles. Section along 1-1 of Fig. 11. Fig. 14. Two cotyledons are still partly fused. Vascular bundles of the cotyledons and the first two leaves are also seen. Fig. 15. The two cotyledons are separate. The plumule with young leaves can be seen through the split. Vascular traces of the cotyledons and the leaves can be seen. Youngest leaf has number of hairs. Section passing along 2-2 of Fig. 11. Fig. 16. Same as above, the young leaf is covered over by hair. Fig. 17. The tip of the 3rd leaf is seen with 3 vascular traces and covered over by hairs. There are generally seven vascular bundles in each cotyledon and they are endarch. Fig. 18. The two cotyledons fused again at a higher level so that even the line of fusion and "Seem" has disappeared. The fused margin is visible to some distance above and then disappears. Section taken along 3-3 of Fig. 11. Fig. 19. Portion of Fig. 10 magnified to show the nature of the vascular bundles—protoxylem A, Metaxylem B, Phloem C, Pith D. M = Mucilage canal. Fig. 20. Section of the young radicle, showing the way the vascular strands orient to change, their position from Endarch to Exarch. Figs. 21, 22, 23. Shows details of the orientation. Px = Protoxylem MX = Metaxylem.

EXPLANATION OF PLATES

PLATE III

Fig. 24 A. Transverse section of a stoma showing the guard cells and accessory cells, × 160.

Fig. 24 B. Longitudinal section of a stoma, × 160.

Fig. 24 C. Two cotyledons of the embryo fusing together and even the "Seem" disappearing. A-A. × 80.

Fig. 24 D. Cotyledonal bundle dividing into three parts at A, × 32.

Fig. 25. Cotyledonal plate just below the hypocotyl showing the proto-stelic condition with two diarch bundles being organised A.A. The tanin cells form a ring all round the stele. B. Mucilage Cells in the cortex C, × 64.

Fig. 26. Section passing at a higher level of the cotyledonal plate showing the Siphonostelic condition. Pith A. Vascular bundles being organised all round the pith B.B. and going out as the cotyledonal bundles, × 64.
Figs. 24–29
FIGS. 30–41
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**Fig. 27 A.** Surface view of the epidermis of the embryo specially at the hypocotyl region showing two stomata, × 80.

**Fig. 27 B.** Tetrarch condition of the stele of a Swollen root of cycas at m, × 16.

**Fig. 27 C.** Triarch condition of the stele of coralloid root branch. Algal layer is seen at, × 64.

**Fig. 27 D.** Apical meristematic region of the root of Cycas, × 80.

**Fig. 28.** Younger stage of cotyledonary plate showing the pitch in the center A and procambial strands being organised into vascular bundles of the cotyledons B.B, × 64.

**Fig. 29.** A single vascular bundle of the cotyledonary plate enlarged to show Protoxylem A, Metaxylem B, Phloem C, Phloem fibres D, × 360.

**Plate IV**

**Fig. 30.** Cycas seeds germinated with the radicle growing out. A = Circle of lenticles, B = Base of the cotyledons united below and above the split C. The plumule and the 1st leaf peeping out. Note the future split and the line of fusion at D.

**Fig. 31.** Cycas seedlings showing 4 different types of roots.

**Fig. 32.** Cotyledonary plate showing the divergence of the vascular strands going to the cotyledons above and meristematic tissue of the root pole, × 32.

**Fig. 33.** Cotyledonary plate at a higher level than the one shown in Fig. 28. The diverging vascular bundle is bifurcating A, × 32.

**Fig. 34.** Cotyledonary plate at a lower level than the one shown in Fig. 25. The diarch nature of the stele at A. Tannin cells form a circle B. Mucilage ducts at C. Root-let trace is formed at D, × 32.

**Fig. 35.** Long section of an young seedling. Armour of leaf-bases A. Central cone of stem tip B. Main Root with lateral coralloid roots C.C. Present years leaf stalk D.

**Fig. 36.** Transverse section of the main roots just below the hypocotyl region. Secondary xylem A. Protoxylem B. Cortex C. Secondary phloem D, × 32.

**Fig. 37.** Transverse section of the main root taken at 9 inches below the soil. Protoxylem A.A. Secondary Xylem B.B. Pith with scattered xylem elements. Secondary phloem C, × 32.

**Fig. 38.** Same as Fig. 28. Two cotyledons with number of vascular bundles AA. first, second, third leaves with their vascular strands, × 25.

**Fig. 39.** Longitudinal section of the young embryo below the cotyledons. Vascular plate giving rise to three traces above going to the cotyledons AAA and two vascular traces BB going down towards the root meristematic region. The root tip C and the broken coleorhiza can be seen at DD, × 25.

**Fig. 40.** Transverse section of the root—Two exarch protoxylem groups at A.A. Separated by the scattered xylem elements and parenchyma cells, surrounded by phloem B.B, × 64.

**Fig. 41.** Longitudinal section of the young Embryo showing stem apex, young leaves, cotyledonary plate giving vascular traces to the leaves above and to the root below. The embryo is still attached to the suspensor since it is younger to that shown in Fig. 39, × 10,