

STUDIES ON PALMS: EMBRYOLOGY OF *PHOENIX ROBUSTA* HOOK.

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ABSTRACT

The paper gives an account of the embryology of an endemic species of *Phoenix* occurring in a corner of Deccan Trap area in the Western Ghats at Mulsi, a taluka in Poona District. It deals with the morphology of male and female flowers, pollen grains, ovule, development of male and female gametophytes, endosperm and embryo in that species.

The tree trunk is 5-7 m high, tessellated as in *Cycas* due to comparatively small size of the old leaf-sheath scars. The plants are dioecious. The pollen grains are round, monocolpate, smooth and thick-walled. They are shed at 2-celled stage. The ovules are bitegmic, crassinucellate and anatropous. Fertilization is porogamous. Endosperm is free-nuclear to begin with but becomes cellular later.

The embryo develops in two ways; according to (1) *Geum* variation and (2) *Polygonum* variation of the Asterad type. Micropyle is terminal in early stages, but shifts to the side of the seed wall later. Previous literature, etc., on the genus has been cited in the previous paper by Mahabale and Biradar (1968) and, therefore, is not given here.

OBSERVATIONS

THIS endemic species was first collected by Hooker (1892) on Parasanath Hills in Bihar, and later by Woodrow (1897) who gave account of it in greater detail. It invited the attention of Talbot (1902) and Brandis (1921), but it was Blatter (1926) who described it again further. In Peninsular India the species is confined to Trap Hills of the Western Ghats around Poona, and Nandgaon (Dist. Nasik) in Maharashtra, and to Parasanath Hills in Bihar. In Poona District it grows at Mulsi on the border of a lake and is highly restricted in its distribution here to an altitude of about 1,000-1,667 m very rarely elsewhere below 667 m. It is abundant between 1,000-1,667 m and forms dense population (Pl. I, Fig. 1). As remarked by Mahabale and Parthasarathy (1963), it probably owes its unique discontinuous distribution to

historical and edaphic factors, and hence has become endemic. Locally the species is known as *Shelu* (in Marathi) and its leaves are used as fodder, or for making brooms. The dark black fruits are eaten by the neighbouring people, being quite sweet.

Morphology.—The trunk of this graceful species is 5-7 m high, 38 cm in diameter and tessellated due to small size of the old adpressed leaf-sheaths on the tree-trunk, which leave diamond-shaped marks on it when fallen as in *Cycas circinalis* (Pl. I, Fig. 2). This is a distinctive character of this species. The light green leaves are 1-2 m long, glabrous, shining, short, thin and smooth. Their basal part 38×12 cm is triangular as in *P. sylvestris*, but the leaflets are quadrifarious and strongly conduplicate.

The inflorescence consists of numerous interfoliar separate male and female spadices, and are borne on different plants. The spadices are 20-25 cm long before opening. The spathes are of about the same length, very coriaceous, almost woody and scurfy. Each of them separates into two boat-shaped valves which are covered all over with dense wooly tomentum. The spathes are persistent. Spikes are numerous towards the anterior face and are simple, 10-12 cm long, slender, flexuose, narrowed at the tip which may or may not bear a flower (Pl. I, Fig. 6).

Male flowers 0.5-1 mm long are alternate, solitary and pale yellow. There are six stamens and three pistillodes in male flowers as in *P. sylvestris* (Mahabale and Biradar, 1968). Filaments are very short and anthers adnate and linear.

The female spadix and spathes are similar to those in the male. The spikes are arranged in distinct groups, 20-30 cm long. Female flowers are distant and round (Pl. I, Fig. 6). Perianth leaves are brown, or greenish-white, the outer ones being shorter than the inner ones. Carpels are three, free and erect. Ovules are solitary. Fruiting peduncle is 0.75-1 m long, smooth, yellow, round, compressed by secondary axes. The peduncle is nodding at the apex due to weight of the fruits. The fruits are sessile, ovoid, cylindrical, 2-3 cm long, 1-1.5 cm thick and apiculate. They are smooth, fleshy and dark black and sweetish when ripe.

Microsporangium, microsporogenesis and male gametophyte.—These are similar to those in other species of *Phoenix* described by Mahabale and Biradar (1968) and Biradar (1968).

Pollen grain and its germination.—The pollen grains are monocolpate, round or oval, smooth and thick-walled. They measure $15 \times 13 \mu$. The

colpus extends as a narrow longitudinal slit as seen in other species (Pl. I, Fig. 3). They germinate readily in 1% sucrose solution with the addition of 0.0001 IAA and Gibberellic acid as in *P. sylvestris* rather than on other media. They start showing signs of germination within an hour, the maximum growth taking place in three hours. The mode of germination was the same as in other species of *Phoenix* observed by Mahabale and Biradar (1968).

In the early stage of germination pollen grains swell considerably. Pollen tube emerges through the colpus and grows for some time and reaches its maximum growth (Text-Figs. 1-7; Pl. I, Figs. 4-5). Its tip bursts releasing contents. Elongation of the pollen tube in this species is comparatively less than in *P. sylvestris*. As a rule the vegetative cell never enters the pollen tube. It degenerates in the grain itself. The generative cell migrates into the pollen tube. The male cells formed are of equal size.

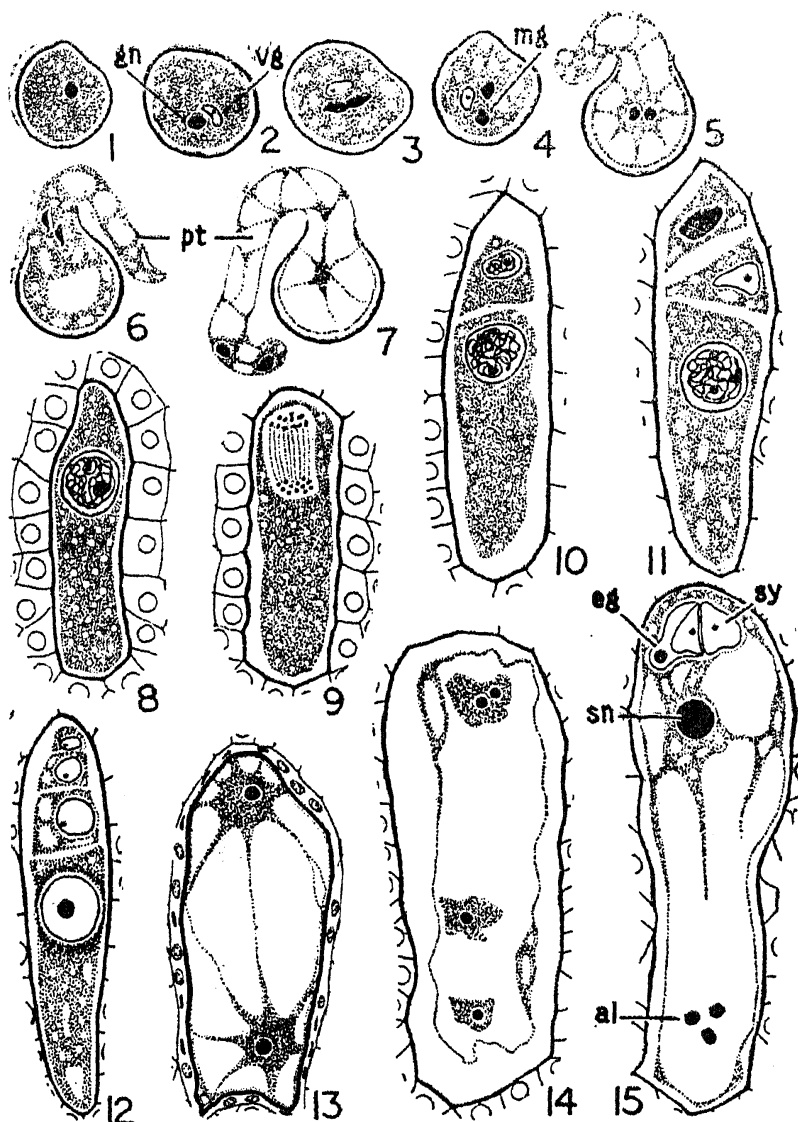
Ovary and ovule.—The ovary is tricarpellate, trilocular, superior, having one ovule in each loculus. Ovules are bitegmic, crassinucellate and anatropous. Micropyle is formed by both the integuments. The outer integument is 5-7-layered and the inner 3-4-layered. The funicular vascular strand ends at the chalazal end of the ovule. It does not send any vascular supply to the integuments.

Tannin is deposited in the inner integument first, covering the whole endostome in mature embryo-sac. It, however, does not close the endostome before fertilization as in *Elaeis guineensis* observed by Kajale and Ranade (1953). Exostome also secretes tannin at maturity. The micropyle, formed by both the integuments, is closed soon after the fertilization due to increased deposition of tannin. The tannin deposition first occurs in each ovule in the chalazal region and extends towards the micropyle encircling the embryo-sac. Its pronounced development is mainly in the chalazal region.

Integumentary tapetum is conspicuous as in *P. sylvestris*, *P. acaulis*, *P. pusilla* (Mahabale and Biradar, 1968; Biradar, 1968). The cells of the innermost layer of the inner integument are palisaded and rich in cytoplasm. In fertilized flowers the integumentary tapetum becomes cutinized owing to deposition of tannin. All the three ovules develop mature embryo-sac, but only one develops into fruit after fertilization.

Megasporogenesis and female gametophyte.—The ovule is crassinucellate and archesporial cell hypodermal. It is conspicuous, due to its large, wedge-shaped appearance, dense cytoplasm and prominent nucleus. It divides periclinally to produce a parietal cell and a primary sporogenous cell.

The primary sporogenous cell does not undergo any division and directly acts as the megaspore mother cell (Text-Fig. 8). The parietal cell divides anticlinally and periclinally to form parietal layers.



TEXT-FIGS. 1-15. *Phoenix robusta* Hook. Development of male and female gametophyte. Figs. 1-12, $\times 750$; Figs. 13-15, $\times 476$. Fig. 1. A microspore. Fig. 2. The same with a vegetative cell *vg* and generative cell *gn*. Fig. 3. The same showing lenticular generative cell. Fig. 4. The same showing vegetative cell *vg* and 2 male gametes *mg*. Fig. 5. Pollen tube formed showing 2 male gametes in the centre. Fig. 6. Male gametes entering pollen tube *pt*. Fig. 7. Two male gametes at the tip of the pollen tube, *pt*. Fig. 8. L.S. of ovule showing megaspore mother cell. Fig. 9. L.S. of ovule showing anaphase division in megaspore mother cell. Fig. 10. Dyad: The micropylar cell is smaller than the chalazal. Fig. 11. Triad. Fig. 12. Linear tetrad. Fig. 13. 2-nucleated embryo-sac. Fig. 14. 4-nucleated embryo-sac. Fig. 15. Mature embryo-sac showing egg, *eg*, two synergids—*sy*, secondary nucleus—*sn* and 3 antipodal nuclei—*al*.

A fully developed megaspore mother cell is tapering and elongated. Its nucleus lies in the micropylar half (Text-Figs. 8-9). Degeneration of the

nucellar cells starts at this stage and becomes pronounced at the chalazal end. Text-Figures 8 and 9 show early drophase and anaphase in the megaspore mother cell. At the end of the first division of the nucleus in megaspore mother cell, two nuclei get organised and a cell plate appears resulting in a dyad, with bigger chalazal cell and smaller micropylar cell (Text-Fig. 10). This is followed by a second transverse division in each dyad cell resulting in a linear tetrad of megaspores. The three micropylar ones are smaller than the chalazal (Text-Fig. 12). Sometimes a second transverse division is omitted in the micropylar dyad and only a triad is formed instead of a tetrad (Text-Fig. 11). Usually the chalazal megaspore is functional and gives rise to mature embryo-sac. The three micropylar cells degenerate.

The functional chalazal megaspore enlarges crushing the surrounding nucellar cells. Its cytoplasm becomes highly vacuolated and nucleus shifts to the centre of the embryo-sac. This division of the nucleus is followed by polarisation of nuclei forming primary micropylar and primary chalazal nuclei (Text-Fig. 13). They divide to form 4-nucleated embryo-sac. At the 4-nucleated embryo-sac stage, the two micropylar nuclei lie side by side and the chalazal nuclei lie one above the other (Text-Fig. 14). The nuclei in 4-nucleated embryo-sac divide and form 8-nucleated embryo-sac. The 8-nucleated embryo-sac shows a group of micropylar and chalazal quartet. The micropylar quartet forms the egg, two synergids and an upper polar nucleus. The lower quartet forms three antipodal nuclei and the lower polar nucleus. The two polar nuclei fuse and form the secondary nucleus (Text-Fig. 15; Pl. I, Fig. 7).

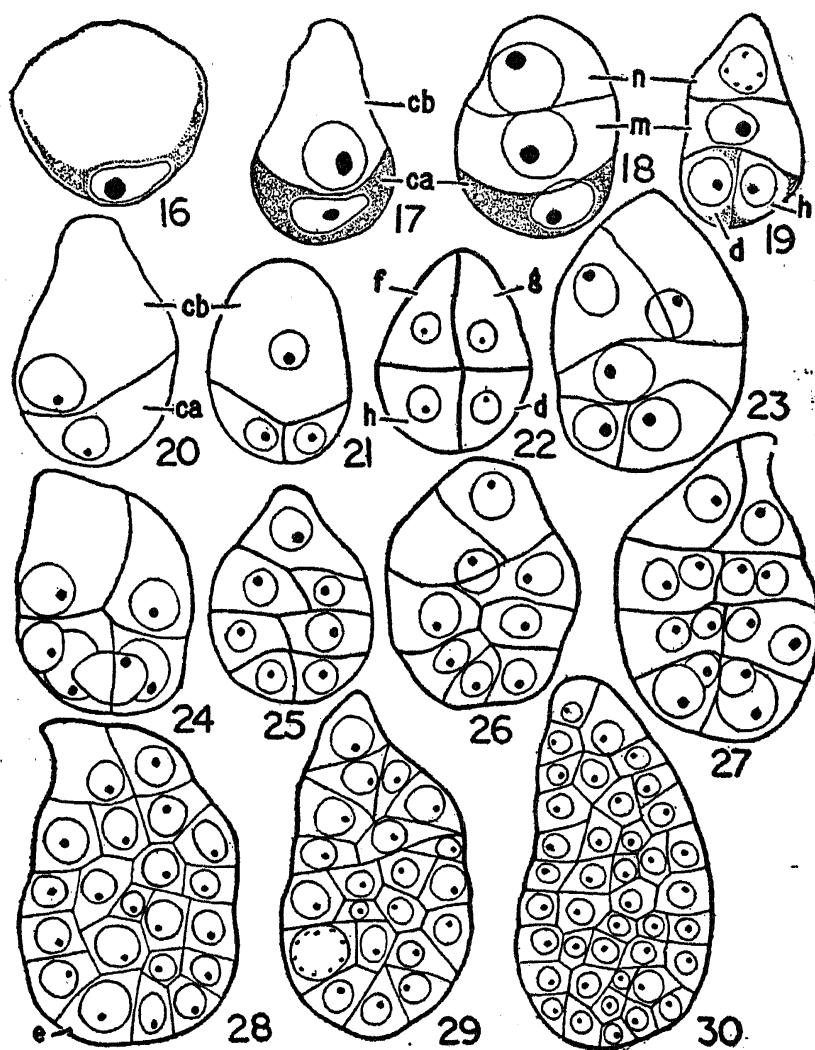
The antipodal cells are ephemeral. They are not organised into cells and degenerate before fertilization of the egg. They remain distinct as three nuclei upto the late mature embryo-sac stage (Text Fig. 15; Pl. I, Fig. 7). The development of female gametophyte on the whole thus conforms to the monosporic 8-nucleated *Polygonum* type.

Fertilization and endosperm.—These are similar to those in other species of *Phoenix* described by Mahabale and Biradar (1968) and Biradar (1968).

Embryo.—It is rather interesting to note that two different variations of the Asterad type of the embryo development were noticed in this species—(1) *Polygonum* variation; and (2) *Geum* variation of Johansen (1950).

The first division of zygote is transverse forming 2-celled proembryo of which the basal cell *cb* is longer than the short terminal cell *ca* (Text-Figs. 16, 17, 20; Pl. I, Figs. 8–9). In the *Polygonum* variation the division in the cell *cb* is transverse resulting in cells *m* and *n* (Text-Figs. 17–18, Pl. I, Figs. 9–10).

Normally the cell *ca* is lenticular and is full of cytoplasm (Text-Figs. 17-18, Pl. I, Figs. 9-10). The cell *ca* divides vertically forming *h* and *d* cells. This results in 4-celled inverted "⊥"—shaped proembryo (Text-Fig. 19; Pl. I, Fig. 13). In the *Geum* variation the basal and terminal cells divide vertically forming 4-celled proembryo consisting of cells *f*, *g*, *h*, *d* (Text-Figs. 20-22). The cell *h* of the lower tier which is comparatively bigger, divides obliquely forming cells *e* and *r*. Cell *e* forms the epiphysial cells, the seat of shoot apex in later stages (Pl. I, Figs. 11-12). Thus both the basal and terminal cells take part in the formation of a mature embryo. From the mode of cell divisions of zygote and subsequent stages of it, the embryo deve-



TEXT-FIGS. 16-30. *P. robusta* Hook. Embryo. All figs, $\times 750$. Fig. 16. Zygote. Fig. 17. 2-celled proembryo with lenticular terminal cell—*ca* and long basal cell—*cb*. Fig. 18. 3-celled linear proembryo showing terminal cell *ca*, and *m* and *n* cells of *cb*. Fig. 19. 4-celled proembryo showing "⊥"-shaped condition, having *d*, *h*, *m* and *n* cells. Fig. 20. 2-celled proembryo showing *ca* and *cb* cells. Fig. 21. 3-celled proembryo. Fig. 22. 4-celled isobilateral proembryo showing *f*, *g*, *h* and *d* cells. Fig. 23. 5-celled proembryo. Fig. 24. 6-celled proembryo. Figs. 25-30. Successive stages in the formation of globular embryo.

lopment seems to conform to the two different variations of Asterad type of Johansen (1950), viz, *Polygonum* and *Geum* type.

A number of periclinal and anticlinal divisions occur in the 8-celled embryo, resulting in a globular mass of embryo (Text-Figs. 23-30). Its further development from globular to mature embryo is similar to that in other species of *Phoenix* in both the types (see Mahabale and Biradar, 1968; Biradar, 1968) (Pl. I, Figs. 14-18).

Mature embryo.—A mature embryo is slipper-shaped. Cotyledon is massive. The nodal plate gives rise to 6 vascular strands as can be seen in T.S. (Pl. I, Figs. 19-20). They undergo a number of divisions and form a ring of 25-30 bundles going to periphery of the elongated cotyledon. Shoot apex is terminal as in all other species. Radicle points towards the micropyle. Hypocotyledonary region is highly condensed. The plumule has a small embryonal apex with a single sheathing leaf (Pl. I, Fig. 18). The micropyle originally is terminal but shifts to the side of the seed wall later as in all other species of *Phoenix*.

CONCLUSION

An endemic species, *Phoenix robusta* grows at Mulshi and Bhorkas in Poona District. There is some uncertainty regarding its origin and systematic position. As suggested by Mahabale and Parthasarathy (1963) it may have arisen as a cross between *P. sylvestris* × *P. acaulis*, both of which grow in Western Ghats. Embryological data supports this view as both species have similar type of embryo development. This is rather interesting as it may serve further as one of the parents for crossing with *P. dactylifera* closely related to *P. sylvestris* and economically important.

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EXPLANATION OF PLATE I

FIGS. 1-20. *Phoenix robusta* Hook. Morphology, development of male gametophyte and embryo.

FIG. 1. *P. robusta* growing on hills at Bhorakas (Dist. Poona).

FIG. 2. A single tree.

FIG. 3. A pollen grain showing colpus and smooth sporoderm, $\times 375$.

FIG. 4. Germinating pollen grain showing vegetative nucleus—*vg* and generative nucleus—*gn*, $\times 280$.

FIG. 5. Pollen grains in germination, $\times 280$.

FIG. 6. A secondary axis of female peduncle: Note a narrow tip ending in a flower, $\times \frac{1}{2}$ N.S.

FIG. 7. 8-nucleated embryo-sac, $\times 70$.

FIG. 8. Zygote, $\times 140$.

FIG. 9. 2-celled proembryo showing lenticular terminal cell—*ca* and long basal cell—*cb*, $\times 280$.

FIG. 10. 3-celled proembryo: Note the transverse division in *cb* cell giving rise to *m* and *n* cells, $\times 280$.

FIG. 11. 3-celled proembryo showing vertical division in terminal cell *ca* differentiating bigger cell *h* and smaller cell *d*, $\times 280$.

FIG. 12. 4-celled proembryo: The cell *h* in Pl. I, Fig. 11 divided obliquely and has formed central cell *e* and *r* cell. Cell *e* functions as epiphysial initial later, $\times 280$.

FIG. 13. 4-celled "L"-shaped proembryo, $\times 280$.

FIGS. 14 & 15. L.S. of globular embryo, $\times 225$ and $\times 140$ respectively.

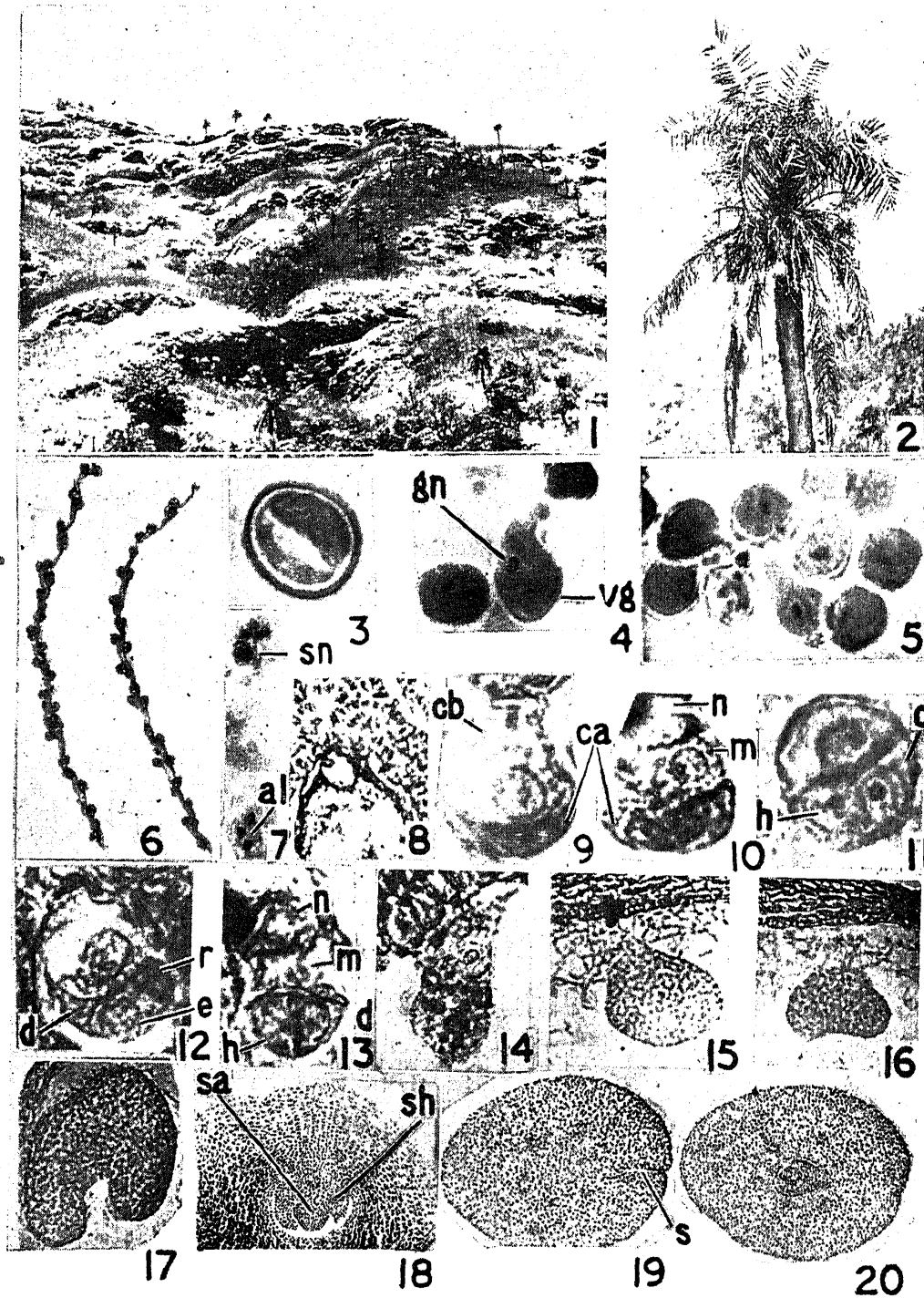
FIG. 16. L.S. of embryo showing the distinct lobing, $\times 80$.

FIG. 17. L.S. of embryo. Two lateral lobes and a terminal meristematic tissue of the shoot apex differentiated, $\times 50$.

FIG. 18. L.S. of mature embryo showing stem apex *sa* and sheathing leaf—*sh*, $\times 23$.

FIG. 19. T.S. of embryo passing below the nodal plate showing 6 vascular strands and lateral slit *s*, $\times 5$.

FIG. 20. T.S. of mature embryo passing through shoot apex showing sheathing leaf, stem apex and 6 vascular strands, $\times 5$.



FIGS. 1-20 *Phoenix robusta* Hook Embryology.