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FLORAL DEVELOPMENT, SPOROGENESIS, AND EMBRYOLOGY IN THE AVOCADO, PERSEA AMERICANA

C. A. SCHROEDER

Introduction

Within recent decades the avocado, *Persea americana* Miller,[1] has become an important crop plant in California, Florida, and other subtropical regions. Consequently, much interest has developed concerning the plant and its fruit. Some preliminary studies have been made on several phases of the anatomy of the whole plant (4) and of the fruit (3), but relatively little information on floral development exists. The present study deals with floral development, sporogenesis, and certain phases of early embryo development in the avocado.

In an early study of microsporogenesis in *P. gratissima* Gaertn. the haploid chromosome number was determined to be 12 (1). Cytological irregularities were also observed such as gigantic microspores which contained two or more nuclei and gave rise to polyploid pollen grains. The occurrence of ovules borne externally on the ovaries has been noted in Rome (2) and in California (9). Anatomical evidence (7) indicates that the six-parted perianth of the flower arises in two whorls and consists of a three-parted calyx and a three-parted corolla. In Florida (8) differentiation of the inflorescence continues over a period of months, but the earliest primordia of individual flowers are first identifiable in January only a few weeks before full bloom.

[1] The nomenclature is that suggested by POPENOE (6).

Material and methods

Materials for the present investigation were obtained from the horticulture orchard on the Los Angeles campus of the University of California and from commercial plantings in various parts of southern California. They consisted mainly of buds, flowers, and young fruits of the Fuerte variety, although similar collections were made of varieties such as Nabal, Matney, Mexicola, Dickinson, Blake, and others. They were fixed in Navashin's solution, after which they were dehydrated and imbedded in paraffin. Sections cut 10–20 μ thick were stained with safranin and fast green, Heidenhain's iron-alum hematoxylin, or Harris' hematoxylin.

Observations

FLORAL DEVELOPMENT.—In longitudinal section the growing apex of the individual flower primordium is first evident as a conical mass of cells in the axial of a bud scale (fig. 1*A*). This mass of meristematic cells becomes slightly flattened, and on the periphery of the disk the perianth parts appear (fig. 1*B–D*). These rudimentary perianth segments elongate and curve inward, arching over the apex of the axis. Stamen primordia arise at the same level but inside the perianth

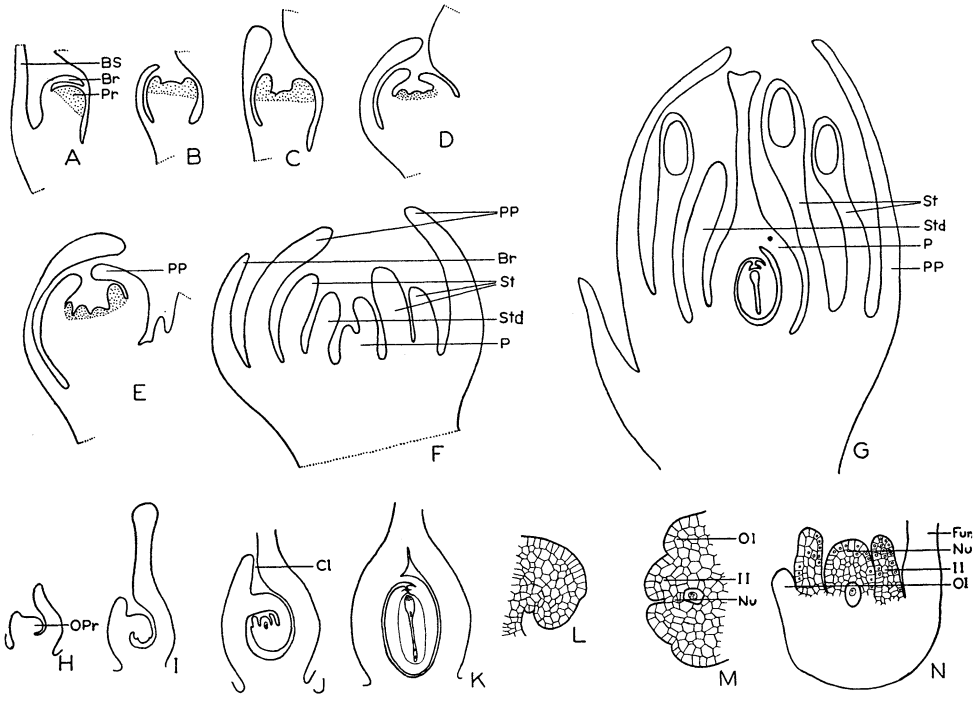

FIG. 1.—*Persea americana*. *A–G*, differentiation of flower parts. *A*, flower primordium in axil of bud scale. *B*, initiation of perianth primordia. *C–E*, initiation of stamens and staminodes. *F*, section through young flower shortly after initiation of pistil. *G*, young flower with all parts differentiated. *H–N*, differentiation of ovule and integuments. *H*, initiation of ovule in young pistil. *I–K*, later developmental stages of ovary and ovule. *L*, differentiation of inner integument in young ovule. *M*, differentiation of outer integument in young ovule. *N*, older stage of ovule with both integuments. *BS*, bud scale; *Br*, bract; *P*, pistil; *PP*, perianth part; *Pr*, bud primordium; *St*, stamen; *Std*, staminode; *OPr*, ovule primordium; *Cl*, cleft in pistil; *OI*, outer integument; *II*, inner integument; *Fun*, funiculus; *Nu*, nucellus.

parts and opposite to them (fig. 1*D*). Inside the stamen whorl appears a third set of primordia, the inner stamens or staminodia (fig. 1*E*). The pistil is the last floral organ to develop (fig. 1*F*, *G*). It first appears as a small, conical mass which by differential growth produces a cuplike structure, one side of which becomes higher than the other. The higher side gives rise to the style and stigma, and the lower, which grows more slowly, differentiates the ovule primordium on its inner surface (fig. 1*F*, *H–J*).

More rapid cell division on the upper side of the funiculus causes the ovule to bend toward the receptacle. Differentiation of integuments and nucellus occurs at this time. The inner integument appears first and is followed by the outer (fig. 1*L–N*). By continued bending the ovule eventually becomes anatropous, and the micropyle is located immediately beneath the funicular attachment (fig. 1*K*).

MEGASPOROGENESIS.—A single hypodermal archesporial cell gives rise to the megaspore mother cell and the primary parietal cell (fig. 2*A*) at about the time of initiation of the inner integument. The megaspore mother cell, distinguished by its central position, large nucleus, and deeply staining cytoplasm, enlarges to four to five times the size of the archesporial cell and becomes imbedded in several layers of nucellar tissue (fig. 2*B*).

By two successive divisions the megaspore mother cell gives rise first to two (fig. 2*C*) and then to a linear row of four megaspores of about the same size. The three micropylar megaspores soon degenerate, leaving the chalazal megaspore to function as the one-cell embryo sac (fig. 2*D*). Before the first division of its nucleus, the functional megaspore increases in size, accompanied by an increase in cell division in the surrounding nucellar tissue. Following the first gametophytic division the two daughter nuclei separate, one migrating to the chalazal end and one to the micropylar end of the elongating and enlarging embryo sac (fig. 2*E*). By two subsequent divisions, these two nuclei produce eight nuclei, a group of four at each end of the now elongate embryo sac (fig. 2*F*, *G*). One nucleus from each of the two groups migrates toward the center (fig. 2*H*). Fusion of these two polar nuclei then occurs (fig. 2*I–K*). As the embryo sac continues to elongate, the three remaining chalazal nuclei migrate further toward the chalazal end and become the antipodals. They are scattered irregularly and generally undergo degeneration very early, sometimes even before the fusion of the polar nuclei. Only traces of the antipodals remain by the time the egg apparatus is mature. The three nuclei remaining in the micropylar end of the embyo sac become the egg apparatus. The nucleus which is centrally located in respect to the micropyle becomes surrounded by a thin membrane to form the megagamete. The other two nuclei, the synergids, are lateral to the megagamete and also become enveloped by distinct membranes.

At maturity the embryo sac is imbedded in nucellar tissue. It is an elongate structure somewhat enlarged at the micropylar end (fig. 2*L*) and measures 650–950 μ in length and 25–75 μ in width.

Cytological irregularities in the embryo sac were observed rather frequently. The most common abnormalities noted consisted of cross walls, irregularity in shape, doubleness, and an increased number of nuclei.

Cross walls were found in several embryo sacs. These walls were about the thickness of the walls of the surrounding

nucellus and extended completely across the sac. Most of the cross walls were confined to the chalazal region, although in some cases they were found at about the level of the polar nuclei. Separation of the antipodal nuclei by wall formation was seen in several instances.

In abnormal embryo sacs the number of nuclei ranged from nine to twenty-five or more. In one case three nuclei were found just lateral to the egg apparatus in the micropylar region, and in another two extra nuclei were noted in the region of the antipodals in the chalazal end. The origin of these nuclei could not be determined. Other embryo sacs contained

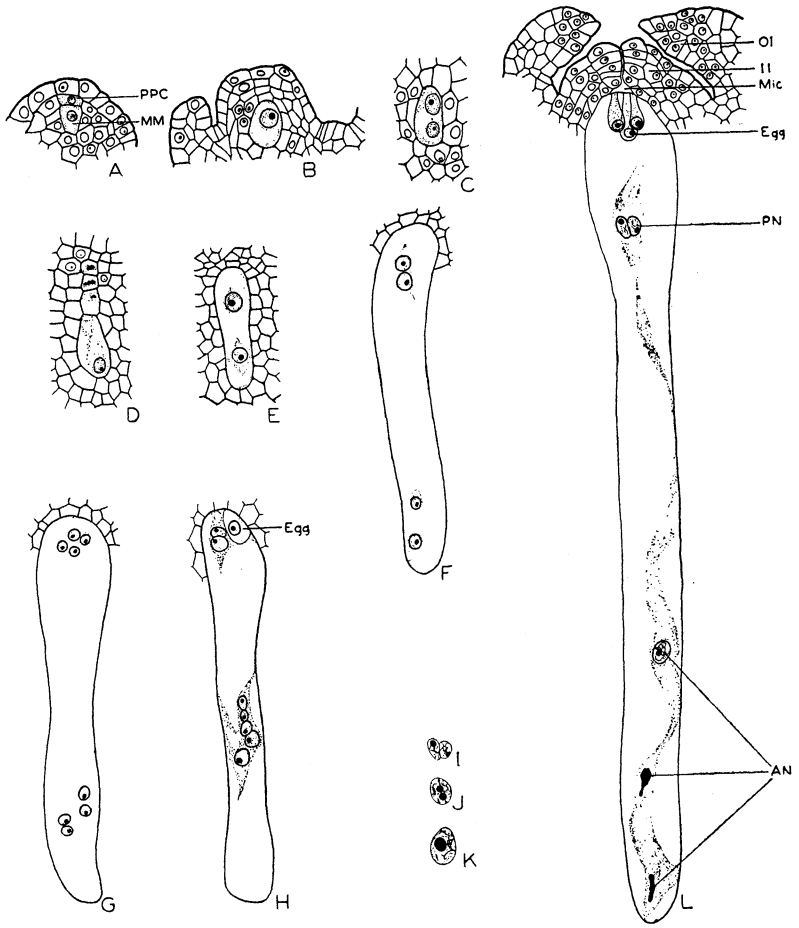

FIG. 2.—*Persea americana*. Megasporogenesis and development of megagametophyte. *A*, section through tip of developing ovule shortly after division of hypodermal archesporial cell. *B*, megaspore mother cell. *C*, daughter nuclei from first division of megaspore mother cell. *D*, linear tetrad of megaspores, showing the chalazal spore becoming functional, the micropylar three disintegrating. *E*, two-nucleate embryo sac. *F*, four-nucleate embryo sac. *G*, eight-nucleate embryo sac. *H*, eight-nucleate embryo sac at later stage showing migration of polar nuclei to center of sac and differentiation of egg cell, synergids, and antipodals. Polar nuclei are central in position in this section. *I*, polar nuclei prior to fusion. *J*, secondary nucleus, with two nucleoli. *K*, secondary nucleus with single, large nucleolus. *L*, mature embryo sac. *PPC*, primary parietal cell; *MM*, megaspore mother cell; *PN*, polar nuclei; *AN*, antipodal nuclei; *OI*, outer integument; *II*, inner integument; *Mic*, micropyle.

many small nuclei in the chalazal end. These numbered from ten to twenty-five or more in each sac. They were very small, approximately 1–3 μ in diameter, and were scattered irregularly.

An ovule containing two sacs was found in a single specimen. This corroborates the observation of polyembryony in avocado seeds.

MICROSPOROGENESIS.—In flower buds about 1 mm. in length, the first evidence of microsporogenesis appears in longitudinal section as a single hypodermal cell which contains a relatively large nucleus and stains deeply (fig. 3*A*). In rapid succession several of the adjacent hypodermal cells exhibit a similar reaction (fig. 3*B*, *C*). Continued cell division in this tissue results in a group of cells, the sporogenous tissue, four to five cells deep and six to ten cells in length just beneath the epidermis (fig. 3*D*, *E*). The cytoplasm of all these sporogenous cells stains deeply, and the single nuclei are conspicuously large.

The tapetum arises from the peripheral layer of the sporogenous tissue (fig. 3*F*). The four-nucleate condition of the cells of this tissue arises as the result of two successive nuclear divisions without ensuing cell-wall formation (fig. 3*G–I*). In the two-nucleate condition the nuclei are often elongated and flattened along the surface of contact and often show two or three nucleoli. After the second division, the four nuclei present in the cell are smaller, more nearly spherical in shape, and possess only one nucleolus each. In prepared materials sometimes the four nuclei are tightly pressed together and thus are distorted from their spherical shape (fig. 3*I*). As this nutritive layer is absorbed during the development of the microspores, the cytoplasm is reduced in amount, the nuclei undergo degeneration, and the cell walls collapse and shrink (fig. 3*J*, *K*). There are no remains of the tapetal layer in the mature pollen chamber.

Shortly after the formation of the tapetum, the spore mother cells undergo meiotic division resulting in the subsequent formation of tetrads (fig. 3*F*, *L–N*). The chromosomes have not been well observed during meiotic divisions. They are small, about 1.5 μ in length, and tend to clump together when ordinary techniques are used. In a few of the better preparations the haploid complement was determined to be twelve.

The microspores remain united in tetrads for only a short time. When they become separated, they assume a spherical shape and have a thin limiting membrane and a large nucleus with one large, spherical nucleolus. The spore wall thickens, and the exine appears (fig. 3*O*, *P*). The pollen grains may be shed in this stage. In most cases, however, the nucleus divides before the pollen is shed. The division results in a large nucleus, retaining a central position, and a smaller nucleus which lies near the periphery of the grain (fig. 3*Q*, *R*). Each nucleus, surrounded by cytoplasm, becomes incased in a thin membrane. Thus in preparations which are slightly plasmolyzed two distinct cells can be identified in the male gametophyte at this stage (fig. 3*R*).

The heavy intine of uniform thickness lies next to the cytoplasm and is covered by a thin exine with short, uniformly distributed conical spines (fig. 3*S*). The exine and intine are easily ruptured when subjected to pressure (fig. 3*T*).

The mature Fuerte pollen grain measures 27–30 μ in diameter. The intine and exine are 2 μ and less than 1 μ thick, respectively. When placed in water or IKI solution, marked swelling of the grain occurs. As there are no germinal furrows

or conspicuous germ pores in the microspore wall, germination of the pollen tube may occur by the splitting of the wall at any point on its surface. The tube nucleus precedes the generative nucleus as they migrate into the elongating pollen tube (fig. 3*U–W*). Shortly after the beginning of germination, the membrane separating the generative and tube nuclei seems to disappear so that the nuclei at least in the early part of their migration down the germ tube are enveloped by a common cytoplasm (fig. 3*U*, *V*). The ultimate fate of either generative or tube

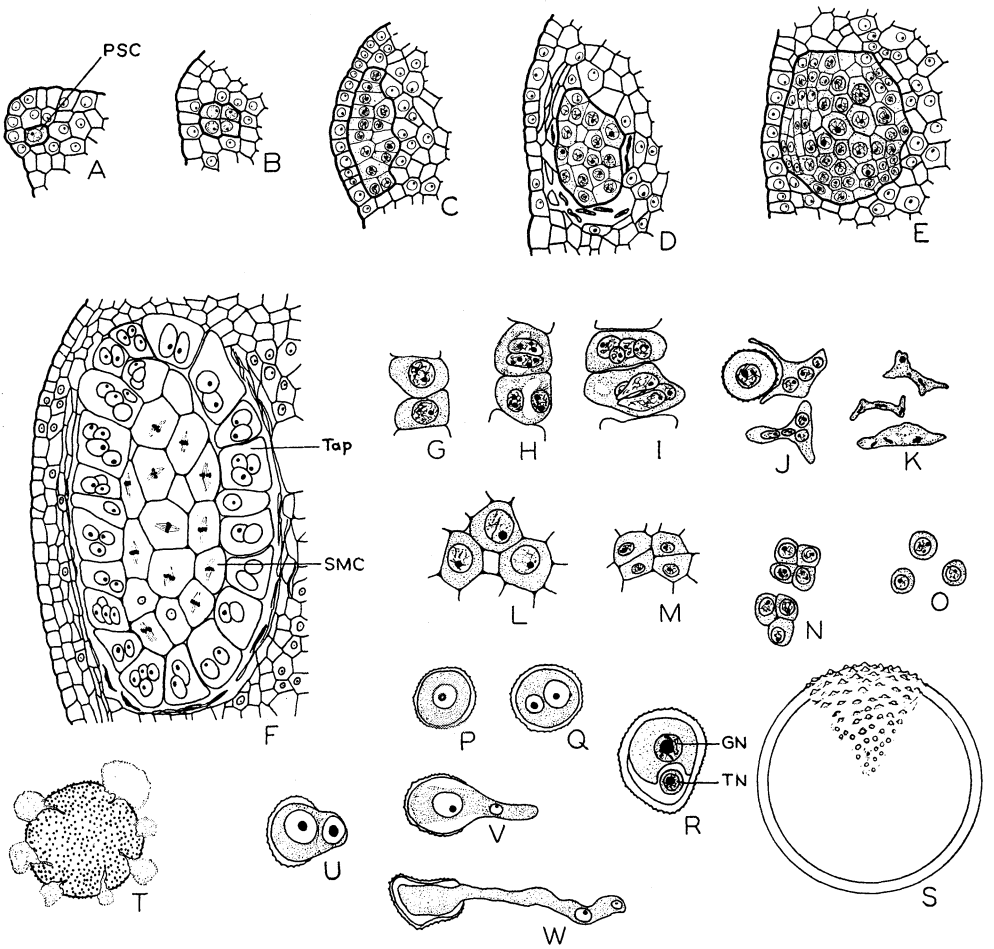

FIG. 3.—*Persea americana*. Microsporogenesis and young microgametophyte. *A*, primary sporogenous cell in young anther. *B–E*, later stages in development of sporogenous tissue. *F*, pollen sac shortly after differentiation into spore mother cells and tapetal layer. *G–I*, one-, two-, and four-nucleate stages of tapetal cells. *J*, *K*, tapetal cells being absorbed by developing microspores. *L*, microspore mother cells. *M*, microspores before second division. *N*, tetrads of microspores. *O*, *P*, young microspores. *Q*, pollen grain with generative and tube nuclei. *R*, mature pollen grain slightly plasmolyzed. *S*, portion of pollen-grain surface showing blunt, conical spines. *T*, bursting of pollen grain when subjected to pressure. *U*, pollen grain starting to germinate; only faint indication of wall separating generative and tube cells remains. *V*, *W*, germinating pollen grains. *Tap*, tapetum; *PSC*, primary archesporial cell; *SMC*, microspore mother cell; *GN*, generative nucleus; *TN*, tube nucleus.

nucleus could not be determined from the materials studied.

ENDOSPERM DEVELOPMENT.—The endosperm tissue apparently results from a triple fusion of chromosome complements, although as yet no fusion stages have been observed. In aceto-carmine smears the chromosome number in thirteen preparations ranged from 27 to 39. The chromosomes are minute (1.2 × 0.6 μ) (fig. 4*A*) and tend to clump, so that they are difficult to count. The diploid complement has been reported to be twenty-four (1, 10). Thus, the observed chromosome number in endosperm tissue is close to the calculated triploid. The occurrence of three nucleoli gives further evidence of the triploid nature of this tissue.

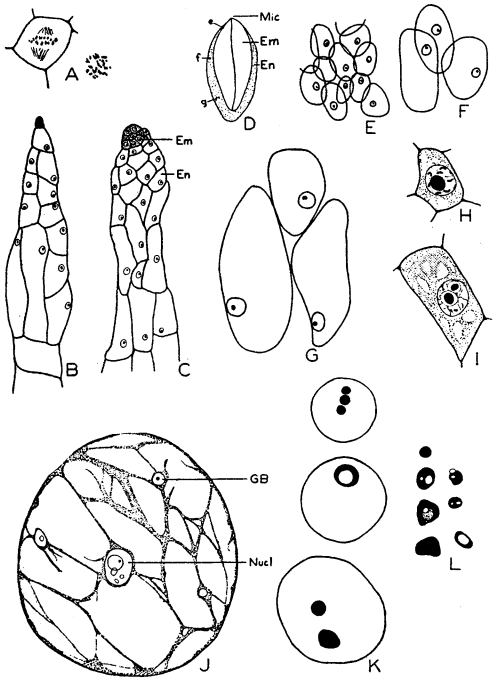

FIG. 4.—*Persea americana*. Endosperm. *A*, mitotic figure in endosperm. *B*, zygote stage of embryo and adjacent endosperm already in cellular stage. *C*, a proembryo stage with surrounding endosperm. *D*, diagram of embryo about 10 mm. long with endosperm. *E–G*, sketch of endosperm cells and nuclei at points *e*, *f*, and *g* of *D* showing relative sizes. *H*, *I*, endosperm cells from near base of embryo. *J*, large nucleus from endosperm tissue showing nature of peripheral chromatin threads, nucleoli, and general structure. *K*, three large endosperm nuclei with different numbers of nucleoli. *L*, types of nucleoli found in endosperm nuclei. *Em*, embryo; *En*, endosperm; *GB*, granular body; *Mic*, micropyle; *Nucl*, nucleolus.

The development of the endosperm precedes that of the embryo. In the earliest stages observed, cellular endosperm already surrounds the zygote or early proembryo (fig. 4*B*, *C*). Whether a free nuclear stage exists was not ascertained. As endosperm development continues, cell divisions occur only in the immediate vicinity of the embryo. The older cells are forced toward the chalazal end of the embryo sac and increase in volume. The isodiametric, meristematic endosperm cells adjacent to the embryo measure 30–60 μ in diameter and contain nuclei which range from 20 to 40 μ in diameter (fig. 4*H*, *I*). The ellipsoidal endosperm cells at the chalazal end may measure as much as 600 by 100 μ in length and breadth, and the extremely large nuclei in these cells range from 60 to 75 μ in diameter and in certain instances have exceeded 100 μ (fig. 4*G*). Reserve materials consist mainly of soluble sugars. No other ergastic substances such as oils, starch, or tannins were observed. When the tissue showed general signs of degeneration, oxalate crystals varying in size and form were noted in cells throughout.

The general appearance of the large resting or prophase nucleus of the endosperm tissue is that of a spherical, homogeneous mass covered on the surface by a thin network of chromatin material of variable thickness (fig. 4*J*). At certain intersections of these chromatin threads, granular bodies appear, and at others nucleoli are found. The central mass appears to be homogeneous throughout,

but occasionally strands of chromatin material appear to traverse the colorless matrix.

The nucleoli in endosperm cells vary in number from one to three (fig. 4*K*). The presence of three nucleoli supports the evidence that the endosperm is triploid tissue, since the number of nucleoli may indicate the number of chromosome complements in the nucleus (5). The nucleoli range in size from 4 to 26 μ in diameter and are generally spherical but may be elliptical or irregular in outline (fig. 4*L*). They are either homogeneous or may contain vacuoles of varying size and shape. The contents of the latter differ in refractive index.

The extremely large size of the endosperm nuclei makes them excellent objects for the study of nuclear morphology; they also may be of value in experiments in nuclear physiology.

EMBRYO DEVELOPMENT.—Development from the zygote (fig. 5*A*) to the multicellular proembryo (fig. 5*B–F*) takes place over a long period of time, probably many days. The earliest mitotic divisions of the proembryo have not been observed, but at the four-cell stage it is already spherical in form (fig. 5*B*). Thereafter, cell division continues in all planes. No suspensor is formed. As the proembryo increases in size, it remains spherical until it attains a diameter of about 0.2–0.3 mm., when cotyledonary differentiation is well marked (fig. 5*G*). The primordia of the cotyledons first appear as two small lobes at the chalazal end and elongate as narrow laminae of thin-walled parenchyma surrounded by an epidermis. The basal lobes of the cotyledons appear later (fig. 5*H*). At this stage the embryo measures about 1 mm. in length; the young radicle and plumule are distinguishable, and provascular tissue can be discerned. The cotyledonary node consists of a mass of meristematic cells distinguished from the surrounding parenchyma by their smaller size, deeply staining protoplasts, and relatively larger nuclei.

Differentiation of provascular tissue near the cotyledonary node and extending into the cotyledons is clearly defined

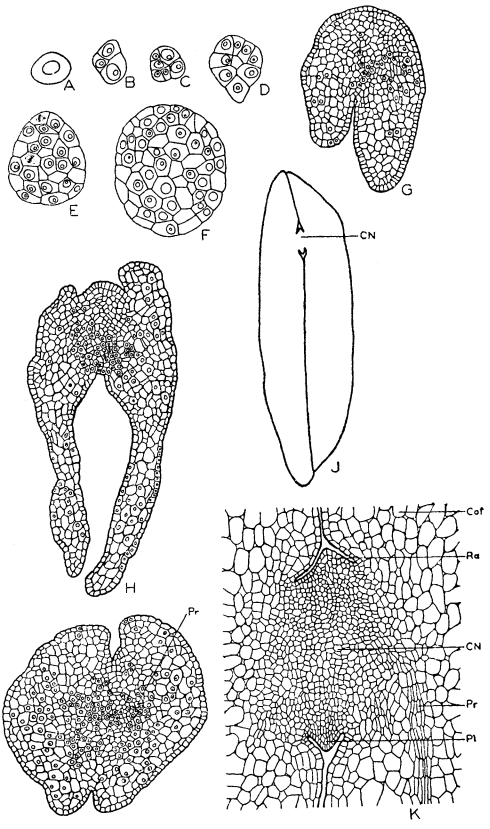

FIG. 5.—*Persea americana*. Embryo. *A–F*, successive stages of embryo development from zygote to multicellular, spherical proembryo. *G*, longisection through embryo about 0.5 mm. long showing early development of cotyledons. *H*, longisection through embryo about 1 mm. long showing cotyledons well differentiated and initiation of radicle. *I*, transection through cotyledonary node of 1-mm. embryo. *J*, later stage of embryo about 4 mm. long in radial section showing relative size of cotyledons, cotyledonary node, plumule, and radicle. *K*, detailed anatomy of cotyledonary node of 4-mm. embryo showing provascular tissue initiated in cotyledon. *CN*, cotyledonary node; *Cot*, cotyledon; *Pl*, plumule; *Pr*, provascular tissue; *Ra*, radicle.

in embryos about 3 mm. long (figs. 5*I*–*K*). Both lobes of the cotyledons are well developed in these embryos, having increased in length and thickness. Differentiation continues until in the mature embryo there are two hemispherical cotyledons with vascular strands located in a plane parallel to their flat faces and close to these surfaces.

Summary

1. In the avocado, *Persea americana* Miller, flower parts develop in acropetal order. The flower is perfect, hypogynous, regular, and trimerous.

2. The ovule is anatropous and has two integuments. Megasporogenesis is initiated with the formation of a hypodermal archesporial cell which is distinguishable shortly after the integuments are evident. By successive divisions a linear row of four megaspores is formed, the chalazal cell functioning as the embryo sac. A normal type of development then follows which results in an eight-nucleate megagametophyte. The mature embryo sac is elongate and bulbous at the micropylar end. Cytological irregularities in the embryo sac, such as cross walls, multinucleate gametophytes, and a double embryo sac, are described.

3. Microsporogenesis begins with a hypodermal sporogenous cell. Primary sporogenous tissue gives rise to a central mass of pollen mother cells surrounded by a tapetal layer, the latter containing four nuclei in each cell. The pollen mother cells undergo meiosis and form tetrads which soon separate into individual spores. Mature pollen grains are spherical with no germ pores and are covered with short, conical spines.

4. The endosperm is a triple fusion tissue which becomes cellular and contains large, conspicuous nuclei. These nuclei sometimes attain diameters of 100 μ.

5. During embryo development no suspensor is formed. Differentiation of cotyledons occurs when the spherical proembryo attains a diameter of about 0.2–0.3 mm.

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