Philippine Journal of Science
131 (1): 23-28, June 2002
ISSN 0031 - 7683

Late Embryogeny of *Pittosporum resiniferum* Hemsl. (Petroleum Nut Plant)

Vivian S. Tolentino*[1] and Prescillano M. Zamora[2]

[1]Department of Biology, School of Science and Engineering
Ateneo de Manila University, Loyola Heights Quezon City
[2]Institute of Biology, University of the Philippines, Diliman, Quezon City

An investigation on the developmental morphology of the embryo of *Pittosporum resiniferum* Hemsl. from the globular to torpedo shapes was carried out using light microscopy. It is aimed to characterize and document the major morphological changes of the embryo in terms of shape and differentiation of tissues in the course of its late embryogeny. The modified paraffin and clearing techniques were used. The embryos were described and classified at different morphological stages.

Stage 1 had embryos which were globular. In stage 2, the embryos were in the early heart-shaped phase with the cotyledons developing at the distal regions on both sides. In stage 3, the embryos were in the mid-heart, with developed cotyledons. In stage 4, the late-heart, the cotyledons were fully developed, elongate, and a deeper depression in-between the two cotyledons was observed, as compared to stage 3. Stage 5, the torpedo shape, the protoderm, procambium and the ground meristems are well defined.

Keywords: globular, ground meristem, procambium, protoderm, torpedo, zygote.

Pittosporum resiniferum Hemsl, commonly called "Petroleum Nut Plant" (Eng.), is widely distributed in the Philippines. It is found on high mountain ridges and forested areas from Bontoc to Sorsogon, Mindoro and Catanduanes particularly in the Cordillera mountains and Benguet (Bakker & Van Steenis, 1972,1985).

P. resiniferum is a potentially important hydrocarbon - containing species due to the combustible property of the fruit's oil extract (Bacon, 1909; Noble, 1978). The oil is commonly used as torch light by the mountain people of Palawan. This was also widely utilized by the Japanese during World War II. Studies on its fuel properties showed that it is quite comparable with that of gasoline. Veracion and Costales (1981), reported that it contains dihydroterpene ($C_{10}H_{18}$), a medicinal and perfumery compound, and heptane (C_7H_{16}), a component of gasoline.

Embryological studies can be used as database in studies in genetics and molecular biology. Stiefel et. al (1996), reported on characterization of genes involved in embryogenesis in maize. They were able to classify the patterns of expression observed during embryogenesis for different genes having defined functions, and analysis of genes tagged by Ac transposon producing mutations during embryogenesis.

The use of molecular approach to isolate genes expressed in Arabidopsis embryos was reported by Thomas et al. (1996),. Results of their in-situ hybridization indicate that one gene (AtS20) is expressed in embryos and seed coats prior to cotyledon stage, and another (AtS35) is expressed from globular stage to late cotyledon stage. KnAt4-11 transcripts are expressed in suspensor and

*Corresponding author: vivian@pusit.admu.edu.ph

endosperm as well. AtSi and AtS3 were isolated by differential display; each of these genes are expressed later during embryogenesis and operationally can be defined as late – embryogenesis abundant genes (lea).

Meijer et al. (1996) studied the role of homeobox genes in regulation of morphogenesis and embryogenesis in rice. They isolated a rice cDNA clone, Oshox1, encoding the first HD-Zip protein from monocotyledonous plant species. Oshox 1 m RNA was also detected in various rice tissues at different developmental stages, with highest levels in embryos, shoots of seedlings, and leaves of mature plants. To investigate whether homeobox genes also have specific functions in regulation of rice embryogenesis, a number of cDNA libraries of rice embryos at well defined developmental stages before, during and after organ differentiation was constructed.

The objective of this study is to characterize and document the major morphological changes and classify into various stages the development of the embryo from globular to torpedo. Results of the study will serve as baseline data in embryology and in the field of genetics, and molecular biology.

Methodology

Mature fruits of *P. resiniferum* were collected from wild populations and cultivated plants from different parts of the Philippines vis-a-vis, Pacdal, Baguio City; Mt. Sto. Tomas, Benquet; Bureau of Plant Industry Economic Garden in Los Baños, Laguna; and Bureau of Plant Industry Research Station in Luisiana, Laguna.

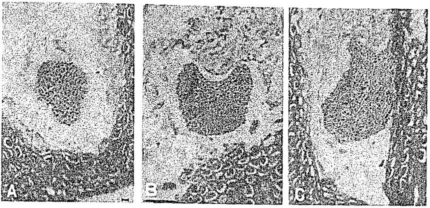
The fruits were fixed in a mixture of 5 ml of 37% formalin, 90 ml of 70% ethyl alcohol, and 5 ml of 99% glacial acetic acid (FAA) and processed using the paraffin technique of Johansen (1940) as modified by Zamora (1992). Seeds were excised from the fruits, decoated by peeling off the seed coat, and cut into desired size. These were dehydrated with a graded series of ethyl alcohol, cleared in xylene series, infiltrated with soft paraffin (melting point 40 - 44°C) changed to medium paraffin (melting point 44 – 50°C), hard paraffin, (melting point 50– 55°C), oven heated respectively, for 12-24 h, and finallly embedded in hard paraffin. The specimens were cut at 8-10 um thick with a rotary microtome. Dewaxed and fixed sections were stained with 1% alcoholic safranin O, counterstained with 0.5% alcoholic Fast Green, and mounted in Canada balsam. Slides were photomicrographed using the phase contrast optics of the BH-2 Olympus epifluorescent microscope.

Whole mounts of embryos were cleared for further documentation on the morphology of the embryo. The seeds were cut longitudinally into halves. The isolated embryos were soaked for two sec in 3% hydrogen peroxide, washed with water, cleared with 50% chloral hydrate, dehydrated with 95% ethyl alcohol, cleared in xylene and finally mounted in Canada balsam. Photomicrographs of the embryos were taken using the phase contrast optics of the BH-2 Olympus epifluorescent microscope. The embryos were characterized and classified to different morphological stages based on the shape, differentiation and development of the cotyledons and primary tissues.

Results

Seeds dissected had an embryo at the micropylar part of the seed, adjacent to the funiculus. Stage 1 embryos were small and globular (Fig.1.A). In this stage, the globular embryo grows transversely than longitudinally, producing an elliptical embryo with a wide apex, on which will arise the two cotyledons. At the end of the globular stage, the cotyledons began to develop at the distal regions on both sides and the globular embryo appears more or less cordate (heart shape). Stage 2 demarcates the earliest "heart-shaped" phase with developing cotyledons (Figs. 2. E,H,I,M,T,U). Stage 3 embryos were in the mid heart-shape with developed cotyledons (Figs.2.D,F,J,L,P). Stage 4, the late heart-shape, the cotyledons are fully developed and elongate, with deeper depression in-between the two cotyledons as compared with stage 3(Figs.2. A,J,Q,R). Stage 5 are torpedo shaped and the embryos (Figs. 1.C,D,E; Figs.2.B,C,G,K,N,O) have developed primary meristems, i.e., the protoderm, procambium and the ground meristem (Figs.1.C,D,E). In most of the embryos, the suspensor was a distinct structure (Figs.2. C, D, G, H, I, with arrow). It is attached to the organogenetic part of the embryo. This structure maybe short-lived as evidenced by its disappearance in some embryos. The embryos were embedded in the embryo sac which was surrounded by the endosperm.

During organ formation of the embryo, the first histogenetic event was the periclinal division in each of the octant cells. The result was the formation of a 16-celled embryo, consisting of eight internal cells that gradually differentiate the primary meristems, the protoderm, procambium and the ground meristem of the hypocotyl and cotyledons. Towards the end of the globular stage, the embryo expands laterally as a result of periclinal divisions in the terminal lateral poles, giving rise to a pair of cotyledons (Figs. 2.E,H,I,M,T,U). At the onset of the cotyledon initiation, the embryo assumed a more or less heart-shaped/cordate stage, (Fig.1. B; Figs. 2.D,F,L,S). At the same time, when the cotyledons were initiated, divisions and differentiation of cells in the basal tier of the embryo gave rise to the hypocotyl. During the cotyledon



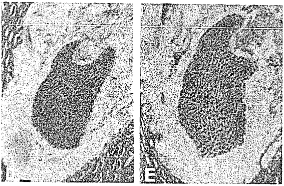
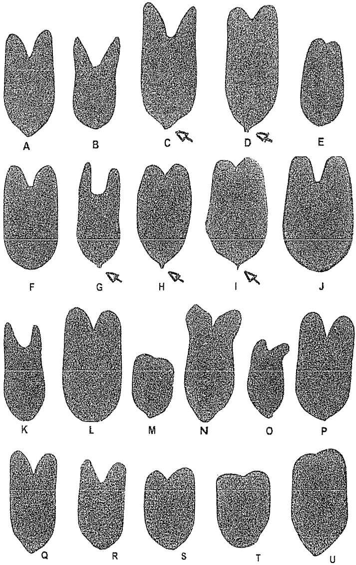


Figure 1. A-C. Paraffin sections of embryos. (A. Globular embryo (Bar=10.8 um). (B. Heart-shaped embryo (Bar=1 8 um). (C,D,E. Torpedo-shaped embryos (Bar=14.4 um).

initiation, a mound of rapidly dividing cells that constitute the future shoot apex was organized in the depression between the two cotyledons. The process of cell differentiation in the cotyledons and hypocotyl were usually accompanied by considerable elongation of these structures, resulting in the formation of the late-heart shaped embryo. The root system of the future embryo was delimited in the form of a root apex at the late-heart stage, increasing in prominence with the further growth of the embryo. Except for the further growth in length of the cotyledons and the hypocotyl, no sharp demarcation was observed when the embryo developed further into a torpedo-shaped embryo (Figs. 1.C,D,E; Figs. 2.B,C,G,K,N,O).

Discussion

Angiosperm embryos exhibit various configuration at different stages of late embryogenesis. Late embryogenesis has been divided into three phases: (1) The globular phase includes all the stages after the pro-embryo stage and before the initiation of the cotyledons. (2) The heart-shaped phase, includes all the stages after the initiation of cotyledons and before the torpedo-shaped phase. (3) The torpedo-shaped phase includes all the events to full establishment of cotyledons and polar meristems (Raghavan 1966). In this study, the heart-shaped phase was further divided into early-heart, which is the initiation of the cotyledons; mid-heart, the development of the cotyledons, and late-heart, the fully developed cotyledons with a



Figures 2. A-U.Cleared embryos at different morphological stages. (arrow points to a suspensor).

deeper depression between the two cotyledons as compared with the mid-heart. The torpedo-shaped with differentiated primary meristems is the most mature in *P. resiniferum*.

As reported by Raghavan (1999), associated with the development of the embryo from the globular to the torpedo stage are: the formation of meristems and embryonic organs, progressive cell divisions, cell expansion, cell maturation, and cell differentiation. These cellular processes mold the external and internal configuration of the developing embryo and lead to the formation of meristems and embryonic organs.

A perusal of earlier literature revealed that the Pittosporaceae and specifically *P. resiniferum*, have not received much attention in the field of embryology. On the other hand, embryological studies on other families were found to be abundant. The family Compositae was studied by Davis (1962), Pullaiah (1979), & Pandey et al. (1986). The family Polemoniaceae was studied by Kapil et al. (1968); and family Orchidaceae by Yasugi (1983). Juncosa (1982) worked on Rhizophora mangle (Rhizopharaceae). Abbe & Stein (1954) studied the embryogeny of maize. Bruck & Walker (1985) worked on the developmental morphology of in-situ and cultured embryos of *Citrus jambri* for cell determination during embryogenesis. In *Amnobium alatum*, Davis (1962) reported that the embryo reaches its maximum expression at the heart-shape stage and then it degenerates. However, in *Datura*, the final stage in the development of the embryo seems to be the torpedo stage. In *Pittosporum resiniferum*, the torpedo shape was observed to be the final and most mature stage.

Poor germinability (5.21%) of seeds from immature fruits of *P. resiniferum* was reported by Noble (1978). An understanding on the developmental morphology of the embryos of *P. resiniferum* will serve as a guide in establishing the viable stage suited for germination of the seed in agricultural management on a large scale. This study will be the first embryological report on *P. resiniferum* which may provide baseline information in the embryology of the Pittosporaceae and related families. Data from this study can also be used in the isolation and characterization of genes in the field of genetics and molecular biology.

Conclusion

The seeds of *P. resiniferum* contained embryos ranging from globular to heart-shaped, to torpedo-shaped. The developmental morphology of the plant was divided into the globular phase, which included all the stages after zygote formation and before initiation of cotyledons. The heart - shaped phase, which was the initiation and development of cotyledons, and the torpedo – shaped, which included all events to full establishment of cotyledons and primary meristems. Most of the embryos isolated were heart-shaped. In this study, the torpedo-shaped is considered to be the most mature embryo, with well developed cotyledons and the fully differentiated protoderm, procambium and ground meristems.

Acknowledgements

The authors thank Dr. G. C. Rivero, Dr. A. Aranez and Dr. N. O. Aguilar for their helpful comments. This research was supported by the UP Natural Science Research Institute, National Research Council of the Philippines, Ateneo de Manila University Faculty Development Program.

References

Abbe EC & Stein OL. 1954. The growth of the shoot apex in Maize. Embryogeny. American Journal of Botany. 4(4):285-293.

Bacon RF. 1909. Philippine terpenes and essential oils. III. Philipp, J. Sci. 4(2):115-118.

Bakker K & Van Steenis CGGJ. 1972. Pittosporaceae. Fl. Males. 6:960-962.

Bakker K & Van Steenis CGGJ. 1985. Pittosporaceae. Fl. Males. 5:345-360.

Bruck DK & Walker DB. 1985. Cell determination during embryogenesis in *Citrus jambhiri*. II. Epidermal differentiation as a one time event. American Journal of Botany 72(10):1602-1609.

Davis GL. 1962. Embryological studies in the Compositae. II. Sporogenesis, gametogenesis and embryogeny in *Amobium alatum* R. Br. Australian Journal of Botany. 65-75.

Esau K. 1977. Anatomy of Seed Plants. New York: John Wiley and Sons, Inc.

Johansen DA. 1940. Plant Microtechnique. New York: Mc Graw-Hill Book Company.

Juncosa AM. 1982. Developmental morphology of the embryo and seedling of *Rhizophora mangle* L. (Rhizophoraceae). American Journal of Botany. 69(10):1599-1611.

Kapil RN, Rustagi PN, & Venkataranam R. 1968. A contribution to the embryology of Polemoniaceae. Phytomorphology. 403-413.

Maheshwari P. 1950. An Introduction to the Embryology of angiosperms. Mc Graw-Hill Book Co. USA.

Meijer AH, Haarsma AD, Ira IGS, Hoge V & JH. 1996. Homeobox genes in regulation of morphogenesis and embryogenesis in rice. In: From single cell to plant - Progress towards understanding zygotic, androgenic and somatic embryogenesis. Abstracts. Centre for applied plant molecular biology, University of Hamburg, Germany.

Narayana LL & Sundari KT. 1977. Embryology of Pittosporaceae. Journal Jap. Bot. 52(7)201-209.

Narayana LL & Sundari KT. 1978. Embryology of Pittosporaceae. III. Proc. Indian Acad. Sci. 87B. (Plant Sciences) 3(8):205-214.

Noble BF. 1978. More secrets of the petroleum nut. Canopy 4(5):4-5.

Pandey AK, Singh C & RP. 1986. Development and structure of seeds and fruits in Compositae: Cosmos species. J. Indian Bot. Soc. 65:362-368.

Pullaiah T. 1979. Studies in the embryology of Compositae IV. The tribe Inulae. Am. J. Bot. 66(10):119-1127.

Raghavan V. 1966. Nutrition, growth and morphogenesis of plant embryos. Biol. Rev. 41:1-58.

Raghavan V. 1999. Developmental Biology of flowering Plants. Springer Verlag, New York.

Stiefel V, Jose M, Roca R, Graziano E, Becerra EL & Puigdomenech P. 1996. Characterization of genes involved in early embryogenesis in Maize. In: Plant Embryogenesis Workshop. From single cell to plant – Progress towards understanding zygotic, androgenic and somatic embryogenesis. Abstracts. Centre for applied plant molecular biology, University of Hamburg, Germany.

Thomas T, Li Z, Hsieh TF & Nuccio M. 1996. Novel genes expressed in *Arabidopsis* embryos. In: Plant Embryogenesis Workshop. From single cell to plant – Progress towards understanding zygotic, androgenic and somatic embryogenesis. Abstracts. Centre for applied plant molecular biology, University of Hamburg, Germany.

Veracion VP & Costales EF. 1981. The Bigger, The More. Philipp. Lumberman. 29:30.

Yasugi S. 1983. Ovule and embryo development in *Dorites pulcherrima* (Orchidaceae). American Journal of Botany. 70(4):555-560.

Zamora CV. 1992. Laboratory Manual in Plant Morpho-Anatomy. University of the Philippines Press. Diliman, Quezon City. 112-114.