

A UNIQUE TYPE OF ENDOSPERM IN *PANAX WANGIANUS* S. C. SUN

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Abstract: *Panax wangianus* S. C. Sun (Araliaceae) is a critically endangered, medicinal plant of north-east India. The objective of this research was to study post-fertilization changes in embryo-sac of *P. wangianus*. A characteristic feature has been observed in the endosperm in *P. wangianus* in which both the nuclear and cellular endosperm remains together in the mature seeds. The embryo is present in the nuclear part of endosperm so that it can draw the nutrition easily. The embryo probably exerts some physical and physiological forces that prevent the nuclear endosperm to become cellular.

Keywords: *Panax wangianus* S. C. Sun, medicinal plant, North-East India, post-fertilization changes, endosperm.

Introduction

The genus *Panax* L. (Araliaceae) consists of 18 species worldwide, of which two species grow in eastern North America and the other species in eastern central Asia. [REUNOV & al. 2008]. The generic name *Panax* is derived from the Greek term meaning “cure all” for its reputed medicinal use in China [ANDERSON & al. 2002]. The Chinese have been using ginseng for over 2000 years as a tonic, a stimulant and a fatigue-resistance medicine [WEN & NOWICKE, 1999].

P. wangianus (syn. *Panax pseudoginseng*) S. C. Sun is a critically endangered [PUSHPANGADAN & NAIR, 2005] herb located in the dense wet forests and bamboo forests of southwestern China, altitude 800-1350 m AMSL [WEN, 2001]. In India, it is native to sub-tropical wet forests of North-East Himalayan regions particularly in Meghalaya sacred groves such as Law Lyngdoh, Smit (Nongkrem), Law Lyngdoh (Mawphlang) and Shillong peak [VENUGOPAL & AHUJA, 2011]. Out of 18 species of the genus *Panax*, the 17 species are monoecious and *P. trifolius* is a dioecious plant [WEN, 2001].

Most of the seeds have persistent endosperm which acts as the repository of reserve food materials in the form of proteins, carbohydrates, fats and vitamins [KRISHNAMURTHY, 1998]. Apart from the importance of endosperm for the plants themselves, they are most important in the food chains of both the man and animals. Probably, endosperm is more valuable to man than any other plant part [VIJAYARAGHAVAN & PRABHAKAR, 1984]. During the course of study of post-fertilization changes in embryo-sac the authors came across a new type of endosperm in *P. wangianus*.

Materials and methods

The bisexual flowers of *P. wangianus* at various stages were collected from the field during the growing season April to September from 2007-2009 and fixed in FAA

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(formalin : acetic acid : 50% ethyl alcohol = 5 : 5 : 90 v/v). The customary methods of dehydration, infiltration, and embedding in paraffin were used [O'BRIEN & McCULLY, 1981]. Samples were sectioned at a thickness of 7-10 μ m by using Leitz rotatory microtome. The sections were stained with Safranin fast green [JOHANSEN, 1940] and observed at 40x under Olympus BX-51.

Results and discussion

P. wangianus grows in a colony of a few plants under shady forest floor with rich leaf litter in Nongkrem sacred grove. *P. wangianus* had a whorl of digitately compound leaf at the summit of aerial shoot; the leaf (prong) consists of three to eight palmately compound leaflets with a petiole, of which the terminal three to five leaflets are larger than the lateral two to three leaflets. Leaves are exstipulate or occasionally with persistent stipules; leaflets are bristly along veins and veinlets on both surfaces, narrowly lanceolate to broadly linear. Rhizome is creeping and elongated with thick internodes [VENUGOPAL & AHUJA, 2011].

P. wangianus is a monoecious plant. The flowers are on umbellate inflorescence (Fig. 1). Flowers are actinomorphic, bracteate, small, often greenish-yellow, perianth biseriate, pentamerous, the calyx inconspicuous, adnate to ovary, petals 5 broad at base, arising from disc and usually valvate. Nectariferous epigynous disc present, covering the ovary top. Ovary is inferior, three to five carpellate with uniovulate. The ovules are unitegmic, pendulous, anatropous, with an enlarged obturator derived from the funiculus.



Fig. 1. An enlarged view of umbel inflorescence of *Panax wangianus*. Bar = 2.0 cm.

The fusion product of two polar nuclei with the second male gamete or sperm cell constitutes the primary endosperm nucleus (PEN) which is triploid in nature located just below the zygote. The PEN shows precocious development than the zygote. It undergoes several mitotic divisions resulting into a nuclear condition; all nuclei are suspended in a cytoplasmic strand around the central vacuole and contain more than one nucleolus (Fig. 2). Nucleolar fragmentation is of common occurrence [GOPINATH, 1944]. Initially the

endosperm is nuclear with two distinct highly folded haustoria when the ovule is young (Fig. 3). Subsequently, centripetal wall formation sets in and the endosperm becomes cellular (Fig. 4). The cells are large, polyhedral, thin-walled and vacuolated with prominent nuclei. At maturity they become packed with reserve food materials mainly starch and proteins in nature as similar in *P. ginseng* [YU & al. 1992]. The starch was localized with IKI (Iodine potassium iodide) and PAS reaction while the protein bodies were stained with methyl green bromophenol blue, which gave positive reaction [O'BRIEN & McCULLY, 1981]. As the ovule enlarges in size, the haustoria are unfolded and absorb nutrients from the integument (testa). The endosperm is very large and occupied entire portion of the seed.



Fig. 2. An enlarged view of haustorium with several nuclei suspended within the haustorium. Bar = 40 μ m.

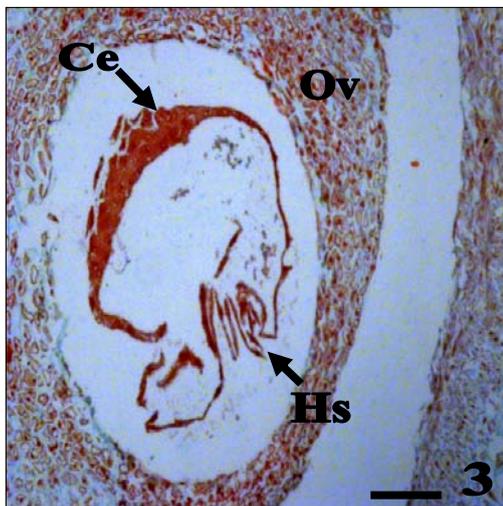


Fig. 3. Longitudinal section of ovule showing the differentiating endosperm proper with two highly folded haustoria. Ov = ovule; Ce = cellular endosperm; Hs = haustoria. Bar = 50 μ m.

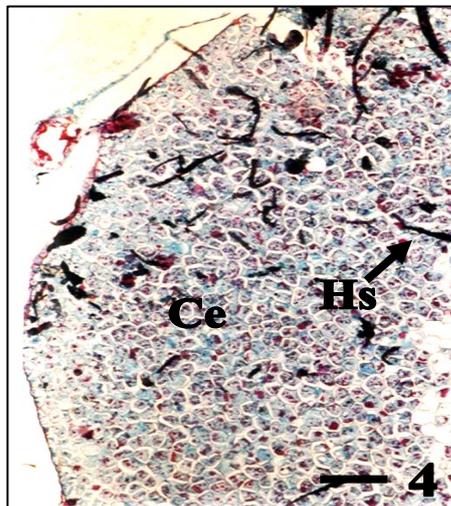


Fig. 4. A portion of cellular endosperm showing the haustoria in the cellular region. Hs = haustoria; Ce = cellular endosperm. Bar = 45 μ m.

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In Araliaceae, the endosperm is first nuclear and later wall formation sets in and it becomes completely cellular e.g. *Panax fruticosum*, *Polyscias pinnata*, *Hedera australiana*, *Heptapleurum venulosum*, *Brassaia actinophylla*, *Tieghemopanax sambucifolius*, *Eleutherococcus senticosus* and *Panax ginseng* [GOPINATH, 1944; MOHANA RAO, 1972; YU & KIM, 1991; ZHURAVLEV & al. 2008]. In the majority of the angiospermic families, the endosperm becomes cellular as seed mature. However, in *Limnanthes* and *Oxyspora paniculata*, free-nuclear condition persists until the endosperm is almost completely consumed by the developing embryo [MATHUR, 1956; SUBRAMANYAM, 1951].

In some families like Brassicaceae, Cucurbitaceae, Fabaceae and Proteaceae, wall formation is no doubt initiated but does not proceed beyond the central region. Thus, the central chalazal region remains free-nuclear. Since the cellular region grows further by cell divisions and the nuclear region by free nuclear divisions, the distinction between the two regions is maintained for some time [VENKATA RAO, 1967]. Cell formation in the nuclear region commences after the cellular part has attained the maximum dimensions [VIJAYARAGHAVAN & PRABHAKAR, 1984]. A characteristic feature has been observed in the endosperm in *P. wangianus* in which both the nuclear and cellular endosperm remains together in the mature seeds. The embryo is present in the nuclear part of endosperm so that it can draw the nutrition easily (Fig. 5). The embryo probably exerts some physical and physiological forces that prevent the nuclear endosperm to become cellular.

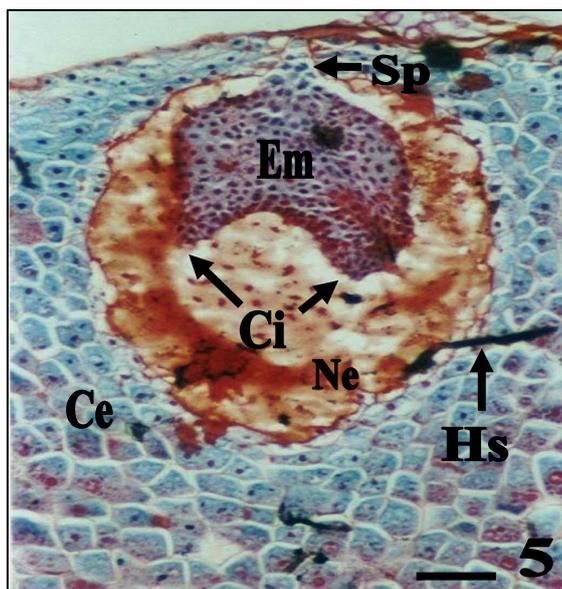


Fig. 5. Longitudinal section of seed showing endosperm with both the nuclear and cellular nature. Note the embryo is present in the nuclear part of endosperm. Ne = nuclear endosperm; Ce = cellular endosperm; Hs = haustoria; Em = embryo; Sp = suspensor; Ci = cotyledonary initials. Bar = 70 μ m.

In *P. wangianus*, the cellular region of endosperm proper produces several haustoria to absorb nutrients (Fig. 4). The integuments (testa) are totally absorbed by the endosperm haustoria. Therefore, in *P. wangianus*, the seed coat is derived by the innermost layers of the locule by undergoing lignifications to protect the embryo and endosperm which is also a significant feature in *P. wangianus*.

Conclusions

During the course of study of post-fertilization changes in embryo-sac we came across a new type of endosperm in *P. wangianus*. The co-existence of nuclear and cellular type of endosperm in the mature seeds is a peculiar feature in *P. wangianus*. The heart-shaped embryo is present in the nuclear part of endosperm so that it can draw easily the nutrition.

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References

- ANDERSON R. C., ANDERSON M. R. & HOUSEMAN G. 2002. Wild American Ginseng. *Native plant J.* **3**: 93-105.
- GOPINATH D. M. 1944. Gametogenesis and embryogeny in a few members of the Araliaceae. *Proceedings of the Indian Academy of Sciences.* **20**(B): 175-186.
- JOHANSEN D. A. 1940. *Plant Microtechnique*. McGraw-Hill Book Company, New York & London: 1-523.
- KRISHNAMURTHY K. V. 1988. Endosperm controls symmetry changes in the developing embryos of angiosperms. *Proceedings of the Indian Academy of Sciences.* **98**(4): 257-259.
- MATHUR N. 1956. The embryology of *Limnanthes*. *Phytomorphology.* **6**: 41-51.
- MOHANA RAO P. R. 1972. Morphology and embryology of *Tieghemopanax sambucifolius* with comments on the affinities of the family Araliaceae. *Phytomorphology.* **22**: 75-86.
- O'BRIEN T. P. & McCULLY M. E. 1981. *The Study of Plant Structure: Principles and selected methods*. Termacarphi Pty. Ltd., Melbourne, Australia, 1-344.
- PUSHPANGADAN P. & NAIR K. N. 2005. Medicinal plant wealth of Meghalaya: its conservation, sustainable use and IPR issues. In: *Biodiversity status and prospects*, Narosa Publishing House, New Delhi, 16-23.
- REUNOV A. A., REUNOVA G. D., ALEXandrova Y. N., MUZAROK T. I. & ZHURAVLEV Y. N. 2009. The pollen metamorphosis phenomenon in *Panax ginseng*, *Aralia elata* and *Oplopanax elatus*; an addition to discussion concerning the *Panax* affinity in Araliaceae. *Zygote.* **17**(1): 1-17.
- SUBRAMANYAM K. 1951. Embryology of *Oxyspora paniculata* DC. *Phytomorphology.* **1**: 205-212.
- VENKATA RAO C. 1967. Evolution of the endosperm in Proteaceae. *New Phytol.* **66**: 755-768.
- VENUGOPAL N. & AHUJA P. 2011. Relationship between age, size, fecundity and climatic factors in *Panax wangianus* an endangered medicinal plant in the sacred grove forest of North-East India. *J Forest Res.* **22**(3): 427-435.
- VIJAYARAGHAVAN M. R. & PRABHAKAR K. 1984. The endosperm. In: *Embryology of Angiosperms*. Springer-Verlag Berlin Heidelberg New York, Tokyo, 319-376.
- WEN J. & NOWICKE J. W. 1999. Pollen ultrastructure of *Panax* (the *Ginseng* genus, Araliaceae), an eastern Asian and eastern North American disjunct genus. *Am J Bot.* **86**: 1624-1636.
- WEN J. 2001. Species diversity, nomenclature, phylogeny, biogeography, and classification of the Ginseng genus (*Panax* L., Araliaceae). In: Punja ZK., ed. *Utilization of biotechnological, genetic and cultural approaches for North American and Asian ginseng improvement*. Proceedings of the International Ginseng Workshop, Simon Fraser University Press, Vancouver, 67-88.

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- YU S. C. & KIM W. K. 1991. Ultrastructural changes and formation of storage materials in endosperm cells during the seed formation of *Panax ginseng* C. A. Meyer. *Korean J Bot.* **34**(3): 201-213.
- YU S. C., LEE C. S. & KIM W. K. 1992. Ultrastructural changes of endosperm cells in ginseng (*Panax ginseng* C. A. Meyer) seeds during after-ripening. *Korean J Bot.* **35**(1): 53-60.
- ZHURAVLEV Y. N., KOREN O. G., REUNOVA G. D., MUZAROK T. I., GORPENCHENKO T. Yu., KATS I. L. & KHROLENKO Y. A. 2008. *Panax ginseng* natural populations: their past, current state and perspectives. *Acta Pharmacol. Sin.* **29**(9): 1127-1136.

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