Studies in the embryology of the family meliaceae. - IV. Fertilisation, endosperm and embryogeny of *Aphanamixis polystachya* (Wall.) Parker (Syn. *Amoora rohituka* W. & A.) - A medicinal plant with a discussion on its taxonomic status and horticulture*

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ABSTRACT

R. B. GHOSH. — Studies in the embryology of the family meliaceae. - IV. Fertilisation, endosperm and embryogeny of *Aphanamixis polystachya* (Wall.) Parker (Syn. *Amoora rohituka* W. & A.) - A medicinal plant with a discussion on its taxonomic status and horticulture *. *An. Aula Dei* **11** (3-4): 396-403.

The process of fertilisation and post-fertilisation stages have been explored in *Aphanamixis polystachya* (Wall.) Parker. Triple fusion and syngamy has not been observed. Endosperm formation is autonomous. Nucellar polyembryony is dominant, a process by which seed formation in the species takes place. The placement of the taxon in the Rutaceae has been suggested on the basis of findings of embryogeny. The occurrence of nucellar mebryony will be a helpful tool to the horticulturists for raising 'nucellar seedlings'.

INTRODUCTION

The family Meliaceae is represented by 800 species and 50 genera distributed in tropical America (LAWRENCE, 1951). The embryological work carried out in the family till 1930 was reviewed by SCHNARF (1931). Since then the important contribution in the

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family was made by WIGER (1935) who studied 40 species spread over 13 genera. Of the Indian works mention may be made of GARUDAMMA (1956, 1957), NARAYANA (1958), NAIR and KUSUM KANTA (1961) and recently of GHOSH (1966).

The bark of the species referred to is used as an astringent. The ripe seeds yield an oil which is chiefly used as a stimulating liniment in rheumatism. The oil of the plant appears to be viscous, clear and yellow brown and such oil is also suitable for soap-making (ANON, 1948).

The present paper deals with the process of fertilisation, endosperm and embryo development in the taxon.

MATERIAL AND METHODS

The material for investigation was collected from the plants growing in the Indian Botanic Garden, Sibpore, Howrah, during the months of February-June, 1963-64. The post-fertilised buds were collected and all superficial parts of the buds trimmed and finally fixed in Formalin-Acetic-Alcohol. Usual methods of dehydration, infiltration and embedding were followed. Sections were cut at thickness between 12 μ-18 μ and stained in Heidenhains haematoxylin as well as destained in Picric Acid. Some slides were prepared using combined stains of Safranin and Fast green.

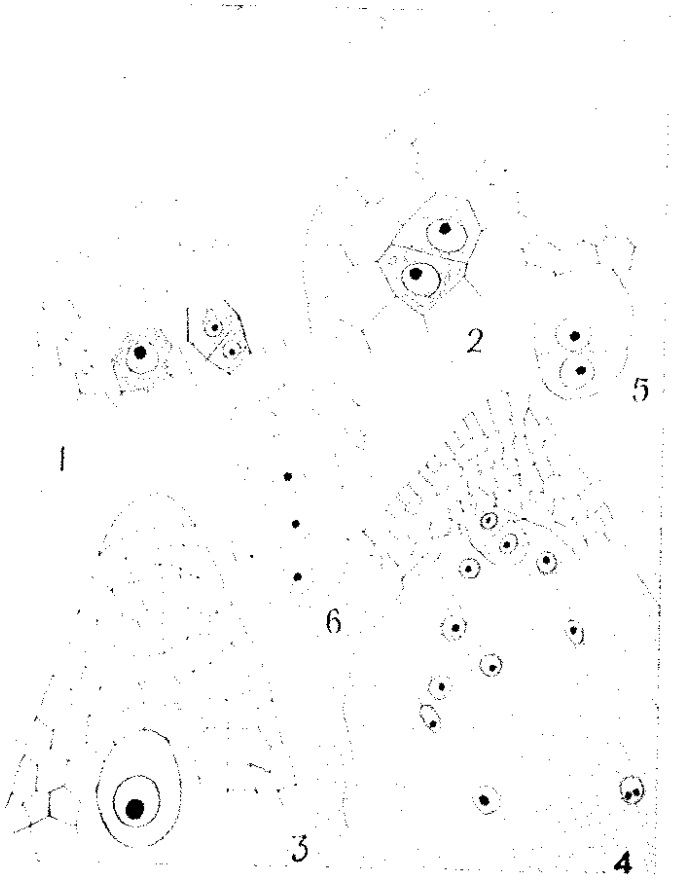
OBSERVATIONS

FERTILISATION AND ENDOSPERM

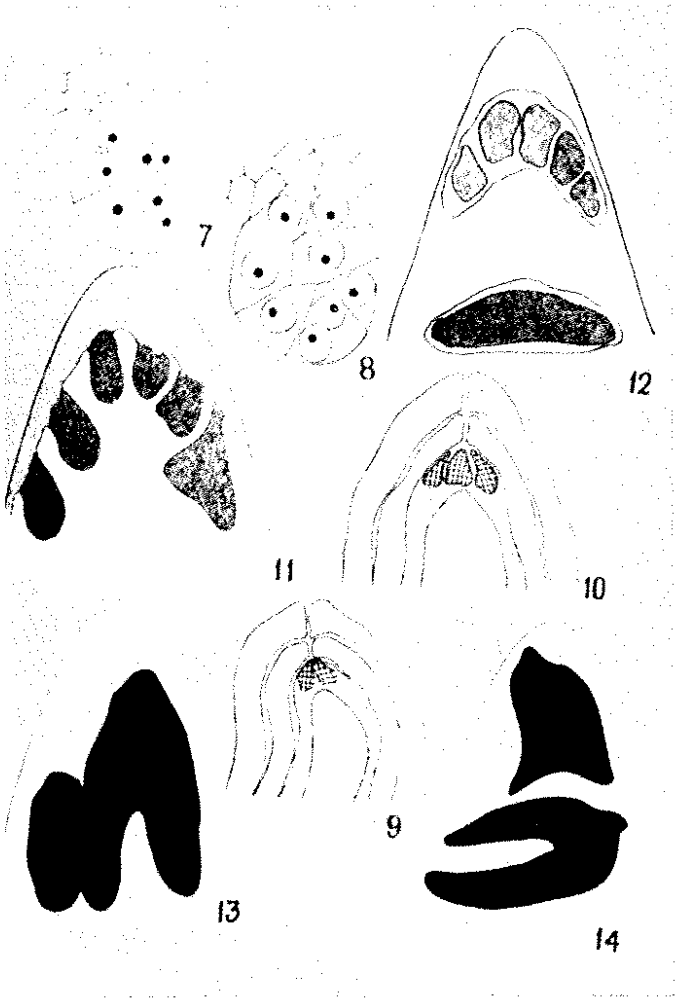
In none of the embryo-sacs examined the entry of the pollen tube was noted and egg apparatus as well as antipodals were found in disorganizing stage except some healthy endosperm nuclei in the sacs. It has been presumed by the author that endosperm development may be autonomous in the taxon as also observed by WIGER (1935) in *Melia* and other species of Meliaceae. The endosperm is free nuclear adequately when there is emergence of a two-celled nucellar pro-embryo near the micropyle (fig. 4).

NUCELLAR POLYEMBRYONY

Although there is lacking formation of sexual embryo in the species, yet the seed-setting device has been adopted by the plant by developing adventitious embryo or embryos from the nucellar cells of the ovules. Of the 300 ovules examined, it has been noted that 1, 2 or more cells from the cells of the nucellus abutting the micropyle are differentiated with deep-stained cytoplasm (fig. 1). These cells either divide obliquely or transversely when still inside the nucellus (figs. 1,2) or after being projected in the embryo sac assuming oval or oblong shape undergo division (figs. 3,5). The nucellar embryo divides primarily into two cells



Aphanamixis polystachya. FIGS. 1-6. — Fig. 1. Portion of nucellus showing two cells with deep-stained cytoplasm, one showing transverse division, x 1200. — Fig. 2. A bit of nucellar tissue showing oblique division, x 1800. — Fig. 3. Portion of nucellus showing enlarged nucellar cell protruded in the margin of the embryo sac, x 1800. — Fig. 4. Portion of mature embryo sac with a bit of nucellus showing 2-celled pro-embryo and free endosperm nuclei, x 1200. — Fig. 5. A 2-celled pro-embryo after first transverse division in the enlarged nucellar cell, x 1800. — Fig. 6. A 3-celled pro-embryo following next transverse division, x 1800.



Aphanamixis polystachya. FIGS. 7-14. Showing different developmental stages of the nucellar embryo and polyembryony, x 1200.

transversely followed by 3-celled stage after another periclinal division either in the lower half or upper half of the 2-celled embryo (figs. 5,6). Sometimes, 2-celled nucellar embryo undergoes two vertical divisions each on the upper and lower half forming quadrant configuration and simultaneously an oblique division takes place in one of the cells of the upper quadrant (fig. 7). After 3-celled stage of pro-embryo, the sequence of divisions are very irregular (fig. 8). Several embryos bud forth from the nucellar tissue (figs. 9,10,11). Fig. 12 shows a mature embryo embedded in the cellular endosperm and five small embryos. The seeds usually contain 2-6 embryos, the maximum number observed was six (figs. 9,11). As a rule, only one of these reaches maturity on ac-

count of mutual competition (fig. 13). It is also interesting to note that in one instance two fully developed embryos were also recorded in a seed (fig. 14).

DISCUSSION

The account of embryogeny so far revealed in the taxon is very irregular and does not either permit or is insufficient to permit any classification of embryonal types (MAHESHWARI, 1950; JOANSEN, 1950). The occurrence of nucellar polyembryony is a dominant feature rather than formation of any sexual embryo in the species, as pollination and fertilisation are not necessary though emdosperm formation is autonomous as reported in *Melia* and other taxa of Meliaceae (WIGER, 1935). GARUDAMMA (1956) after having investigated embryogeny in *Azadirachta indica*, closely allied taxon of Meliaceae featured the development of embryo in 5th group of Megarchetype of the first period of Soueges classification but later on NAIR and KUSUM KANTA (1961) could not typify the embryo of the some taxon as described by GARUDAMMA (1956).

The origin of one additional embryo as recorded by GARUDAMMA (1956) in *Azadirachta* was undertermined but NAIR and KUSUM KANTA (1961) have been able to trace out the origin of additional embryo i.e. synergid embryo in the sane taxon after re-investigation in contrast to nucellar polyembryony in *Aphanamixis polystachva* as recorded by the present author. The sequence of nucellar embryo development in the species under study somewhat simulates with the development of *Xanthoxylum oxyphyllum* (In Press), *Citrus* and *Murraya* (CHAKRAVORTY, 1935-1936), the members of Rutaceae closely beset with Meliaceae under Rutales (RENDLE, 1952).

Taxonomic status

The placement of the taxon referred to in the Meliaceae on the basis of polyembryony is more or less justified and as such the family has been placed in the order GERANIALES by BENTHAM and HOOKER (1862-1893), ENGLER and DIELS (1936) and LAWREN-

CE (1951), as most of the embers of the order show similar occurrence of polyembryony. The present author suggests a more close relationship of the taxon with the members of Rutaceae for showing similarity in the methods of formation of nucellar polyembryony and hence it may be placed in the family Rutaceae along with Meliaceae, Simaroubaceae and Burseraceae which are included in the order Rutales (RENDLE, 1952). The phyletic classification of the order Geraniales (LAWRENCE, 1951) and Rutales and Meliales of HUTCHIUSON (1959) may be reviewed exploring embryology of all the taxa by the present day embryologists.

Horticulture

The Gardeners as well as horticulturists are often fond of cultivating the plant investigated in the gardens as a medical preserve. Frequently, difficulty is encountered in its propagation through seed germination as because the species is apomictic. Sexually formed seeds are abortive leading to failure of germination. So, it is better to adopt means of propagation of the plant by air-layering and cuttings which give better progenies of the species. As raising of rootstocks of *Aphanamixis polystachya* (Wall.) Parker is difficult from seeds, the gardeners, horticulturists, agriculturists as well as nurserymen and medical men interested in medicinal plants may rectify the situation for developing uniform clonal rootstocks, which may be secured from *'nucellar seedlings'* raised from polyembryonic plants of the taxon as prenomenon of polyembryony is profusely met with.

RESUMEN

Se ha estudiado el proceso de fertilización y post-fertilización en *Aphanamixis polystachya* (Wall.) Parker. No se ha encontrado triple fusión ni singamia. La formación del endospermo es autónoma. La poliembrionía nucelar es predominantemente el proceso por el que se realiza la formación de la semilla en esta planta. Se sugiere la colocación del taxon dentro de la familia de las Rutáceas, basándose en dos resultados embriogénicos obtenidos. La presencia de

embrionía nucelar, puede servir de ayuda para producir "plántulas nucelares" de esta especie, dada la dificultad de su propagación por semilla por ser especie apomíctica.

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