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Development of Ovule, Embryo Sac and Embryo in Three Species of Mulberry (*Morus L.*)

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Abstract

This article reveals the comparative studies on development of ovule, embryo sac and embryo of three species of genus morus namely, *Morus cathyana*, *Morus laevigata* and *Morus nigra*, through microtone sections. Commonly, in all the three species, the pistil is bicarpellary, syncorpous having unilocular ovary with a bitegmic ovule on marginal placenta. The matured ovule is anatropous in *M. nigra* and hemitropous in *M. cathyana* and *M. laevigata*. The micropyle is formed by both outer and inner integuments in *M. nigra* while it is formed only by the inner integuments in other two species. In *M. Laevigata*, embryo sac develops only in 12% of the ovules. Occasionally, the antipodal cells become hypertrophied with large vacuoles in *M. nigra*. The hypostase is not conspicuous in *M. nigra* unlike other two species. The phylogenetic significance of these variations in embryological features are discussed in comparisons with other related species and genera.

Keywords: Mulberry (*Morus* spp.); Ovule, Embryo sac; Embryo

Introduction

Various workers have emphasized the role of embryology in systematic and evolutionary studies. There are several examples where embryology has played a decisive role in solving the taxonomic problems of plant taxa. In depth knowledge of plant reproductive biology is also crucial in Germplasm conservation and hence, reproductive biology studies have to be integral features of all conservational projects [1].

Despite of high socio- economic importance of mulberry, studies on its embryology and reproductive biology are scanty. They are mostly restricted to a few species namely, *Moru alba*, *Morus indica, Morus multicaulis* etc. The perusal of literature clearly showed that embryological information is completely absent in *M. cathyana* [2]. Except short reports on microsporogenesis [3], anther dehiscence [4], occurrence of adventives embryony [5] and Megasporogenesis [6], no other information is available in *M. laevigata*. Hence in the present report, detailed embryological studies in three important species namely, *M. cathyana, M. laevigata* and *M. nigra* are made.

Materials and Methods

Three mulberry species were used for present study are *M. cathyana*, (Acc. No. ME- 0018), M. laevigata (Acc. No. ME- 0178) and *M. nigra* (Acc. No. ME- 0008) collected from, Central Sericultural Germaplasm and Resource Centre (CSGRC), Hosur and maintained in the Department of Life Science, Bangalore University, Bangalore. For embryological studies, ovaries and ovules of *M. cathyana*, *M. laevigata* and *M. nigra* at different stages of their development were fixed in 3:1 mixture of ethanol and acetic acid for 24 hours. Fixed materials were stained with a fresh solution of Delafields Haematoxylin for 8 hours, dehydrated in ethanol-xylol series and embedded in paraffin. Cleaning the paraffin infiltration embedding, cutting and mounting as per routine methods. Large numbers of microtome sections of paraffin embedded material were cut into 12-15 microns thick and stained with Haidenhai’s Iron alum haematoxylin and counter stained with 0.5% fast green for 3-5 minutes. Proper orientation of the stained material during embedding is possible and the mounting of a paraffin ribbon with stained sections are easier. The observations were recorded with microphotographs.

Results

Three species of genus morus were used to study, the development of ovules, embryo sac and embryo. Histological analysis of the ovaries at different stages of ovary was made. Longitudinal sections through ovules at this stage showed the pistil is monocarpellary, having unilocular ovary with a bitegmic anatropous ovule. At the tip of the young ovule a group of 3-4 hypodermal cells develop as archesporial cells**.** Each archesporial cell undergo divide periclinally to give rise to outer parietal cells and inner primary sporogenous cells. A single primary sporogenous cell functions as megaspore mother cell. At this stage, the ovule is Orhotropous and the inner integument usually projects well beyond the outer integument**.** The megaspore mother cell undergoes meiotic divisions to produce a linear tetrad of megaspores**.** The chalazal megaspore becomes functional and the remaining three degenerate**.** The rapid growth of embryo sac on one side renders the ovule hemi anatropous/anatropous.

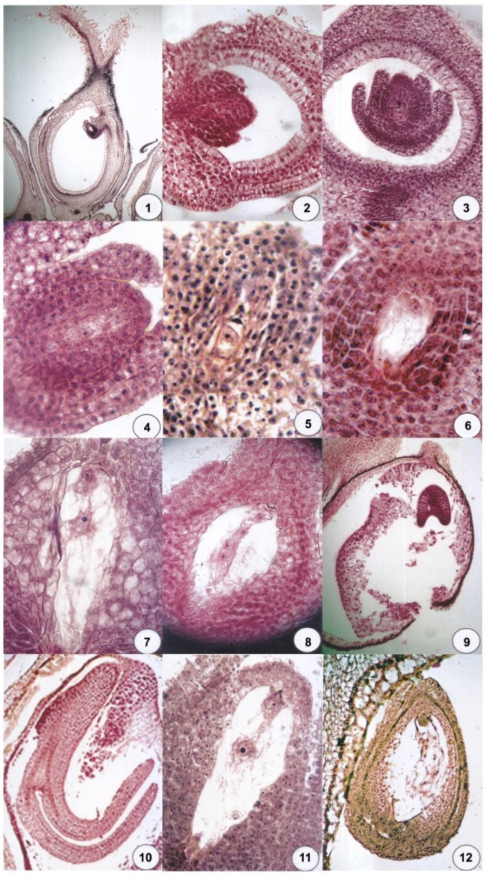

Figure 1: L/S of ovary/ovule showing development of ovule, embryo sac and embryo of three Morus species. 1- Female flower showing unilocular ovary with single anatropous ovule on marginal placenta. 2- Ovule with integumentary initials. 3- Young ovule showing inner and outer integumentary initials. 4- Linear tetrad of megaspores. 5- Functional megaspore and degenerating micropylar megaspore of tetrad. 6- 4 nucleate embryo sac. 7- Organised embryo sac. 8- Zygote with few endosperm nuclei. 9- Tarpedo embryo. 10- Matured embryo. 11- Degenerating of egg and endosperm of embryo sac in *M. laevigata*. 12- Ovule with micropyle formed of both the integuments in *M. nigra*.

Monosporic and Polygonum type of embryo sac cells and egg cells were especially clear. Most of the ovaries showed degenerating synergids and antipodal cells soon after fertilization. Recently, Song et al. [7] confirmed the persistent of three antipodal cells after double fertilization in *Arabidopsis* by expression of florescence of reporter genes.

All other cells of the embryo sac except the egg cell degenerated just before egg division. Hence it is confirmed that the gynogenic embryo originates by division of the egg cell. The secondary nucleus becomes the primary endosperm nucleus. The hypostase is well developed. The endosperm formation is of nuclear type. The embryo development follows the normal pattern. In mature seed the embryo lies curved in the endosperm.

In *Morus laevigata* majority of the ovules showed degeneration of egg and endosperm at different stages of development. Only 12% of the ovule showed further development of chalazal megaspore into embryo sac. Only few matured ovules showed embryo sac. In *M. nigra*, both the integuments participate in the formation of micropyle. Sometimes antipodal cells of this species become hypertrophied. The hypostase is not conspicuous in *M. nigra* unlike other two species **(Figure 1).**

Discussion

Most of the studies on the embryogenesis of Moraceae describe the embryo sac development of as of the Monosporic Polygonum type such as in *Agave* tequilana [8]. In mulberry, gynogenetic plants emerged from the egg cell. Origination of gynogenetic plants from the egg cell has been reported in *Beta vulgaris*. The mode of development of gynogenesis in mulberry is via direct embryogenesis. About 4% of the responding ovaries retained the polar nuclei or secondary nucleus and divided along with egg cell to for free- nuclear endosperm. The number of endosperm nuclei ranged from 10-40; they occupied the embryo sac along with gynogenic embryo.

A similar mode of regeneration is reported in *Allium cepa*. In contrast to these observations there are reports of gynogeneic callus and embryo development from synergids and antipodal cells.

Majority members of the family Moraceae, the ovules are anatropous [2,6,9]. But the mature ovule is hemi anatropous in *M. cathyana* and *M. laevigata* while it is anatropous in *M. nigra*. Unlike *M. indica* all the three species showed multicelled archesporium [10]. The mode of female gametophyte development in all the species confirms to the Polygonum type. This type of embryo sac is common in all the families of order Urticales except in Ulmaceae, where it is of the Adoxa type or intermediate between Adoxa and Drusa type. The degeneration of embryo sac at different stages of development in *M. laevigata* may be due to high degree of meiotic irregularity during sporogenesis as it as a triploid genotype. The endosperm is of nuclear type in the beginning but later it becomes cellular in all the species which is in agreement with similar to previous report *M. multicaulis* [11]. The adevntive embryony observed by Srivastava & Tandon [5] in *M. laevigata* is not found in the present study. While, Gonzalez-Gutierrez et al. reported the endosperm of *Agave tequilana* as of the helobial type [12].

Conclusion

The aim of this work was to study and charecterise such processes in order to generate basic knowledge for its use in taxonomy and production of haploid plants among other uses. And excepting one or two minor variations, there are no major differences in the ovule, embryo sac and embryo of *M. cathyana*, *M. laevigata* and *M. nigra*. This is highly useful in the systematic investigations of mulberry germaplasm and conservation of mulberry biodiversity.

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