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SCAN ME

Research Paper

DEVELOPMENT OF ANTHER AND MICROSPORE IN *Morus indica* VARIETY MYSORE LOCAL MALE

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Abstract

Pollen development is a critical step in plant development that is needed for successful breeding and seed formation. The reproductive nature and fertility, development of anther and microspore in *Morus indica*, Variety Mysore local male was studied through microtome sections. Pollen mother cells of *M. indica* var. Mysore Local male revealed 14 gametic chromosomes (n=14) and exhibited normal meiosis. Simultaneous cytokinesis in both the daughter cells ultimately leads to the formation of isobilateral or tetrahedral tetrad. The pollen grains are of uniform size (19.36 µm) and showed smooth exine and two pores in their wall. The pollen attainability was found very high.

Key words: Mulberry, embryology, anther, meiosis, microspores.

INTRODUCTION

Embryological characters such as development and nature of ovule, integument, embryo sac, nucellus, endosperm, seed and seed coat in female flowers and development of anther wall, anther tapetum, dehiscence of anther, nature of pollen grains etc. in male flowers, are less subjected to adaptive stress, climatic changes, transplantation, hybridization or polyploidization.

Various workers have emphasized the role of embryology in systematic and evolutionary studies as well as genetic improvement programmes. The importance of embryological data in solving the problems of taxonomy was first emphasized by [13]. [8] has indicated that the embryological characters can be taken as an evidence for determining and confirming the interrelationship of doubtful genera and families. According to [10], the embryological characters can be employed as any other

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morphological characters in taxonomy. [9] stated that the embryological characters can be utilized for determining the limits and also for evaluating any scheme of classification. Anther and pollen development have been widely studied in Arabidopsis [17]. It is a complex and important process that leads to the release of viable pollen and plant fertilization.

There are several examples where embryology has played a decisive role in solving the taxonomic problems of plant taxa. Despite of high socio-economic importance of mulberry studies, on its embryology and reproductive biology are scanty, compared to many other economically important crops Plants. Cytological information is an important parameter in identifying and evolving promising cultivars. Most of the cultivated varieties of mulberry are diploid with 2n=28 chromosomes, a few are polyploids [15]. The present communication reported an anther and microspores development in Mysore local variety of mulberry.

MATERIALS AND METHODS

Morus indica variety Mysore local were grown and maintained in the department of Life Science Bangalore University, Bengaluru. Male inflorescences at different stages were harvested between 9.00 A M to 10.30 A. M. And fixed in 3:1 ethanol and glacial acetic acid after 24 hrs of fixation they were washed in 70% alcohol and preserved in the same.

Anther development

The samples were dehydrated through tertiary butyl alcohol and xylol series following standard procedure [7] and embedded in paraffin wax having a melting point between 52^0C and 54^0C. Samples were sectioned at 5-10µm thickness using AO Spencer Rotary Microtome. Sections were stained with Iron alum- Haemotoxylin and counterstained with 0.5% solution of erythrosine dissolved in clove oil. The stained sections were mounted with DPX. Photomicrographs were taken with Leitz Microscope fitted with Sony digital camera.

Meiotic studies

At the time of study, anthers were smeared on a clean glass slide in 2% Aceto-carmine and a cover slip was placed on the smear. Gentle pressure was applied over the cover

slip to get uniform spreading of chromosomes. After scanning PMCs in division, selected PMCs at divisional stages were photographed with Leitz Microscope fitted with Sony Digital Camera.

RESULTS AND DISCUSSION

A very young anther arises as a homogeneous mass of cells bounded by a well defined epidermis and assumes a four-lobed appearance (Fig. 01). Each lobe lodges 6-8 densely cytoplasmic, large nucleated hypodermal archesporial cells. These cells divide periclinally, producing primary parietal and primary sporogenous layers. The primary parietal layer by a similar division produces two layers of cells, the inner of which directly develops into the glandular tapetum. The outer, on the other hand gives rise to 2-3 middle layers and one layer of endothecium. The tapetal cells enlarge radially and become bi-multinucleate (Figs. 02 & 03) as vacuole appears in the cytoplasm. Later the middle layers are consumed and endothecium acquires a characteristic fibrous thickenings. In the meantime the cells of the primary sporogenous layer divide and form a group of microspore mother cells which undergo meiosis to form haploid microspores (Fig. 04).

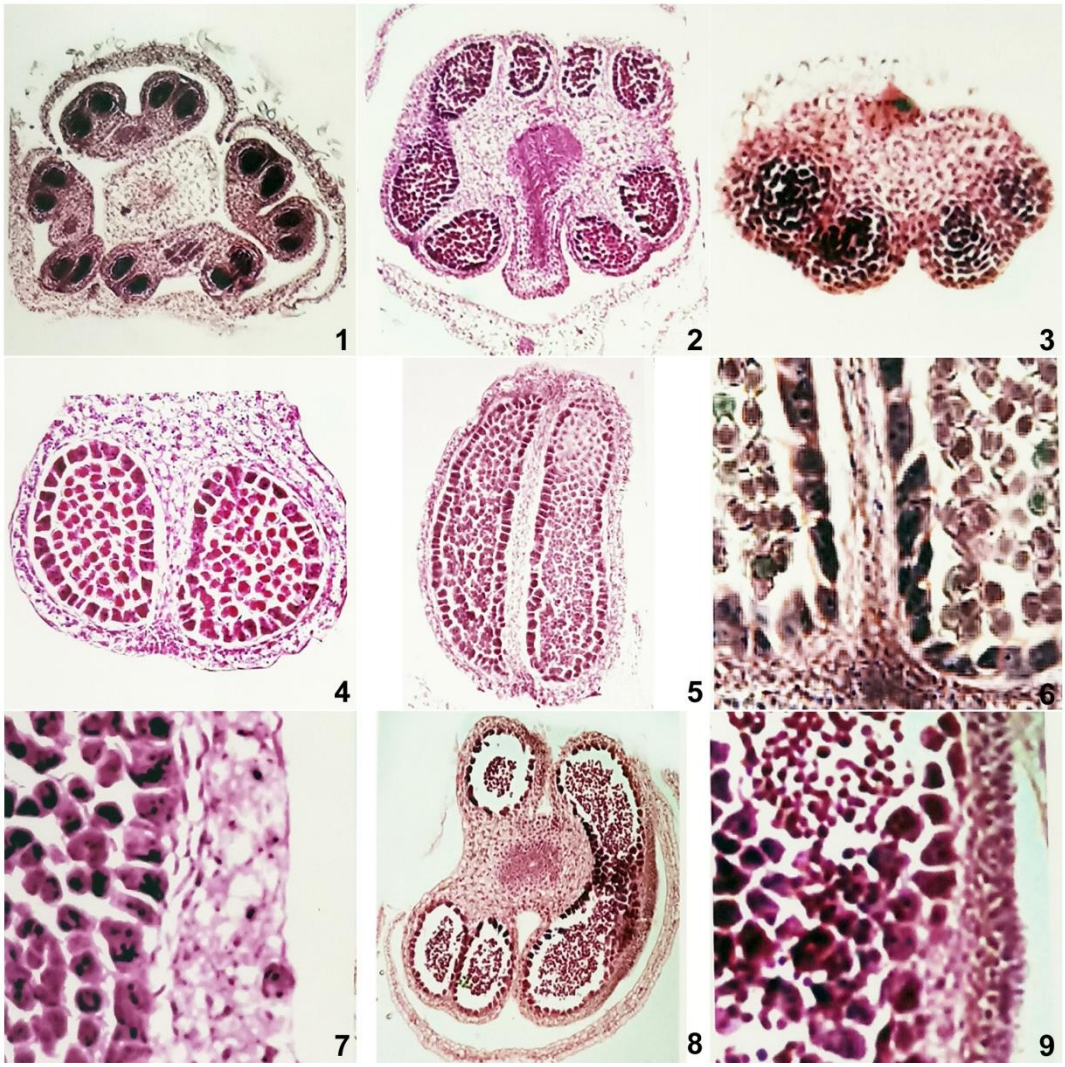
The development of anther wall conforms to the basic, (type I), dicotyledonous (type II), monocotyledons (type III) and reduced (type 1V), [2]. In some families have more than one type, e.g. Solanaceae (type I & II) [1] and the Commenlinaceae (type I & III) (Hardy and Stevenson 2000). In the basic type, all sporogenous cells divide periclinally and differentiate to form the four layers, while in the dicotyledonous type only the outer sporogenous cell layer divides periclinally [1]. It comprises an epidermis, followed by an inner layer of endothecium, 2-3 middle layers and a single layered tapetum (Fig.05). The most conspicuous anther cell layer is the tapetum, which is singer layer of metabolically active cells encasing the developing pollen. The degeneration of the tapetum is required for release of pollen wall materials onto the developing pollen [12], but it also important for normal dehiscence of anther. Epidermal cells are greatly stretched; the endothelial cells enlarge, radially elongated and develop fibrous bands in their tangential walls; the cells of the middle layers become flattened and generally crushed during meiosis. The tapetum becomes glandular with dense cytoplasm and two to many nuclei of varied size. At maturity a stomial groove develops in the wall. The

anther wall becomes fragile along the stomial groove (Figs. 6 & 7). The endothecial cells limiting the stomial groove begin to lose cytoplasm and the cells of the middle layer gets degenerated. The anther wall ruptures along the stomial groove liberating the pollen grains (Figs. 8 & 9).

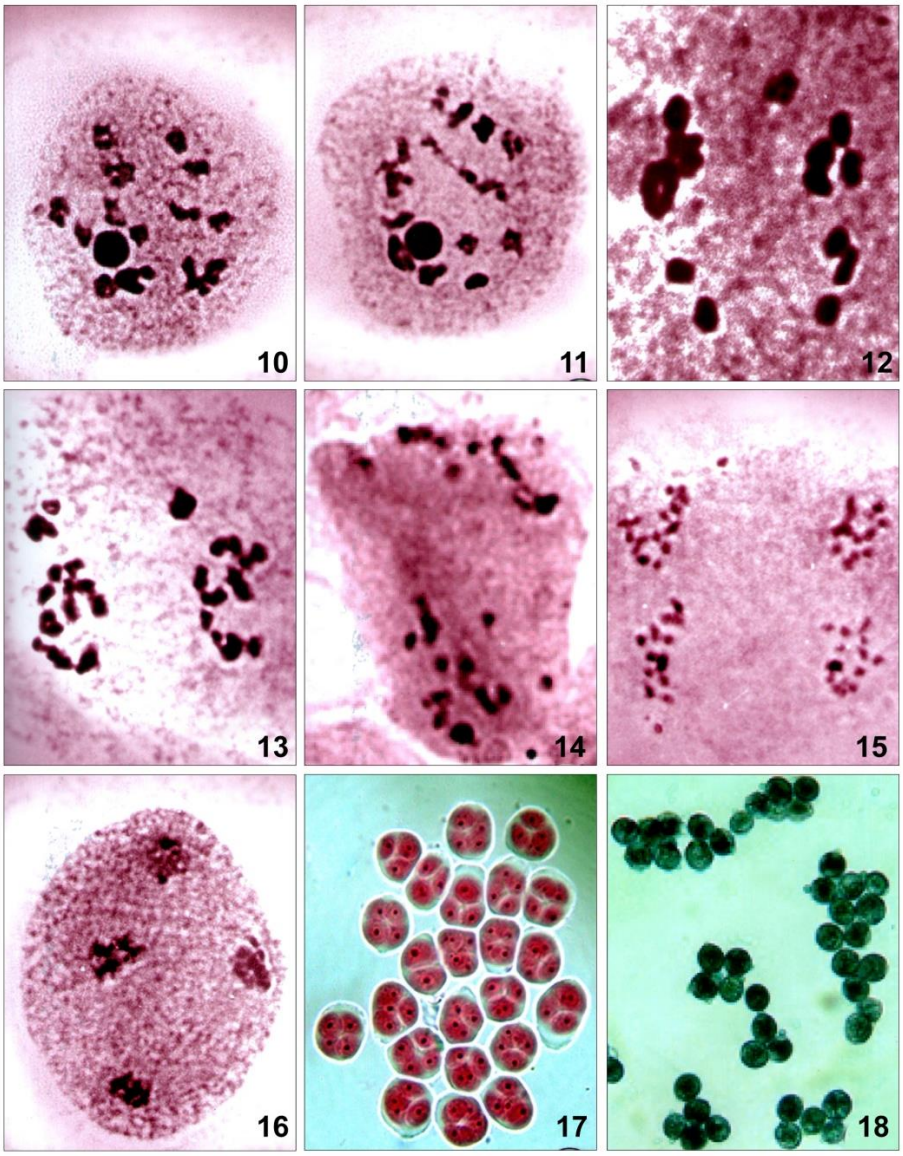
Pollen mother cells or meiocytes undergo meiosis to form tetrads of haploid microspores [11]. *M. indica* var. Mysore Local male revealed 14 gametic chromosomes (n = 14) and exhibited normal meiosis. Regular occurrence of single large nucleolus (Fig. 10) and mega-chromosome pair was found associated with nucleolus (Fig. 11) at diakinesis. At metaphase-I, 12 bivalents and one quadrivalent were found scattered in cytoplasm (Fig. 12). Depending upon the pairing of chromosomes, bivalents and quadrivalent showed different configurational shape. Like wise anaphase I showed equal separation of chromosomes moving towards opposite poles in the ratio of 14:14 and in unequal separation chromosomes were distributed in the ratio of 13:15 (Figs. 13 &14). In Meiosis II, separation of chromosomes at anaphase II was regular and equal number of chromosomes in the ratio of 14:14 observed at each poles (Fig. 15). Telophase II was also observed (Fig. 16). Simultaneous cytokinesis in both the daughter cells ultimately leads to the formation of isobilateral or tetrahedral tetrads (Fig. 17). Majority tetrads showed four spores with uniform in size while, others tetrads were two and three spores. The pollen grains are of uniform size (18.96 µm) and showed smooth exine and two pores in their wall (Fig. 18). The pollen stainability was found very high (94.84%).

The taxon investigated in the present study, *Morus indica* var. Mysore local male displayed diploid chromosome number of 2n=2x=28. Microsporogenesis is quite normal and showed regular pairing of chromosomes in majority of PMC's. The reduction in pollen fertility, association of chromosome, presence of univalents, trivalents and various other meiotic abnormalities were observed in triploid mulberry varieties [16]. Genus *morus* has wide range of chromosome number from 2n = 28 to 2n = 308 and ploidy from x to 22x. [14] has reported 2n = 28 for *M. alba* and *M. indica*. The present findings are also in agreement with the previous report [4 & 15]. Hence the highest chromosome number of 2n= 308 (22x) was reported by [6] in *M. nigra*. Regular occurrence of single large nucleolus and association of megachromosome pair to the nucleolus at diakinesis indicates that two homologous nucleolar organizers of diploid

complement organize a large nucleolus. Presence of 14 bivalents in most of the PMCs and rarely one quadrivalent at metaphase I confirms the diploid nature of the Plant. One quadrivalent was found bigger in comparison to other as also reported by [4] and [3] in the genus *Morus*.



Figures. 1-9: 1. Cross section of a male flower showing four anthers each with four micro sporangia, 2. L/S of a stamen showing tetrasporangiate anther, 3. Cross section of young anther showing four lobes, 4. Cross section of an anther lobe showing wall Layers, 5. L/S of an anther lobe showing wall layers, 6. L/S of micro sporangia showing pollen tetrads and wall layers, 7. L/S of an anther (enlarged) showed wall layers, 8. L/S of a mature anther lobe showing wall layers. 9, L/S of anther lobe- a part enlarged showing the degeneration cells along the stomial groove.



Figures. 10-18: 10. Diakinesis showing chromosome association and nucleolus, 11. Diakinesis-one megachromosome pair associated with nucleolus, 12. Metaphase-I showing 12 bivalents and one quarivalent, 13. Anaphase-I showing equal separation of chromosomes, 14. Anaphase-I showing unequal separation of chromosomes, 15. Anaphase –II showing equal separation of chromosomes, 16. Telophase-II regular orientation of spindle fibers, 17. Pollen tetrad, 18. Pollen grain (Viable and non-viable).

(Scale Bar 6µm).

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