



## Histochemical studies on male gametophyte of *Adansonia digitata* Linn. - An endangered species of Karnataka, India.

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**Abstract:** The distribution of proteins in the developing anthers of *Adansonia digitata* Linn. was investigated. The results indicate that the possible importance of histochemical substances which are present in the tapetum and meiocytes and their derivatives in pollen formation has been correlated. The proteins and their metabolism seems to be essential for the development of fertile pollen. The anthers are tetrasporangiate. The development of anther wall follows the basic type, the epidermis, endothecium, 2-3 middle wall layers and tapetum. The tapetum is bilayered and glandular with multinucleate structure shows rich content of proteins at all stages of its development. At early meiotic of the anther development the meiocytes are rich in total proteins. The cytokinesis is of simultaneous type which results in the formation of tetrahedral tetrads. At subsequent stages of meiosis, the concentration of protein in the meiocytes reduces. The early microspores the quantity of proteins decline and subsequently at pollen grain stage, proteins gradually increase and persists in the mature pollen. The present work seems to be first contribution to the knowledge of protein composition in the developing anther of *Adansonia digitata* Linn.

**Key words:** *Adansonia digitata* Linn.; Microsporogenesis; Meiocytes; Proteins; Tapetum; Tetrads; Pollen grains.

### Introduction

The sexual reproduction process in nature maintains their genetic diversity to adapt to the external environment. As a matter of fact, that it is important to understand the reproductive biology for protection of endangered plants. In most angiosperms, formation of viable pollen is achieved by a series of complex events that require cooperative interaction between gametophytic and sporophytic tissues in the anther (Mascarenhas, 1989; Goldberg *et al.*, 1993). The earlier workers have made numerous studies on the development of pollen along with the localization of different histochemical substances on different plants by Rudramuniyappa and Panchaksharappa (1985), Yeung *et al.*, (2011), Vardar and Unal (2011), Liu *et al.*, (2011, 2012), Vardar *et al.*, (2012), Ekici and Dane (2012), Aybeke (2012), Galati *et al.*, (2012).

Although *A. digitata* Linn. is of economically and ecological important very limited data is available on its reproductive biology. Previous studies have mainly focussed on its morphology and distribution of plant, flower and the development of anthers and ovule, pollen and pollination and reproduction efficiency and its systematic status. (Roa, 1954b) But no data is available on the biochemical aspects of origin and differentiation of anther tissues. However, there is few reports available on anther ontogeny (Rao, 1954b) of some members of the same family, Malvaceae, *Labea divaricata* Mart. (Lattar and Galati *et al.*, 2012), *Eriolaena candollei* Wallich. (Ya Tang *et al.*, 2009), *Excentrodendron bsiennu* Chun WY, How FC, Chang HT, Miao RH. (Ya Tang *et al.*, 2006).

*Adansonia digitata* Linn. is an important arboreal species belonging to the family Malvaceae. It has

been reported that this plant is facing the crisis of survival and hence enlisted as an Endangered Species in "Red data book". (Sughandha *et al.*, 2013). In Karnataka only 11 to 12 trees are available. It is generally called African baobab because it is native to South Africa, Mali, Benin, Senegal, Ivory Coast, Sudan and it is exotic to India. It is said that Muslim traders were credited to their introduction to India.

The plant is a multipurpose tree, as it produces food, non food product. There is also a considerable interest in testing baobab products for evidence-based medicine by many cosmetic pharmaceutical industries in Africa. (Sidibe and William's 2002). Clinical investigation and other researchers suggest that extracts of baobab fruits, flowers and leaves have multiple health effect including antibacterial, antioxidant, antifungal, anti malarial, antipyretic, and almost all parts of the body are used for healing purpose. Active ingredients found in baobab includes steroids, saponins, triterpenes in the (Donatien *et al.*, 2011) fruit pulp. The tree has mythological significance and is known as "Kalpavriksha" in India because every part of the tree is reported to have numerous medicinal uses. It serves as an important source of income for both rural and urban people. (Gebauer *et al.*, 2002).

To the best of our knowledge, no report is available on light microscopic histochemical aspects of anther development in *A. digitata* Linn. In the present study, the events taking place during the anther development in *Adansonia digitata* Linn. were documented with a special attention to the distribution of total proteins.

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## Material and Methods

The flower buds of *Adansonia digitata* Linn. at different stages of their development were collected between 7.00-9.00 am, fixed in FAA for 24 hrs. Following fixation materials was processed using conventional microtechnique methods of dehydration using alcohol butanol series and embedded in paraffin. Sections were cut at 6  $\mu$ m thickness with Leica rotary microtome, and they were mounted on glass slides by using gelatin adhesive. The staining procedure for proteins followed from the "Botanical Histo-Chemistry". (Khasim, 2002). The proteins were localized using Amido Black 10B method. Finally, the stained sections were observed and microphotographed using ProGress C5 ZEISS Imager M2 Bright field/ Fluorescent Modular microscope. Proteins appear bluish colour. The assessment of total proteins in the cells and tissues is qualitative. The results presented here are principally based on the intensity of the colour in the cytoplasm and nuclei normal, rich and intense.

## Results

The young anther primordium is uniformly rich in total proteins (Fig.1A). During subsequent growth the differential distribution of total proteins becomes evidently different in the four lobes of anther primordium. The cells of the lobes are densely cytoplasmic which corresponds to higher concentration of proteins. Both archesporial cells, primary parietal and primary sporogenous cells are rich in cytoplasmic proteins. Further the divisional products of primary parietal cells the endothecium, middle layer and tapetum maintain uniformly rich protein concentration (Fig.1B).

The early sporogenous cells are surrounded by 5 wall layers i.e. epidermis, endothecium, 2 middle wall layers and tapetum, all are having rich cytoplasmic and nuclear proteins in them. (Fig.1C, D). As sporogenous cells increase in their number rich content of proteins persists until they transform into Pollen mother cells (Fig 1E).

When sporogenous cells transform into meiocytes a slight reduction in the protein content is seen in the latter. However, the anther wall layers lose slightly the protein content in them, but the tapetum does not lose its protein content (Fig.1F).

As the meiotic process progress in the meiocytes, further reduction in the cytoplasmic proteins is observed, but the tapetum retains rich content of proteins in both cytoplasm and nuclei. (Fig.1G).

The PMC's have a prominent nucleolus which reacts very strongly for proteins. At prophase the chromatin in the nucleus was thin, thread like and scattered around the nucleolus and reacts

moderately for proteins. The tapetal nuclei undergo multiply become bi-nucleate (Fig.1G).

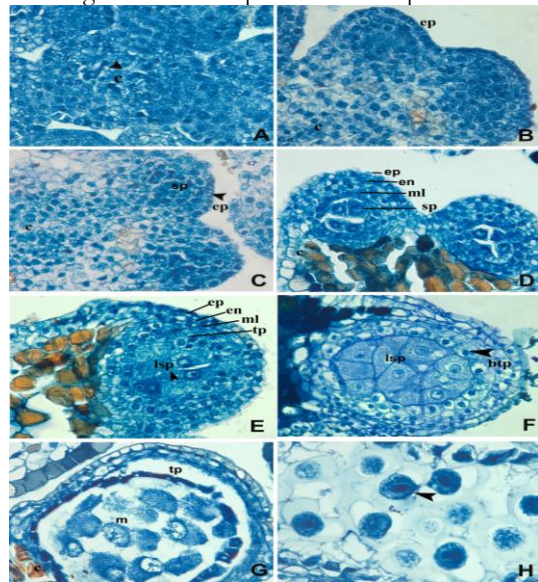
Further, the chromosomes become shorter and thicker reacting for proteins. At metaphase I, the nuclear membrane and nucleolus disappeared spindle fibre appeared and paired homologous protein rich chromosomes were arranged on the equatorial plate of the MMC's. As compared to nuclei the cytoplasm contains less protein. (Fig.1H).

At the end of Meiotic-I, division of two daughter cells are formed with no cell plate between them, then two cell are called dyad cells (Fig.2A). Following meiosis-II division prophase I, the two dyad cells give rise haploid microspores (Fig.2B). In both dyad cell and microspores nuclear protein are high. At anaphase and telophase, the, homologous chromosomes separate from each other moving towards the opposite poles (Fig.2A). At all stages of meiosis, the tapetal cells retain rich content of total proteins, while the middle layer, endothecium and epidermis lose much of the protein content in the cytoplasm. However, the nuclei in all these tissues react more for proteins. (Fig.2C). The young microspore tetrads are formed showing rich proteins and are surrounded by a common callose wall. During this period the middle layers gradually degenerates and the tapetum is fully developed and shows intense concentration of proteins in both the cytoplasm and nuclei. (Fig.2C).

When microspores are released from the tetrad, they are uninucleate and the nuclei is present at the central position. The microspores are rich both in cytoplasmic and nuclear protein content. The vacuole starts appearing (Fig.2D, E) and as a result of this the nuclei displaced towards the periphery of the microspores. During this stage the tapetum begins to degenerate (Fig.2F).

The cells of the endothelial layer enlarge and show very less cytoplasmic protein (Fig.2E, F). The endothelial thickenings differentiate in to endothecium (Fig.2G, H). These thickenings react feebly with amido black 10B. Tapetal cells starts emptying their contents into the anther locule. Both exine and intine of the pollen are protein positive. Similarly, the cytoplasm of the mature pollen is also rich in protein (Fig.2H). The stomium breaks and the pollen grain are set free.

**Figure 1:** T.S of *Adansonia digitata* Linn. anther showing distributional pattern of total proteins.



(ep-epidermis; en-endothecium; ml-middle wall layers; tp-tapetum; sp-sporogenous tissue; m-meicyte; ttd-tetrad; msp-microspores; p. g-pollen grains; c-connective).

Fig.1A: Anther primordium is rich in total proteins; Fig.1B, C: Four lobed anther showing primary parietal and primary sporogenous cells rich in total proteins; Fig.1D: The early sporogenous cells and differentiating tapetum are rich in total proteins.; Fig.1E: Late sporogenous tissue and tapetum are rich in proteins. The anther wall layers contain rich cytoplasmic and nuclear protein content.; Fig.1F: At meiocyte tissue stage, the fully differentiated meiocytes and bilayered glandular tapetum shows lack protein content.; Fig.1G: Microspore mother cells at prophase I show chromatin which are thin, thread like and scattered around the nucleus and shows moderate deposition of protein content.; Fig.1H: Metaphase I of microspore mother cell rich in total proteins. Fig.1A-H: (10X x 40X).

**Figure 2:** *Adansonia digitata* Linn.

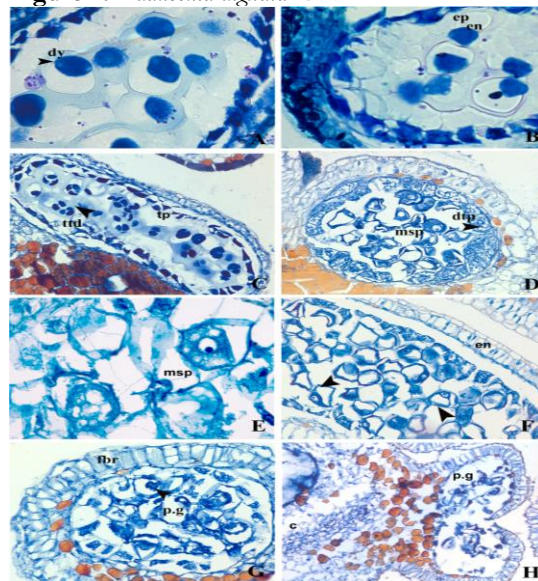


Fig.1A: At Anaphase I, of meiosis of the microspore mother cell showing the homologous chromosomes at the opposite poles (10X x 40X); Fig.1B: At telophase II of the microspore mother cell, tapetum is rich in total proteins whereas, other wall layers show moderate deposition of protein in them (10X x 40X); Fig.1C: Microspore tetrads and tapetal cells are rich in

total proteins. (10X x 20X); Fig.1D: Mid vacuolate microspores and degenerating tapetal cells cytoplasm shows moderate amount of total proteins (10X x 20X); Fig.1E: Late vacuolate microspores cytoplasm and nuclei are rich in total proteins (10X x 40X) Fig.1F: At vacuolate microspores stage, the epidermis, endothelial thickenings lack protein content. Pollen grains are rich in proteins (10X x 20X) Fig.1G: Pollen grains and remnants of tapetum are rich in proteins (10X x 20X). Fig.1H: Dehisced anther showing protein rich pollen grain but anther wall layers lack protein (10X x 10X).

## Discussion

The synthesis, accumulation, and transformation of biochemical substances form a nutritional basis required for the initiation of successive growth phases in the life cycle of a plant. The macromolecules are essential for growth and are metabolically unstable as they are to be broken down in the individual cells. In other words, synthesis and breakdown of biochemical substances show spatial and temporal distribution. The energy is generally utilized for the variety of biosynthetic activities in the cell, as well as other vital cell components. In this regards proteins are vital and take part in the cell metabolism, growth and development.

The present investigation also reveals that, in addition to the requirements of energy the morphogenetic is also needed to evoke developmental reactions. The occurrence of specific biochemical substances in the specific tissue at different stages of anther development, which are under control.

In the present study anther wall prior to maturation consists up to 5 cellular layers. Its development in *Adansonia digitata* Linn. was reported to be basic type which is in accordance with the other members of the family Malvaceae such as *Luebea divaricata* Mart. (Elsa *et al.*, 2012); *Eriolaena candollei* Wallich.; *Excentrodendron hsienmu* Chun WY, How FC, Chang HT, Miao RH. (Tang *et al.*, 2006); *Corchorus argutus* Kunth; *C. birtus* L.; *Luebea candicans* Mart.; *Heliocarpus popayanensis* Kunth. (Lattar *et al.*, 2012).

The sporogenous tissue and the anther wall layers at early stages of differentiation synthesises rich amount of total proteins needed for their growth. The rich proteins in the sporogenous cells suggest that the subsequent event of growth and differentiation of anther are dependent on these substances and also reflects their mitotic and metabolic activities (Goldberg, 1993).

Prior to meiosis, meiocytes resemble sporogenous cells in their protein composition. This suggests that the metabolic status of the meiocytes to undergo further differentiation. Further the reduction in the cytoplasmic proteins in the meiocytes as well as in anther wall layers appears to be a general feature recorded for number of

plants. (Panchaksharappa and Rudramuniyappa, 1974). Perhaps this reduction has relevance with callose deposition. The meiocytes before and during meiosis are generally enclosed by the self-synthesized, refractive layer namely, the callose. The presence of a callose wall around meiocytes is widely regarded as a prerequisite for meiosis in flowering plants. The callose provides a sort of isolation to the meiocytes, keeping them away from the influence from the surrounding sporophytic tissues.

In the *A. digitata* Linn. the cytokinesis is of simultaneous type and the MMC's are rich in proteins. At early prophase the concentration of proteins decline to low level. However, at late meiosis I and II, proteins are resynthesized gradually as seen in the meiotic products reaching rich concentration in the tetrads. Once again, as the microspores are released from the additional callose wall a substantial fall in the concentration of proteins are noticed in the young free-microspores. However, as the microspores differentiate and undergo pollen mitosis, resynthesis of proteins takes place and in the mature pollen grains rich protein content is seen. The shedding pollen grains show maximum concentration of proteins.

#### Tapetum:

The tapetum essentially plays an essential nutritive role. Its role is essential in the formation of viable pollen grains (Echlin, 1971). The tapetum of *A. digitata* Linn. is glandular type (Secretory tapetum) and this character is same with other members of the subfamily Bombacoideae, Such as *Bombax malabaricum* DC., *Eriodendron anfractuosum* DC., *Pachira aquatic* Aubl., *Ochroma lagopus* Sw. (Rao, 1954b) and *Ceiba insignis* Kunth. (Galati and Rosenfeldt, 1998). But the amoeboid type of tapetum is seen *Eriolaena candollei* Wallich; (Tang *et al.*, 2009) which was not in accordance with that of other members of Malvaceae.

According to Bhandari (1984), despite the presence of tapetum formed by two or more cellular layers, the presence of a single layer is more common among angiosperms. The tapetum is uniseriate in most parts, but biseriata in some (present study) which is same that of in *Excentrodendron hsienmu hsienmu* Chun WY, How FC, Chang HT, Miao RH. and *Luebea divaricata* Mart. At the early stages, the tapetal cells possess binucleate cells during microsporocyte meiosis. (Roa, 1954b).

The tapetal cells of *A. digitata* Linn. having a large nucleus and a dense cytoplasm at almost all stage of its development. At premeiotic stages the nucleus of the tapetal cells divides into two results into binucleate tapetal cells. This is in contrast to other members of the family, *Excentrodendron hsienmu* Chun WY, How FC, Chang HT, Miao RH.

(Tang *et al.*, 2006); *Eriolaena candollei* Wallich.; (Tang *et al.*, 2009) where the tapetal cells remain uninucleate at the initial stage and the cells become binucleate at later stages of its development. The bilayered glandular tapetum with multinucleate structure shows rich content of proteins at later stages. But in *Luebea divaricata* Mart. it has been reported multinucleate tapetal cells and this character state are shared with some species of the Malvaceae. The tapetal cells start degenerating at post-meiotic stages, while degenerating the cells become slightly elongate radially with many nuclei, the condition is similar to that of *Eriolaena candollei* Wallich. (Tang *et al.*, 2009).

In most of the angiosperms, at the tetrad stage, the tapetal cells reach their maximum development and shows rich amount of biochemical substance including proteins and this can be correlated with the high rate of metabolic activity in the tapetal cells that are caused by the synthesis of callase (Sevil, 2013). The cells of the tapetum in *A. digitata* Linn. were in contact with the middle layer until the tetrad stage and become highly vacuolated and irregularly shaped from the free microspore stage, and were fully disintegrated before shedding.

The programmed cell death of the tapetum is like that of other plants with a Secretory tapetum (Taylor and Osborn, 2006; Liu and haung, 1999). The present paper provides the first information on the timing of the tapetal degeneration in *A. digitata* Linn.

At the free microspore stage, the degradation of the tapetal cells takes place (Raghavan, 1997) in all angiospermic plants, However *L. divaricata* Mart. it takes place at mature pollen grain stage and in *A. digitata* Linn. at the pollen grain stage the tapetal cells will degenerate. However, in *Jacaranda mimosifolia* D. Don. it takes place at bicellular pollen grain stage (Galati and Strittmatter, 1999). The pollen grains increase in size and spherical in nature and are 2-celled at the stage of release. In the course of enlargement of the pollen grains the tapetal cells are gradually absorbed, and completely disintegrated when the pollen grain matures. Thus at times tapetal degeneration could be correlated with the normal functioning of pollen, as also observed in the present study.

#### Conclusion

The present investigation reveals histochemical features of the anther tissues during the development of pollen in *A. digitata* Linn. and this study is mainly focussed on the changes seen in the biochemical substances with reference to proteins in correlation with the physiological activities taking place during the anther ontogeny. It exhibits the following features; Anther wall development is of basic type. Endothecium

develops fibrous bands of thickenings at the time of anther dehiscence. Middle layer is ephemeral. Tapetum is bilayered and glandular type and degenerates after the microspores are formed. Degeneration of tapetum during microspore development indicates the tissue has provided the required nutrition. Microspore tetrads are tetrahedral. Mature pollen shed at two celled condition.

Rich content of proteins is present in the sporogenous tissue and tapetum. During meiosis, proteins content in PMC are comparatively less. Regain of protein is observed in pollen tetrads. Synthesis and degradation of proteins is observed in specific stages of anther development. So it can be concluded that there is a mutual interaction and utilization of biochemical substances in the anther during formation and differentiation of pollen grains.

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