Chapter-I
INTRODUCTION
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Anther is an important structure because of the central role played by it in plant reproduction and agriculture. The anther produces the male gametophyte, which gives rise to the haploid male gamete during sexual reproduction. The anther also consists of sporophytically derived tissues that nourish and protect the male gametophyte. Many processes are involved in the anther development such as establishment of pattern, lineage specific cell and nuclear division and programmed cell death. These processes are exemplified by events such as the redistribution of organelles and biochemical activities in the microsporocyte and male gametophyte during anther development.

Majority of the angiosperm species produce pollen grains in monads (individual pollen grains). However, occurrence of composite pollen grains (permanent tetrads, polyads or pollinia) is reported in Angiosperm families such as Mimosae, Asclepiadaceae and Orchidaceae. Composite pollen grains are formed by the aggregation of the individual pollen grains. The aggregation of
pollen grains is brought about by the synthesis of a common wall layer (Fitzgerald et al., 1994).

Plants possessing composite pollen grains have a selective advantage in reproduction because they provide an efficient means of dispersal of pollen grains by pollinators (Faegri and van der Pijl, 1976; Kress, 1981). In case of composite pollen grains, single pollination event ensures maximum seed set (Kenrick and Knox, 1982).

The members of Asclepiads and Orchids show some unusual synorganization between parts and organs of flowers. This has led to the evolutionary origin of new type of floral organs that are not present in other Angiosperm plants. Synorganization of corolla and androecium has led to the origin of ‘corona’ and complicated canal system for nector deposition. Synorganization of androecium and gynoecium has led to the formation of ‘gynostegium’ and the ‘pollinaria’. Synorganization of neighboring stamens has led to the formation of guide rails (slits) to attach pollinaria to the pollinator body.
In Asclepiadaceae, the anthers are postgenitally fused with the style head to form gynostegium, while in Orchidaceae the androecium and gynoecium are congenitally fused to form gynostemium. In monandrous orchids there is only one fertile anther, borne on the top of the gynostemium. In diandrous orchids there are two fertile anthers, which are borne subterminally on the two sides of the gynostemium (Endress, 1994).

The pollinarium, consisting of a stalk upon which the pollinia are present, attaches to the pollinator with the help of viscid-like substance. In Asclepiadaceae pollinarium is morphologically heterogeneous because its translator and two pollinia are both gynoecial and androecial in origin. The pollinia are formed in the stamens, while the translator is secreted at the surface of stylar head (stigmatic secretion). The sticky secretion on the stylar head functions as glue for pollen transport.

In Orchidaceae, pollinia and stalk are formed in the anther, whereas the viscidium is produced at the end of the rostellum. Rostellum is a modified apical part of the median carpel. Pollinia
are connected to the viscidium by stalk. In lower orchids the stalk is differentiated from the sterile sporogenous cells of the anther tissue called caudicle (Rasmussen, 1986). In advanced orchids, such as Epidendroideae, the stalk of the pollinium is derived from the tissue of rostellum and is called stipe (Rasmussen, 1986).

Thus, the new organ- pollinarium, in Asclepiadaceae and Orchidaceae, differentiates from the synorganization of originally different floral parts. Although several studies are made on microsporogenesis and gametogenesis, only little attention is paid towards the study on the developmental aspects of entire pollinium (Endress and Stumpf, 1990).

That the flowers of Asclepiadaceae and Orchidaceae have attained a peak in pollination biological adaptation is exemplified by unusual structural and histochemical features of stamens (Hegde et al., 2000). Different authors have defined the pollinium in different way. According to Dressler (1993) pollinium is more or less compact and coherent mass of pollen surrounded by the anther wall layers. According to Freudenstein and Rasmussen (1996) this
definition means even a single massula is a pollinium, which in fact is a one sector of pollinium. Freudenstein and Rasmussen (1996) define the pollinium in more restrictive way as 'a cohesive mass of pollen that is spatially delimited from other such masses by a sterile tissue'. But one pollinium may be connected to other pollinium by extension of modified polliniferous tissue. The inclusion of extensions (caudicle), which function as stalks that attach pollinia to pollinators, again paves way to ambiguity. The stalk or extension may be slender or similar in relation to the size of the main pollinia. Depending upon the relative amounts of pollen and viscid material, caudicles may be distinct in colour and composition from the pollinia, but they may be similar also. Thus, there is a possibility to have a continuum of morphologies from a pair of separate pollinia, through two pollinia connected by caudicles, to a single lobed pollinium (Freudenstein and Rasmussen, 1996). In the present thesis, any contiguous mass of pollen, formed in a common loculus, is considered as pollinium and connections between pollen masses or anther loculus are not taken into consideration.
Aim and Scope of the present work:

There are some excellent papers on the pollinium ontogeny. On the contrary, data on histochemistry of developing pollinium is very scarce (Vijayaraghavan and Shukla, 1976, 1977; Vijayaraghavan and Cheema, 1978; Hegde and Rudramuniyappa, 1986; Arora and Kapil, 1989; Fitzgerald et al., 1994; Hegde et al., 2000). Papers on pollinium contain a great deal of information on structural changes, but not on the underlying mechanism that causes such changes. Due to this lacuna, the developmental processes leading to the production of pollinium or polyads are not well understood.

All higher organisms are multicellular. The collective shapes, functions and interactions of its component cells determine the structure and functions of a particular tissue or organ. Therefore knowledge of how individual cells function and how they change their biochemical activities is very important to understand the processes of development. Anthers are heterogenous in structure consisting of epidermis, endothecium, middle layer(s), tapetum and reproductive cells. Each anther cell-type attains specific functional
ability by virtue of its biochemical composition. In other words the biochemical specialization of cells reflect their developmental potential. The developmental potentials of cells do not remain same throughout the development of the anther. By employing a technique for in situ localization of metabolites, biochemical characterization of cell and tissue-types can be assessed. In this regard histochemical technique provides both structural details as well as a picture of changing chemical composition of cell- and tissue-types during development. Histochemical studies carried out on the anthers of flowering plants have shown that qualitative and quantitative histochemical composition of cells prove critical and responsible to set the course of development of anther. Such information on the pollinium development is lacking. Keeping this in view, present ontogenetical and histochemical studies on the anthers of *Moringa oliefera* Lam, *Dregea volubilis* (L. f.) Benth. ex Hk.f. and *Dendrobium ovatum* (Willd.) Kranz. are undertaken with the aim to identify the histochemical basis of anther development and function in these plants.
The present study tries to address the following intriguing questions about anther development by assessing:

1. The temporal and spatial domains of syntheses and degradation of specific histochemical substance(s).

2. Structural and histochemical variations in anthers that produce monad and polyad pollen grains.

3. Structural and histochemical variations in the anthers of Asclepiadaceae and Orchidaceae members.

4. Nature of mechanism(s) responsible for bringing aggregation of pollen grains in Asclepiadaceae and Orchidaceae members.

The reasons to select *M. oliefera*, *D. volubilis* and *D. ovatum* include (1) lack of information on histochemistry of anther development in these plants (2) easy accessible of these plants and (3) histochemical basis of anther development can be generalized from these plants to other plant species.

In the present investigation, *in situ* localization of insoluble polysaccharides, cellulose, callose, sporopollenin, ascorbic acid, lipids, RNA and total proteins has been carried out because of their
vital role in growth and development. This does not imply that other metabolites have no role to play in the growth of anther. In the present thesis, histochemistry and ontogeny of reproductive cells and surrounding anther wall layers are studied. The structures that connect the two pollinia are not included in the present investigation.

The present work confines to qualitative histochemistry only. Despite the importance of quantitative histochemistry, it is not attempted in the present work due to non-availability of cytophotodensitometer in Karnataka University or in the nearby research stations. However, based on the relative differences in staining intensity, an attempt has been made to describe the histochemical data on arbitrary quantitative terms. In the histochemical tests the staining intensity is directly proportional to the quantity of substance present in the cells. Since uniformity is maintained in the thickness of anther sections, the differences in staining intensity are taken as indications of quantitative differences in the substance.