FLORAL AND REPRODUCTIVE BIOLOGY STUDIES IN *Spathiphyllum wallisii*

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FLORAL AND REPRODUCTIVE BIOLOGY STUDIES IN *Spathiphyllum wallisii*

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Dedicated to

My Family

DIVISION OF HORTICULTURE

UNIVERSITY OF AGRICULTURAL SCIENCES

BANGALORE

CERTIFICATE

This is to certify that the thesis entitled " FLORAL AND REPRODUCTIVE BIOLOGY STUDIES IN Spathiphyllum wallisii " submitted by Ms. SAVITHA.H.V., in partial fulfilment of requirements for the degree of *Master of Science (Horticulture)* in *FLORICULTURE* of the *University* of Agricultural Sciences, Bangalore, is a bona fide record of research work carried out by her during the period of her study in this University under my guidance and supervision and that no part of this thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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INTRODUCTION

I INTRODUCTION

Floriculture trade is one of the most rapidly expanding and dynamic global enterprises. In India, commercial floriculture is a recent development. The ornamental plants have expanded their horizons from being objects of hobby to products of lucrative business. The potted plants and flowers can earn foreign exchange of more than Rs 100 crores, if their export potential is efficiently tapped. In addition, the Indian floriculture industry is least capital intensive. The ornamental potted plants constitute an important part of floriculture trade. These account for 48 per cent of India's export of floriculture products. The major customers of Indian potted plants are Middle east and western Europe. Apart from export, the demand for these plants in the local market also is increasing specially in urban areas.

In the foliage industry, 1/3 of the plants belong to the family of Araceae (Henny,1988). It has versatile house plants and plants for various situations in the landscape. The members of this family are native to tropical regions. The family has 110 genera and 250 species (Croat,1979). The ornamental aroids are valued either as flowering, foliage or flowering foliage plants. Eleven genera of ornamental aroids are important, namely *Aglaonema, Alocasia, Anthurium, Caladium, Dieffenbachia, Epiprenum, Monstera, Philodendron, Spathiphyllum, Zantedeschia* (Henny,1988). Among these plants, *Anthurium* and *Zantedeschia* have beautiful coloured Spathe and Spadix, the inflorescences of which are highly valued as cut flowers. Other plants are valued for their varied range of colourful foliage.

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Aroids are mostly tropical herbs with fleshy woody stems, others root climbers with variable leaf forms. They are characterised by inflorescences composed of densely flowered spadix, subtended by a spathe or bract, often coloured and showy (Graf, 1982).

A major distinguishing trait of ornamental aroids is their floral structure which consists of a spadix subtended by a modified bract or spathe. The spadix is a central fleshy spike covered with many flowers and is enveloped by the spathe till anthesis. The flowers are relatively small, may be bisexual or unisexual . *Anthurium, Epipremnum, Spathiphyllum* have bisexual flowers while the rest of the genera have unisexual flowers. In case of species with bisexual flowers, the spike in the spadix is completely covered with flowers and in those with unisexual flowers, generally the pistillate flowers are on the lower 1/3 of the spadix and staminate flowers on the upper one or two thirds. The central region is sterile.

Spathiphyllum wallisii is one of the ornamental plants belonging to the family "Araceae". It is commonly called as "Peace lily". It is popular as a flowering foliage plant because of its beautiful, glossy green foliage and inflorescence having white spathe. The inflorescences can be used as cutflowers. This plant is becoming more and more popular these days because it is a hardy plant easy to propagate that blooms through out the year. In addition, there are no serious pests and diseases that threaten its cultivation and maintenance.

Research concerning the culture of ornamental aroids exceeds that pertaining to breeding partly due to lack of basic information on breeding methodology and long

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breeding cycles. As a result, most new cultivars of ornamental aroids have originated from mutation of established cultivars or introduction of new species collected from the wild (Henny 1988).In *Spathiplyllum wallisii* also there is need to take up improvement work in order to obtain plants with varied forms and colours of the foliage, which can increase its popularity as a potted plant. Improving the forms and colour of the spathe can increase its popularity as a cut flowers. Also, there is need to incorporate resistance to certain diseases as Cylindrocladium rot, Dasheen mosaic virus. To take up such a work, the basic information on floral and reproductive biology of the plant is required. With this background, the present study was carried out with the following objectives:

To study (i) Floral biology.

(ii)Microsporogenesis and male gametophyte development.

(iii)Megasporogenesis and female gametophyte development.

(iv) Vase life of cut Spathiphyllum flowers.

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REVIEW OF LITERATURE

II REVIEW OF LITERATURE

In the family "Araceae" the work on reproductive biology has been reported in many of the members. Reports on the study of floral biology aspects in the members of the family are very meagre. The work on floral and reproductive biology in the Genus "Spathiphyllum" has not been carried out. A brief review of the work done with respect to reproductive biology, floral biology and vase life of ornamental aroids and a few other crops has been presented here:

2.1 FLORAL BIOLOGY

Croat (1980) studied the flowering behavior in the genus *Anthurium*. He observed marked protogyny in many species belonging to this genus. Considerable differences in the rate of development of flowers, the precence, source and amount of stigmatic nectar and the method of pollen presentation were observed. In all the species studied, it was noted that the lateral pair of stamens emerged first, followed by the anterior and then the posterior stamen of the alternate pair. Variations in the degree of exertion of stamens,disposition with respect to stigma, degree of retraction, changes in pollen colour among different species were also reported. Some species had stamens which are retracted completely after opening, while a few species were observed to merely force the pollen out in the form of long ribbons. Considerable differences in the flower aromas with both fly and bee pollination syndrome were noticed.

The members of the genera, *Spathiphyllum* and *Anthurium*, produce seeds readily

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if pollinated when the stigmatic surfaces appear to glisten (Henny, 1989). He also has reported about using GA for induction of flowers, factors affecting pollination, genetics of ornamental tropical aroids and also the hybridisation work carried out in the genera, *Aglaonema, Anthurium, Dieffenbachia* and *Spathiphyllum*.

Galil (1990) reported that pollen germination in *Arisarum vulgare* occurred in two phases, the aeration phase and the germination proper phase. According to him, aeration is a crucial prerequisite for germination. He observed that in the absence of aeration, the pollen scattered on the inner wall of the inflorescence chamber failed to germinate. The decrease in relative humidity, with in the chamber at noon, increased the aeration while the increase in humidity at night promoted germination.

In anthuriums, anthesis occurs between 8-10am on a fairly sunny day. The anthers project out of the spike and dehisce to release the pollen. All anthers in an inflorescence emerge in 4-8 days. Anther emergence is comparatively less in March and July. A well fertilized inflorescence sets 100-200 fruits as reported by Mercy and Dale (1994). The authors have given information about systematics and morphology, cytogenetics, floral biology, pollination and fertilization, cultivation, disease and pest control in Anthuriums.

2.2.HISTOLOGICAL STUDIES IN THE FAMILY ARACEAE.

2.2.1. Anther

In the young anther primordium, the archesporium is differentiated in the hypodermal layer consisting of a vertical file of cells in *Theriophonum minutum*

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(Parameswaran, 1959). The pollen mother cells(PMC's)undergo reduction division in successive manner. The tetrads can be isobilateral, decussate, or tetrahedral (Maheswari and Khanna, 1956). When microspores are being formed, the cells of the tapetum lose lateral contact with one another and cytomixis occurs. The *en masse* protoplasts start expanding centripetally, ensheathing the developing microspores forming a "Periplasmodium". The presence of periplasmodium has been reported in many of the plants belonging to Araceae as, *Dieffenbachia seguine* (Campbell, 1900), *Symplocarpus foetidus, Peltandra undulata* (Duggar, 1900), *Typhonium trilobatum* (Banerji, 1947), *Aglaonema versicolor* (Gow, 1908 b) *Peltandra virginica* (Goldberg, 1941), *Arisaema wallichianum* (Maheswari and Khanna,1956) and *Theriophonum minutum* (Parameswaran, 1959).

The pollen grains are spheroidal or echinulate (Maheswari and Khanna,1956), shed at two celled stage. Exine is relatively thin and shows tiny spinescent sculpturing. The epidermis of the anther wall remains intact at maturity (Parameswaran, 1959). Longitudinal dehiscence of anthers has been reported in *Arisaema wallichianum* (Maheswari and Khanna, 1956). The pollen grains were reported to be two celled at dispersal stage in *Arisaema, Dieffenbachia, Spathyema, Symplocarpus, Synandrospadix* and *Theriophonum*.Pollen grains, were shed at three celled stage in *Aglaonema, Arum, Peltandra*, and *Zantedeschia* (Kapil,1967).

2.2.2 Ovule

In *A. wallichianum* the ovule is bitegmic and crassinucellate (Maheswari and Khanna,1956). In *Theriophonum minutum* it is tenuinucellate. During development the inner and outer integuments originate from the base of the nucellus. Though initial growth

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of the inner integument is relatively rapid, the outer integument overgrows the inner during later ontogeny (Parameswaran, 1959). But in case of *Agaloenema versicolor* both the integuments appear to be more or less of the same height (Gow, 1908b). The nucellar epidermis, by periclinal divisions produces a "nucellar Cap" (Johri *et al* 1993). In *Theriophonum minutum* the nucellar cap develops at four nucleate embryo sac stage and its remnants persist upto the late zygote stage (Parameswaran,1959).In aroids,the cells of the inner integument that line the embryo sac, become radially elongated in a plane perpendicular to the long axis of the embryo sac and differentiate into "Endothelium" (Maheswari and Khanna, 1956).

The archesporial cell is hypodermal in *Arisaema wallichianum* and sub epidermal in *Theriophonum minutum* (Maheswari and Khanna, 1956, Parameswaran, 1959).Early degeneration of nucellar cells has been reported in *A. wallichianum* that makes the embryo sac come in direct contact with the inner integument. The nucellar cells at the micropylar end form the nucellar cap and those at the chalazal end undergo division to form a short column. After fertilization the nucellar column is absorbed as a result of haustorial activity of the basal apparatus. The disintegration of nucellar remains during early stages in the development of the endosperm has been reported in many other representatives of the family eg., *Lysichiton* (Campbell, 1899), *Dieffenbachia seguine* (Campbell, 1900), *Aglaonema commutatum Spathicarpa sagittaefolia* (Campbell, 1903) *Anthurium violaceum* var leucocarpum (Campbell,1905) *Arisaema triphyllum* (Gow, 1908a)*Typhonodorum lindleyanum* (Boodle and Hill, 1929), *Peltandra virginica* (Goldberg, 1941) and *Typhonium trilobatum* (Banerji, 1947).

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After meiosis of the archesporial cell, generally the chalazal megaspore functions in *Arisaema wallichianum* and *Theriophonum minutum*, giving rise to a 'polygonum' type of embryo sac(Maheswari and Khanna,1956, Parameswaran,1959). Polygonum type of embryo sac is found to occur in other members of the family also.

The synergids in *Arisaema wallichianum* are pear shaped. Fusion nucleus is situated close to the antipodals(Maheswari and Khanna,1956).The polar nuclei fuse before fertilization. The antipodals are ephemeral (Johri *et al,* 1993). But they are persistent in *Theriophonum*. They enlarge in *Arum orientale* and *Symplocarpus foetidis* and multiply in *Aglaonema*, *Lysichiton* and *Xanthosma* (Johri *et al* 1993).

2.2.3 **Endosperm and Embryo**

In Araceae development of the endosperm is of the cellular type. The division of primary endosperm nucleus is followed by formation of a wall separating the micropylar chamber from chalazal chamber.The micropylar chamber develops into cellular endosperm. The chalazal chamber remains uninucleate and becomes haustorial with a hypertophied nucleus. (Johri *et al*, 1993). This is referred to as the "Basal apparatus". This is reported to be a common feature of 'Araceae' (Maheswari and Khanna,1956). Out of the twenty four species in Araceae, eleven species have been reported to have an *ab initio* cellular endosperm. In the remaining spices, the endosperm is either nuclear or helobial(Parameswaran,1959). Helobial type of endosperm has been observed in *Artopsis peltata* (Gowda,1980).

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Zygote undergoes a transverse division. The basal cell does not divide, but elongates and forms a suspensor (Johri *et al*,1993). The terminal cell undergoes repeated divisions and forms an embryonal mass in *Theriophonum minutum* (Parameswaran, 1959). In *Pistia stratiotes,* the epicotyl and cotyledon develop side by side from the embryonal apex. (Haccius and Lakshmanan, 1966).

The seed is operculate (Buell, 1935) with a cylindrical embryo in *Theriophonum.* The testa is multiplicative, parenchymatous and contains raphides. Some of its cells and the cells of the inner epidermis of the tegmen are tanniferous(Johri *et al*, 1993).

2.3 HISTOCHEMICAL STUDIES IN OTHER CROPS..

The review of research work done on histochemical localisation of macromolecular substances at successive stages of anther and ovule development in different crops is presented here:

2.3.1 Anther

Taylor (1959) after auto-radiographic studies in *Lilium longifolium*, reported that large amount of total proteins was present in the sporogenous tissue in both nucleus and cytoplasm. Labeled RNA accumulated in tapetum during DNA synthesis and also after late nuclear division. Proteins accumulated in tapetum after nuclear division as it was necessary for the microspore wall formation.

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In Maize anther, tapetal RNA synthesis increased during meiotic metaphase and further accumulation was in tetrad, proteins accumulated upto mid meiotic prophase in tapetum. In meiocytes, proteins accumulated through the early prophase. There was no synthesis during meiotic mitosis and proteins accumulated in the developing spores(Moss and Heslop-Harrison,1967).

The pollen and pollen tubes of *Pennisetum typhoidium* Rich. were rich in starch, proteins , lipids and polysaccharides but not reducing sugars. The male cells were rich in RNA, protein and lipids (Panchaksharappa and Rudramuniappa, 1972).

Panchksharappa and Syamasunder (1974) reported the presence of rich cytoplasmic and nucleolar RNA in sporogenous tissue, PMCS, dyads, tetrads and microspores in *Iphigenia pallida.* It is suggestive of high rate of metabolism, where as the insoluble polysaccharides and proteins were at low level in sporogenous tissue and PMC'S. Large number of starch grains appeared in the vegetative cell of the young two celled pollen grains which disintegrated and formed densely PAS positive cytoplasm in the shedding pollen grains. The cytoplasm of the PMC's, dyads,tetrads, microspores and pollen grains was rich in RNA. The tapetal cells were rich in RNA and considerable concentration of protein and polysaccharides was noticed in these cells. The fibrous endothecial thickenings were lignified.

Comparitve histological and histochemical studies of anther development in cytoplasmic male sterile lines and normal lines of Sunflower(Helianthus annuus L.) revealed that the development in both was similar till the on set of meiosis. As meiosis progressed, there was abnormal enlargement of binucleate tapetal cells evetually crushing the meiocytes. These hypertrophied tapetal cells of CMS anther were significantly low in

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RNA and proteins. At PMC stage, there was starch accumulation and aberrant, thin callose deposition around meiocytes in these lines in contrast to low amount of starch and thick callose deposition around PMC's in normal anthers (Vittala Raya Kini, 1981).

In mango(*Mangifera indica*), the sporogenous cells in the anther had rich polysaccharides, RNA and intense protein content indicating high rates of metabolism in them. The differentiation of the glandular, binucleate tapetum was evident at tetrad stage. The tetrads, microspores had rich polysaccharide, intense RNA and protein content (Anitha Karun,1989).

Low cytoplasmic polysaccharides and RNA were observed in anther primordia of *Pyrostegia venusta.* But they increased to high level in PMC's, as reported by Aswath *et al* (1989). The tapetum at tetrad stage remained unchanged in size indicating its hyper activity which prevented proper development of microspores in to pollen grains.

2.3.2 **Ovule**

In *Lilium longifolium*, the megaspore mother cell (MMC) had reducing sugars, lipids and high RNA content. Rise in protein and RNA content was noticed at the early stages of development in the embryo sac(Miki-Hirosige, 1964).

Pritchard (1964) reported that in *Stellaria media*, the archesporium and MMC had PAS positive cell wall, PAS negative cytoplasm and high RNA. Rich protein content was noticed in archesporium. While MMC had low proteins. The egg cell had PAS positive

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cytoplasm which was also high in protein content. Synergids possessed PAS positive cytoplasm, low RNA and proteins. Central cell cytoplasm was weakly PAS positive but rich in RNA and proteins. Antipodal cells had low RNA and proteins. Around 2 and 4 nucleate embryo sac stage starch grains were accumulated.

According to Jensen, (1965a,b) the egg cell in cotton contained a few starch grains. It had very high RNA and intense protein content. Central cell possessed rich starch, RNA and intense protein content.

Histochemical study of embryo sac development in *Vanda* revealed that the archesporial cell had PAS positive cell wall and PAS-Negative cytoplasm. MMC showed PAS negative cytoplasm and was low in RNA content. Megaspores had PAS negative cytoplasm and were rich in RNA and protein. PAS negative cytoplasm was observed in 2, 4 and 8 nucleate embryo sacs. Egg cell cytoplasm had PAS positive nucleolus and high RNA but low protein content. Synergids had PAS positive cytoplasm,and were rich in RNA and proteins. Antipodal cells had PAS positive cytoplasm but were low in RNA and proteins. The central cell had low PAS positive cytoplasm which was rich in RNA and proteins (Alvarez and Sagawa, 1965).

In *Dipcadi montanum* the insoluble polysaccharides were at very low level in archesporium, MMC,dyads, tetrads and functional megaspore while RNA and proteins were at high level in all of them. The cytoplasm of young embryo sac and the central cell in the mature embryo sac had low concentration of polysaccharides, RNA and proteins. In the organised embryo sac the egg cell had low concentration of cytoplasmic polysaccharides and RNA while proteins were at high level. The Synergids had densely PAS positive

filiform apparatus and rich proteins and less RNA content. Antipodal cells had rich polysaccharides and proteins. The cells of hypostase and those of the integuments had rich RNA and proteins(Panchaksharappa and Syamasunder, 1975).

In *P.venusta*, the archesporium and MMC were found to be poor in insoluble polysaccharides and intense in protein content. The archesporium was found to be intense while the MMC was rich in RNA content. The megaspores showed the accumulation of starch but were poor in RNA and protein content indicating decreased metabolic activity. The megaspores degenerated in all the ovules studied, and there was no development of the female gametophyte (Aswath *et al*, 1989).

2.4 VASE LIFE STUDIES

2.4.1 Vase life Studies in Araceae

There are hardly any reports regarding the vase life studies in Spathiphyllum. In case of another ornamental aroid, 'Anthurium' which is quite popular as a cut flower, there are some reports on the vase life studies. They are presented here:

Watson and Shirakawa (1967) studied shelf life of anthurium flowers in relation to gross morphology. They suggested that picking the flowers when the stigmas are receptive should be avoided as water loss would be greatest at this stage.

The vase life of eight clones of anthurium flowers cut at different stages was

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studied by Kalkman (1983). He recorded longest vase life with flowers cut when the spathe was almost completely white. Avo-cintha and Avo-Ingrid had longest average vase life amounting to 31.3 and 25.4 days respectively in winter and 29.1 and 25.4 days respectively in Summer.

Phytotoxicity resulting from HCN fumigation, a potential disinfestation treatment was measured for various cut flowers and foliage by Hansen *et al* (1991) Midori anthuriums and wet ozaki anthurium showed phytotoxicity at 4600 ppm of HCN.

Temperatures below 13^0C caused bluing of red anthuriums, dark red ones being most susceptible as reported by Sytsema and Barendse (1974). Preservatives were found to have no effect on bluing. They also observed that flowers that were picked too early, wilted very quickly. The sugar content, respiration rate and spathe quality of cut flowers of *Anthurium andreanum* Cv scarlette, was studied after storage at chilling temperatures. It was found that storage at chilling temperature of 4^0C tended to slow the loss of reducing sugars probably due to slower rate of respiration. The spathe quality (bluing) was not directly related to the content of reducing sugars (Pritchard *et al*, 1991).

Akamine and Goo (1972) studied the vase life of anthuriums in relation to physical characteristics and found that vase life was inversely related to petiole length, diameter, and flower weight.

Pulse treatment with silver nitrate was found to extend the vase life of anthuriums Cv Ozaki red. Maximum vase life was observed in flowers treated with 4μM silver nitrate for twenty minutes with in 12 hours of harvest. Silver thiosulphate complex was less

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effective than silver nitrate (Paull and Goo,1982). The use of waxes for extending the post harvest life of anthuriums was studied by Paull (1983). He reported that FMC-819, carnauba based wax was most effective in increasing the vase life from 18 days in control to 36 days and also that it imparted high gloss to the flowers. Paull and Goo (1985) observed a reduction in ethylene production when stems were treated with amino ethoxy venyl glycine or cobalt chloride (1μM). They also reported that continuous bubling of vase solution with N_2 or co_2 reduced autocatalytic ethylene production from stems and increased vase life by eleven and four days respetively.

2.4.2 **Vase life studies in other crops.**

Changes in water contents, water and osmotic potential, turgor pressure and solutes in the petals of carnation flowers kept in water and sucrose solution was studied by Acock and Nichols (1979). They found that in water, turgor pressure decreased rapidly after seventh day because of decrease in tissue solid content. In sucrose soluetion, loss of solutes were delayed. This, according to them was probably because of maintenance of membrane integrity, as sucrose provided a respiratory substrate. In these cells, due to accumulation of sucrose and water there was decrease in water potential and osmotic potential. The solutes and water were lost at about day 15 and turgor pressure was lost.

Gowda and Gowda (1990) recorded longest vase life of cut *Gladiolus* flowers Cv Black Jack with 1.0μM aluminium sulphate (18.3 days) followed by 3 per cent sucrose (17.0 days) shortest vase life was with control (9.1 days). It is suggested that the increase in vase life with aluminium sulphate may be due to a lowering of the petal pH, stabilization of anthocyanins, and acidification of holding water thus reducing bacterial

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growth and improving water uptake. The increase in vase life due to sucrose was attributed to decrease in moisture stress, and improvement in water balance.

The vase life of cut roses Cv Marina was reported to be influenced by vase water management. Longest vase life in this study was recorded with flowers kept in tap water, which was changed every day. The combination of 5 per cent sucrose and 300 parts per million (ppm) aluminium sulphate gave a vase life 5 days of while control (Tap water) gave a vase life of 3 days (Ahn and Um, 1991).

Nagarajaiah and Reddy (1991) studied the effect of calcium, zinc and sucrose on the post harvest behaviour of cut 'Queen Elizabeth' Roses. They recorded longest vase life of 7.66 days with flowers kept in vase solutions with 2.0μM calcium chloride and 4 per cent sucrose. The metal salts used and sucrose were found to improve the water balance and thus maintain the fresh weight of the cut roses.

Rath *et al* (1991) reported that silver nitrate and potassium aluminium sulphate soultions at varying concentrations inhibited the vascular blockage in the stem of *Rosa hybrida* Cvs Laura, Love and Landora. Increased water uptake, reduced water loss and water loss and water uptake ratio by the cut flowers; all these helped in increasing the vase life of the roses.

Aluminium sulphate slightly reduced water uptake and slightly extended vase life compared to controls in cut roses Cv Sonia, as reported by Put *et al* (1992). They also reported that aluminium sulphate had weak biocidal activity towards *Bacillus subtilis* at $pH \leqslant 4$.

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MATERIAL AND METHODS

III MATERIAL AND METHODS

The present studies were carried out during the year 1993-94 at the Horticulture Research Station, Division of Horticulture, Gandhi Krishi vignana Kendra, University of Agricultural Sciences, Bangalore.

Studies were made partaining to the histological and histochemical changes during micro and megasporogenesis, floral biology and vase life of cut *Spathiphyllum* flowers. *Spathiphyllum* is a tropical herb, also called as 'Peace lily'. The plant is vigorous in growth. Leaves are glossy green, thin leathery, oblong-lanceolate. The inflorescences are borne on reed like stems (fig 3.1). The inflorescence is composed of densely flowered maze like spadix with small bisexual flowers, subtended by a white spathe. The spathe is ovate, 8-10 cm long (fig 3.3). One of the charcetoristic features of the plant is that, the spathe turns green from white with age (fig 3.2). The plant flowres through out the year and is propagated commonly through suckers, even though there is seed set observed.

3.1 FLORAL BIOLOGY

The development of the inflorescence from emergence to seedset was studied.

3.1.1 Flower development in the inflorescence

Fifteen inflorescences were tagged at the stage of visible initiation and the average number of days required for opening of the spathe, Spathe to turn green from white, Fruit set in the inflorescence were recorded.

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3.1.2 **Stigma Receptivity**

The average number of days required for the stigma to become receptive from the stage of visible initiation and the duration of Stigma receptivity were observed. the Stigma was considered receptive when it was fully developed with a white sticky mass at the tip of the pistil. The stigmatic surfaces of fifteen inflorescences were examined with the help of a hand lens.

3.1.3 **Anther dehiscence**

The average number of days required for the first anther to dehisce in an inflorescence was observed. The time at which anthers dehisced in an inflorescence, the pattern of anther dehiscence and also the average number of days required for completion of anther dehiscence in an inflorescence were recorded by tagging fifteen inflorescences.

3.1.4 **Pollen viability**

The stain for estimaiton of pollen viability was prepared by adding the various constituents given below, shaking after the addition of each item. The prepared stain was stored in coloured reagent bottles for 8-10 days before use.

Ethyl alcohol (95%)	20ml
Malachite green	20mg
Distilled water	50ml
Glycerol	40ml
Acid Fuchsin	100mg
Phenol	5g
Lactic acid	4ml

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Pollen was dusted on to 1-2 drops of stain, stirred well with the help of a needle, warmed gently over a flame for 4-5 times. A cover slip was mounted on it and examined after letting the slide stand for 4-5 minutes (Alexander,1980).

3.1.5 **Pollen Germination**

The different media tried for pollen germination are as follows:

16% Sucrose + 200ppm Boric acid

17% Sucrose + 200ppm Boric acid

18% Sucrose + 200ppm Boric acid

19% Sucrose + 200ppm Boric acid

20% Sucrose + 200ppm Boric acid

These media were prepared by dissolving 16g Sucrose + 20mg Boric acid, 17g Sucrose + 20mg Boric acid, 18g Sucrose + 20mg Boric acid, 19g Sucrose + 20mg Boric acid, 20g Sucrose + 20mg Boric acid in 100ml of water each, respectively. Hanging drop method was followed for pollen germination. The percentage germination obtained in each medium was observed and recorded.

3.1.6 **Artificial Pollination**

Pollen collected from inflorescences in the anther dehiscence stage was dusted on to the inflorescences having flowers with receptive stigmas. Artificial pollination was done for five inflorescences and the number of fruits set in these infloresences was recorded and compared with that in a naturally pollinated inflorescence.

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3.2 HISTOLOGICAL AND HISTOCHEMICAL STUDIES

3.2.1 Materials

The plant material for study, the inflorescences were collected from the plants maintained in pots at the Floriculture unit, Horticulture Research Station, G.K.V.K, UAS Bangalore.

3.2.2 Sampling

The inflorescences were grouped into different classes or stages depending on their size. Sampling was done in such a way as to include all the representative stages in the development of anther and the ovule (fig 3.4).

3.2.3 Fixation

The inflorescences were fixed in Carnoy's B fixative which has six parts of ethanol, three parts of chloroform, and one part of acetic acid by volume. The inflorescences were fixed by treating them with the fixative for fifteen minutes.

3.2.4 Dehydration

The fixed inflorescences were dehydrated by taking them through grades of ethanol and n-butanol after washing them with 70 per cent ethanol. The material was left in each grade for a period of three hours.

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PLATE 2

Fig 3.3 : Developing stages of the inflorescence.

Fig 3.4 : Inflorescence of *S. wallisii* at various stages of flowers development from visible initiation to fruit set.

Dehydration using ethanol and n-butanol grades

	Ethanol	n-butanol
01	70%	--
02	80%	--
03	90%	--
04	100%	--
05	75%	25%
06	50%	50%
07	25%	75%
08	--	100%
09	--	100%

3.2.5 **Infilteration and Embedding**

The medium of n-butanol containing the dehydrated material was added with wax (a mixture of nine parts paraffin wax and one part of Bee's wax) of 58-60°C melting point.The material was kept in an oven and maintained at 60°C. Later changes with fresh molten wax were given at three hours interval to remove the trace of butanol and replace it with wax. Subsequently the material was embedded in paraffin wax by employing the paper boat technique. (Jensen 1962).

3.2.6. **Microtomy**

Uniform sections of 8µm thickness were taken with ERMA rotrary microtome.

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3.2.7 **Affixing the Sections to slides**

For affixing the sections to the slide, gelatin (0.8%) with little potassium dichromate was used as an adhesive. A few drops of gelatin was placed on the slide. The paraffin ribbons cut to convenient length were placed on the surface of the adhesive. The slides were then warmed to 45°C on a warming plate to fecilitate stretching of the paraffin ribbon. The excess adhesive was poured out after complete stretching of the ribbon. The slides were later dried in a dust free environment for 72 hours at room temperature.

3.2.8 **Deparaffinising and hydrating the Sections**

The slides with paraffin ribbons were treated with xylol for removal of the paraffin around the sections of the plant material. These slides were then treated with n-butanol and 100 per cent ethanol to eliminate the traces of xylol left on the secctions. Later a coating of celloidin 0.05 per cent in 50 per cent ether was given to prevent washing out of the sections during subsequent histochemical staining procedures. The sections were either hydrated before staining or directly subjected to staining procedures depending upon the requirement.

The steps followed for deparaffining were:

$xylol_1$	5 min
$xylol_2$	5 min
$n\text{-}Butanol_1$	5 min
$n\text{-}Butanol_2$	5 min
100% Ethanol	5 min
Water	

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3.2.9 **Histochemical Staining Procedures**

For the localisation of different cellular components viz., insoluble polysaccharides, insoluble proteins, and ribonucleic acid, the sections were subjected to different histochemical staining Procedures.

The assessment was based on the intensity of staining which involved specific reaction between the stain and the corresponding compound, and it was based on visual obsevation.

After histochemical staining, the sections were subjected to dehydration using alcohol, n-butanol grades, cleaned in xyol and mounted in DPX.

3.2.9.1 **Total Insoluble Ploysaccharides**

Periodic Acid Schiff's (PAS) mehtod was employed for localisation and assessment of insoluble polysaccharides (Hotchkiss, 1948).

Preparation of Schiff's Reagent (Longley, 1952)

This stain was prepared by mixing 1g of basic fuchsin and 1.8g of potassium metabisulphate with 100ml of 0.15 normal hydrochloric acid in an airtight container and shaking the contents repeatedly for 24 hours. A straw yellow coloured solution was obtained. Activated charcoal, at the rate of 100mg was added to this solution, shaken and filtered. The colourless filterate was stored in an amber coloured container and used as the reagent.

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Staining Procedure:

(a) the slides were deparaffinised and hydrated

(b) treated with periodic acid for fifteen minutes

(c) washed thoroughly in running water

(d) kept in Schiff's reagent for five minutes at room temperature.

(e) rinsed in water for differentiation.

Excess stain was removed by treating with a bleach which was prepared by adding 5ml of 1 normal hydrochloric acid and 5ml of 10 per cent potassium metabisulphate to 90ml of distilled water.

(e) washed in running water

(g) dehydrated by passing through n-butanol

(h) cleaned in xyol and mounted in DPX.

3.2.9.2 **Ribonucleic acid**

For localising and staining ribonucleic acid, the dye toluidine blue was used. The stain was prepared by dissolving 0.5g of toluidine blue powder in 100ml of water. The following procedure was followed for staining the sections.

(a) Deparaffinising and hydration

(b) Staining for 30 seconds by immersing in toluidine blue solution

(c) Removal of superfluous stain by vigorous shaking in water

(d) Dehydration in n-butanol, cleaning in xylol.

(e) Mounting in DPX.

The sites of RNA took characteristic purple colour, the intensity of which is a measure of the amount of RNA present in the tissue.

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3.2.9.3 **Total Proteins**

The Mercuric Bromophenol Blue method (Mazia et al, 1953) was employed for the assessment of total proteins.Only the insoluble proteins were stained as all the soluble protein was assumed to be lost during the processing of the material. The stain was prepared by dissolving 10g of mercuric chloride and 100mg of bromophenol blue powder in 100ml of 100 per cent ethanol. The staining procedure was as follows:

(a)deparaffinising the sections and passing them through n-butanol and 100 per cent ethanol.

(b)treating them with mercuric bromophenol blue stain for 10 minutes.

(c)differentiation by thorough shaking in acidic water.

Differentiation produced the final blue colour at the sites of proteins.

(d)dehydration in n-butanol, cleaning in xylol.

(e)mounting in DPX.

3.2.10 **Cytochemical Assessment**

Based on visual observations of the degree of cytochemical reactions with specific reagents for various cellular compounds, the grading was given as,

(a) Intense	(+++)
(b) Rich	(++)
(c) Poor	(+)
(d) Absent	(-)

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3.2.11 **Micrometry**

The size of different tissues of the anther and the ovule at different stages of development was measured.

Observations were taken using a calibrated ocular micrometer. The value of each division on the ocular micrometer was standardised using a stage micrometer. The ocular micrometer readings were converted into actual values by multiplying with the standard value.

3.2.12 **Photomicrography**

The photographs were taken with the help of PENTAX photomicrographic equipment.

3.3 VASE LIFE STUDIES

In this experiment, the inflorescences about to open were harvested along with leaves and leaves were removed. Each of these inflorescences were placed in a 500ml dextrose bottle containing 200ml of tap water or other vase solutions with different chemicals after recording the initial weight. Freshly prepared vase solutions were used for vase life studies.

The experiment was conducted employing completely randomised design with seven treatments replicated thrice along with control.

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Treatment: a) control - tap water

b) Sucrose - 0.5%

c) Sucrose - 1.0%

d) Sucrose - 1.5%

e) Sucrose - 2.0%

f) Aluminium Sulphate - 25 ppm

g) Aluminium Sulphate - 50 ppm

h) Aluminium Sulphate - 100 ppm

Design : Completely Randomised Design.

Replications : 3

Number of spikes in each replication : 2

Total number of spikes : 48

Preparation of vase solutions

Tap water was used as control, sucrose solutions 0.5 per cent, 1.0 per cent, 1.5 per cent and 2.0 per cent were prepared by dissolving 0.5g, 1.0g, 1.5g and 2.0g of Sucrose in 100ml of water each respectively. Similarly Aluminium Sulphate 2.5mg, 5mg and 10mg was dissolved in 100ml of water each to get 25ppm, 50ppm and 100ppm solutions respectively.

Observations

a) Fresh weight of the inflorescence (g)

Before the inflorescences were kept in the vase solution, the fresh weight was recorded.

b) Uptake of the vase solution (ml)

The amount of vase solution in the bottle was measured every alternate day. The difference between the consecutive values gave the volume of the vase solution taken up.

c) Final Weight (g)

The weight of the inflorescence was recorded at the end of its vase life ie., when the spathe turned brown.

d) Vase Life (days)

The inflorescence was considered fit to be kept in the vase till its spathe turned brown. The number of days taken for the spathe to turn brown was recorded by daily observation. This period represents the vase life of the inflorescence in days.

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EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of the present study are described in this chapter, under appropriate headings.

4.1. **FLORAL BIOLOGY** :

In *Spathiphyllum wallisii*, the spadices have tiny bisexual flowers which are closely arranged on cylindrical spikes (Fig. 4.1). The number of flowers per square centimeter is about 13.80, and the diameter of each flower is about 3.94 mm. The flower is sessile with six tepals, six stamens and a pistil with trifid sessile stigma. The perianth is in two whorls, trimerous, polytepalous and imbricate. The stamens are in two whorls of three each, the members of the outer whorl older than those of the inner whorl. The anthers measure 1.15 x 1.20 mm on an average. These stamens which are hidden, enclosed by the perianth (Fig. 4.3) emerge out and dehisce immediately to release the pollen. The pistil projects beyond the perianth lobes and is surrounded by the stamens (Fig. 4.1, 4.2). The diameter of the ovary is about 2.40 mm. The gynoecium is tricarpellary, syncarpous with two rows of anatropous ovules attached to the axile placenta. The glandular outgrowths arise from the axis and also from the inner walls of the ovary contiguous to the axis. The flowers are protogynous; the stigma being receptive first, at the pistillate phase (Fig. 4.4,4.4a,4.4b). The emergence of the first anther from the perianth whorl is only after the stigma loses its receptivity, as evidenced by the drying up of the stigmatic surface (Fig.4.5). The fruit set is about 5.7 per cent in the inflorescences left for open pollination.

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4.1.1. **Flower Development:**

From the stage of visible initiation, on an average 25.50 days were required for the spathe to start opening and 30.10 days were required for complete opening of the spathe. The pistillate phase viz. stima receptivity was noticed after 30.50 days of visible initiation, followed by anther dehiscence which started 35.57 days after visible initiation. Spathe required 67.00 days on an average to turn green from the stage of visible initiation. An average of 88.00 days were required for fruit set in an inflorescence from the stage of visible initiation and 155.50 days for fruit ripening. (Table 1.).

4.1.2. **Stigma receptivity**

The average number of days required for the stigmas to become receptive from the stage of visible initiation was observed to be 30.50. Once the stigmas became receptive, they remained so for 3.30 days on an average.

4.1.3. **Anther dehiscence**

The first anther to dehisce in an inflorescence required 35.57 days on an average from the stage of visible initiation. Anther dehiscence started in the basal 1/3rd of the inflorescence and proceeded in the direction of the apex. More than one such cycle was observed in an inflorescence as each flower had anthers at different stages of development. Completion of anther dehiscence in an inflorescence required an average of 7.8 days. Maximum number of anthers dehisced between 8 a.m and 11 a.m. The data on anther dehiscence is presented in Table 2.

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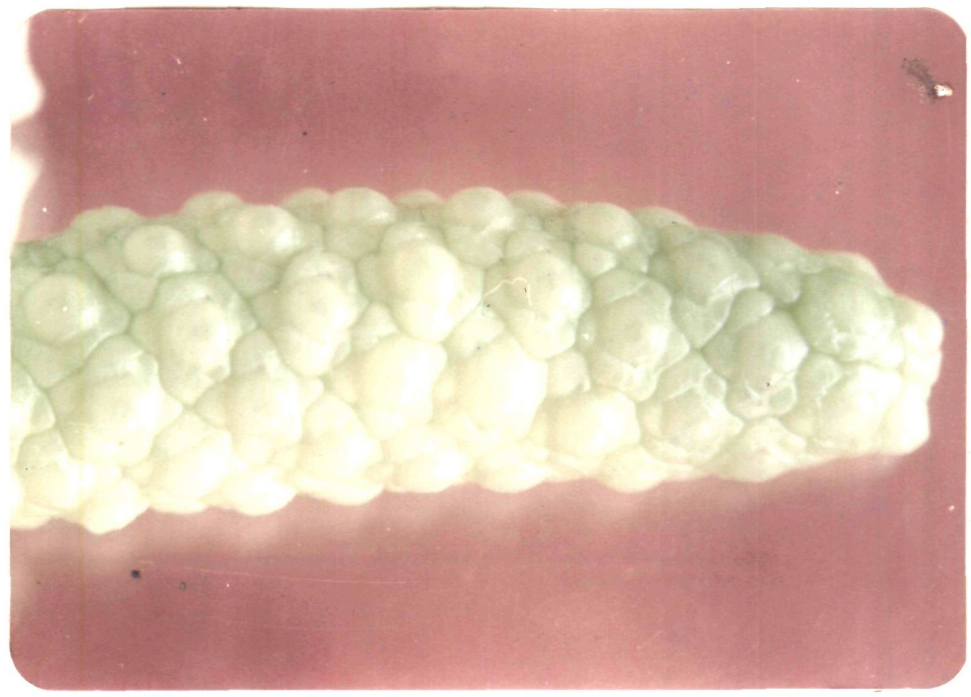
PLATE 3

Fig 4.1 : Inflorescence of *S. wallisii* at early stages of development showing the arrangement of stamens and tepals around the pistil x25

Fig 4.2 : Inflorescence at later stages of development showing trifid sessile stigma. x25

Fig 4.3 : Transverse Section of the inflorescence showing the anthers enclosed by the perianth lobes and section of the pistil x25



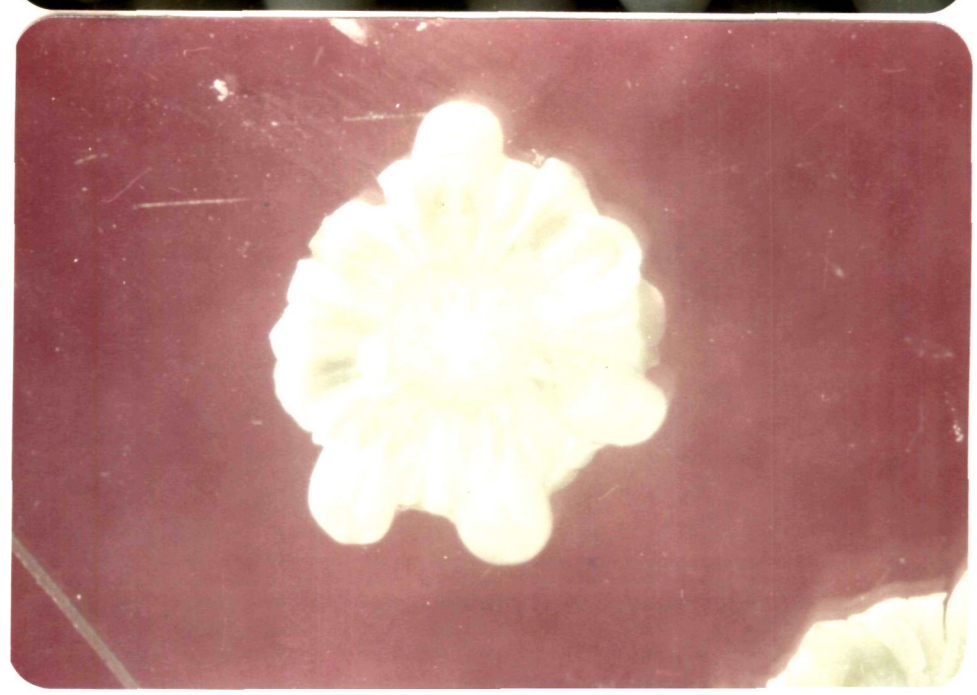


PLATE 4

Fig 4.4 : Inflorescence of *S. wallisii* at the Stigma receptivity.

Fig 4.4a : Spike showing the flowers at Stigma receptivity. x25

Fig 4.4b : Flowers at Stigma receptivity showing white glandular Stigmatic papillae x50

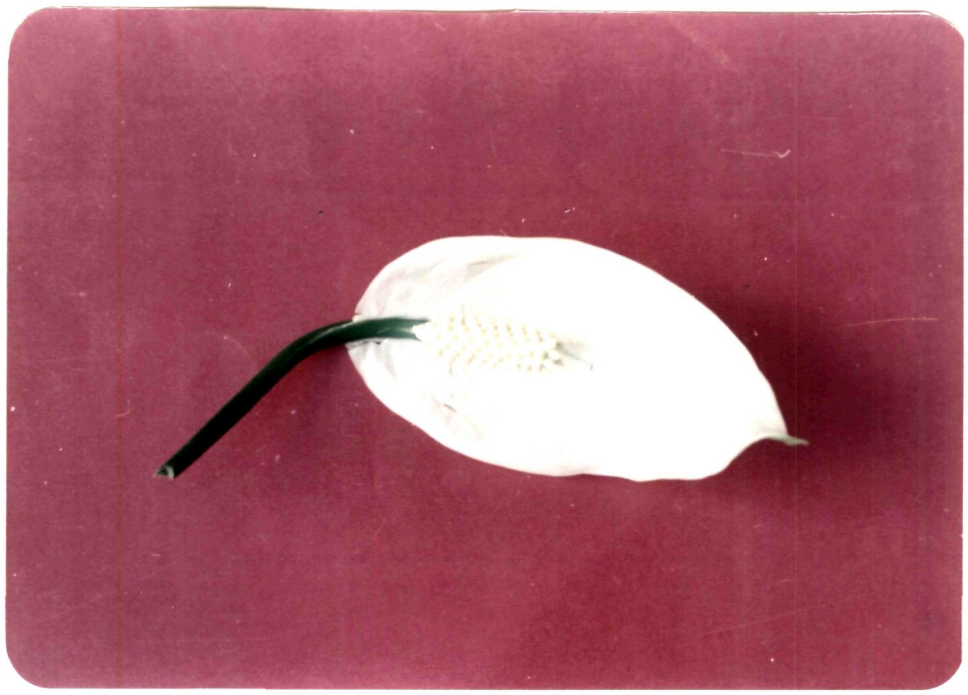


Table 1: Floral Biology of *S. wallisii.*

	Number of days required
Visible Initiation	0
Beginning of spathe opening	25.50
Complete opening of the spathe	30.10
Stigma receptivity	30.50
Anther dehiscence	35.57
Greening of spathe	67.00
Fruit Set	88.00
Fruit Ripening	155.50

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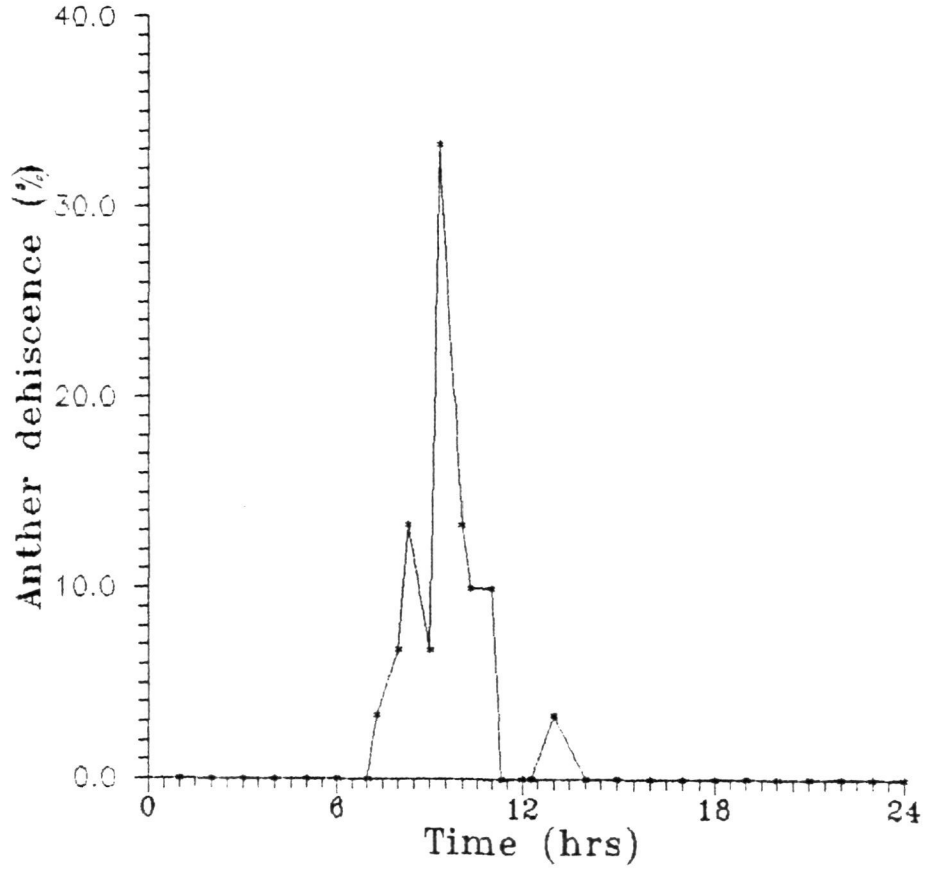

Figure 1: Time of anther dehiscence in S. wallisii

Table 2: Time and per cent anther dehiscence

Time of the Day (hrs)	No. of Anthers dehisced	per cent Anther dehiscence
01.00	00.00	00.00
02.00	00.00	00.00
03.00	00.00	00.00
04.00	00.00	00.00
05.00	00.00	00.00
06.00	00.00	00.00
07.00	00.00	00.00
07.30	01.00	03.33
08.00	02.00	06.77
08.30	04.00	13.33
09.00	02.00	06.77
09.30	10.00	33.33
10.00	04.00	13.33
10.30	03.00	10.00
11.00	03.00	10.00
11.30	00.00	00.00
12.00	00.00	00.00
13.00	01.00	03.33
14.00	00.00	00.00
15.00	00.00	00.00
16.00	00.00	00.00
17.00	00.00	00.00
18.00	00.00	00.00
19.00	00.00	00.00
20.00	00.00	00.00
21.00	00.00	00.00
22.00	00.00	00.00
23.00	00.00	00.00
24.00	00.00	00.00

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4.1.4. **Pollen Viability**

Pollen viability was estimated immediately after extraction of pollen from the inflorescence. It was found to be hundred per cent.

4.1.5. **Pollen Germination**

Among the different media tried, maximum percentage of pollen germination (98%) was recorded with a medium containing 20 % sucrose and 200 ppm Boric acid. (figs 4.6a,4.6b). The data on pollen germination is presented in Table 3.

4.1.6. **Pollination and Fruit Set**

The percentage fruit set observed in a naturally pollinated inflorescences was 5.71 and that in an artificially pollinated inflorescences was 23.8. The average of the number of fruit set in five naturally pollinated inflorescences was 4.00 and that of five artificially pollinated inflorescences was 29.20. The number of fruits set in both naturally and artificially pollinated inflorescences are presented in Table 4.

4.2. HISTOLOGICAL CHANGES IN ANTHERS DURING MICROSPOROGENESIS AND MALE GAMETOPHYTE DEVELOPMENT IN *S.wallisii.*

The data on histological changes observed during microsporogenesis and male gametophyte development in *S.wallisii* and micrometric observations at successive stages of anther development are presented in Table 5 and are described as follows :

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PLATE 5

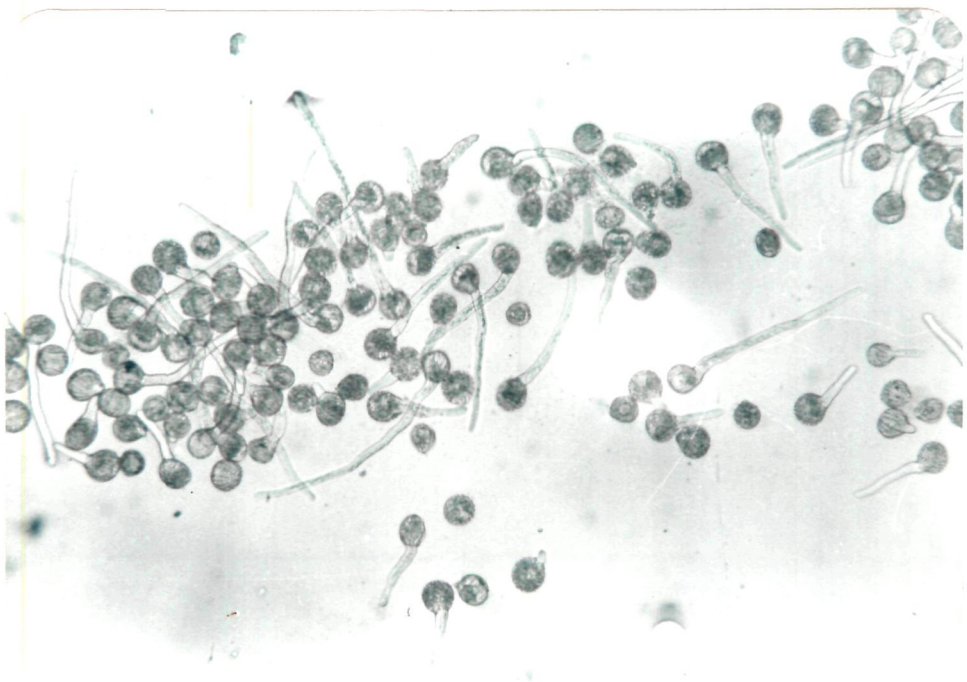
Fig 4.5 : Inflorescence of *S. wallisii* at the stage of anther dehiscence showing anthers emerged out of the perianth.

Note: The dried stigmatic surfaces of the flowers. x25

PLATE 6

Fig 4.6a : Germinating pollen grains x100

Fig 4.6b : Germinating pollen grains x400



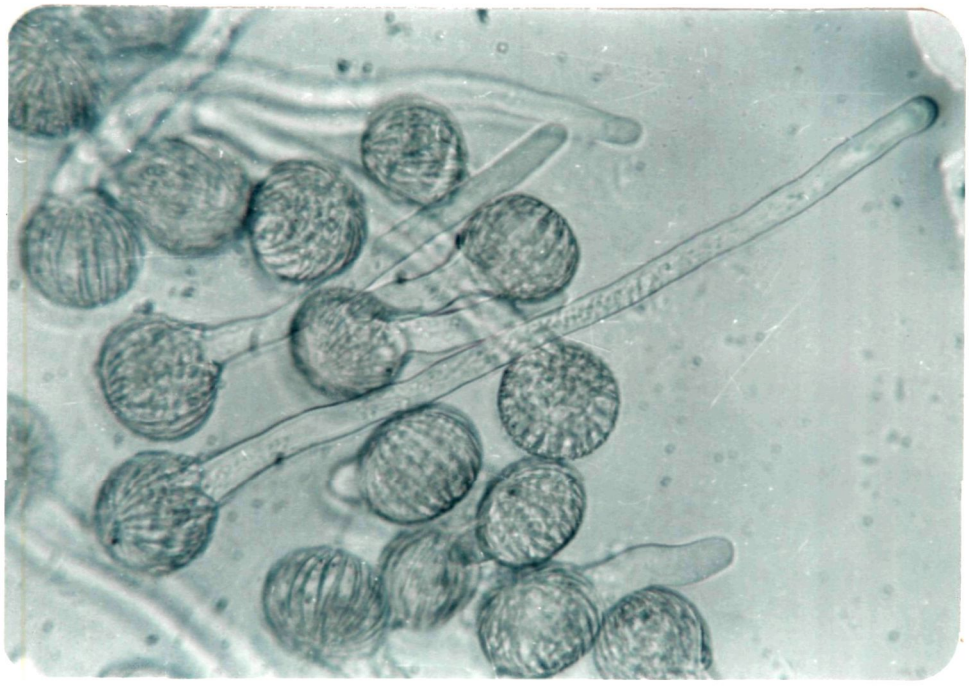


Table 3. Media and percentage of Pollen germination

Sl. No.	Constitution of the medium	per centage Germination
1.	16 % Sucrose + 200 ppm Boric acid.	0
2.	17 % Sucrose + 200 ppm Boric acid.	0
3.	18 % Sucrose + 200 ppm Boric acid.	0
4.	19 % Sucrose + 200 ppm Boric acid.	10 %
5.	20 % Sucrose + 200 ppm Boric acid.	98 %

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Table 4. Fruitset in Naturally and Artificially Pollinated Inflorescences

Sl.No.	Number of Fruits set	
	Naturally pollinated inflorescences	Artificially pollinated inflorescences
1.	1	26
2.	2	32
3.	5	30
4.	5	28
5.	7	30
Average	4	29.20
%	5.71	23.8

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The cross section of anthers at the stage of sporogenous tissue showed four microsporangia in the corners connected by a central connective tissue. The diameter of the pollen sac was 34.20 µm. Thickness of the wall layers was 7.35 µm at this stage, the thickness of the epidermis was 3.00 µm.

As the pollen mother cells (PMCs) differentiated from sporogenous cells, callose deposited around them, separating sister meiocytes. The cells of the innermost wall layer increased in size, became densely cytoplasmic and got differentiated into "Tapetum". The thickness of the tapetum was 10.99 µm on an average. The tapetal cells proximal to the connective were 14.40 µm and those distal to the connective were 7.60 µm in thickness. The pollen sac diameter was 89.40 µm and the number of PMCs in a section of the microsporangium was 24.25 on an average. The diameter of individual PMC was 9.90 µm.

The PMCs underwent meiosis I and II. At the end of meiosis II, four haploid microspores were formed. Cytokinesis was successive forming either isobilateral or decussate microspore tetrads. The tapetum further increased in thickness to 13.80 µm. The tapetal cells proximal to the connective were 18.60 µm and those distal to the connective were 9.00 µm. The pollen sac diameter also increased and was 116.80 µm at the tetrad stage. The number of tetrads in a section microsporangium was 28.5. The tetrads were 12.45 µm in diameter. The middle wall layers were 5.70 µm thick at this stage and later they degenerated.

With the disappearance of callose, the microspores got released from tetrads. The tapetum was of invasive, amoeboid or plasmodial type. As the microspores got released

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from tetrads, the tangential and radial walls of the tapetal cells began to disintegrate and the tapetal protoplasm diffused into the anther locule forming a "periplasmodium" (Figs.4.16,4.28). The contents of these protoplasts disappeared as the microspores developed further. The pollen sac diameter at this stage was found to be 124.20 µm. The diameter of individual microspore was 9.90 µm and 117.00 microspores were found in a section of the microsporangium on an average. The epidermis which persisted upto this stage was 6.00 µm thick, later it degenerated. The endothecium was 9.6 µm in thickness at this stage.

The microspores released from tetrads developed into pollen grains with concomitant increase in diameter which was 11.40 µm at this stage. The average number of pollen grains in a section of microsporangium was found to be 76.00. The pollen sac diameter at this stage had increased to 144.00 µm. The thickness of the endothecium increased to 13.20 µm. The endothecial cells, by this time, had developed the fibrous wall thickenings. Along with the cells of the endothecium, three or four layers of connective cells contiguous to the endothecium developed fibrous wall thickenings which were similar to the endothecial thickenings (Fig.4.20,4.22,4.30,4.34,4.44)

At the anther dehiscence stage, the diameter of the pollen sac further increased to 201.00 µm. The number of pollen grains per section of microsporangium decreased slightly, it was 55.50 on an average. The average diameter of pollen grains was found to be 11.52 µm. The endothecium at this stage was 13.80 µm in thickness. The pollen grains were shed at the two celled stage.

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Table 5. Micrometric Observations during Microsporogenesis and Male gametophyte development in *S.wallisii.*

Stages	Pollensac diameter (µm)	No.of cells	Individual cell size (µm)	Tapetum thickness (µm)	Middle wall layers (µm)	Endothecium (µm)	Epidermis (µm)
Sporogenous tissue	34.20	45.50	5.55	1.95	2.40	3.00	3.00
Pollen mother cells	89.40	24.25	9.90	10.99	3.30	7.20	4.20
Tetrads	116.80	28.50	12.45	13.80	5.70	9.05	7.80
Microspores	124.20	117.00	9.90	-	-	9.60	6.00
Pollen grains	144.00	76.00	11.40	-	-	13.20	-
AntherDehiscence	201.00	55.00	11.52	-	-	13.80	-

4.3. HISTOCHEMICAL CHANGES DURING MICROSPOROGENESIS AND MALE GAMETOPHYTE DEVELOPMENT IN *S.wallisii*

4.3.1. Total Insolubale Polysaccharides.

The PAS reaction was employed to localise the insoluble polysaccharides. The changes in insoluble polysaccharides observed at various stages of microsporogenesis are presented in Table 6 and described as follows :

During the early stages of development the sporogenous cells, cells of the tapetal primordium, were very poor in cytoplasmic polysaccharide content. (Fig.4.7).

At PMC stage, the tapetal cells showed rich polysaccharide content. The PMCs were poor in polysaccharides. The middle wall layers and epidermis were poor in polysaccharides while endothecium showed rich polysaccharide content (Figs.4.8,4.9 & 4.10).

The tetrads formed after meiosis I and II were found to have rich polysaccharide content, so also the tapetum. The middle wall layers, endothecium and epidermis were poor in cytoplasmic polysaccharide content. (Figs.4.11,4.12).

As the microspores started getting separated from the tetrads the tapetal contents rich in insoluble polysaccharides started getting diffused into the anther locule (Figs.4.13, 4. 4, 4.15). The microspores had rich polysaccharide content. At this stage, the tapetal cells which had diffused into the anther locule were poor in polysaccharide content (Figs.4.16,4.17). The connective had rich cytoplasmic polysaccharide content (Figs. 4.13,4.14,4.15,4.16,4.17).

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Table 6. Changes in Insoluble Polysaccharides content during Microsporogenesis and Male gametophyte development in *S.wallisii*.

Stages	Sporogenous tissue	Tapetum	Middle wall layers	Endothecium	Epidermis	Connective tissue	Vascular strand
Sporogenous tissue	+	+	+	+ +	+ +	+ +	
Pollen Mother cells	+	+ +	+	+ +	+	+	
Tetrads	+ +	+ +	+	+	+	+	+
Microspores	+ +	+ (diffused)	crushed	+	+	+ +	+
Pollen grains	+ + +	diffused	crushed	+ +		+ +	+
Anther Dehiscence	+ + +	diffused	crushed	+ +		+ +	+

- => Absent
+ => Poor
+ + => Rich
+ + + => Intense

PLATE 7

Fig 4.7 to 4.22 : Transverse sections of anthers tested with Periodic Acid Schiff's (PAS) reagent to localise insoluble polysaccharides at successive stages of microsporogenesis and male gametophyte development in *S. wallisii.*

Fig 4.7 : Sporogenous tissue with low polysaccharide content in Sporocytes. x400

Fig 4.8 : Pollen mother cells (PMC's) low in insoluble polysaccharide content and the tapetum with rich insoluble polysaccharide content. x100

Note: Tapetum thicker towards the connective than towards distal portion of the pollen sac.

Fig 4.9 : Pollen mother cells low in insoluble polysaccharides surrounded by PAS positive callose deposition, tapetum richly stained for insoluble polysaccharides. x400

C -- Connective

Cl -- Callose

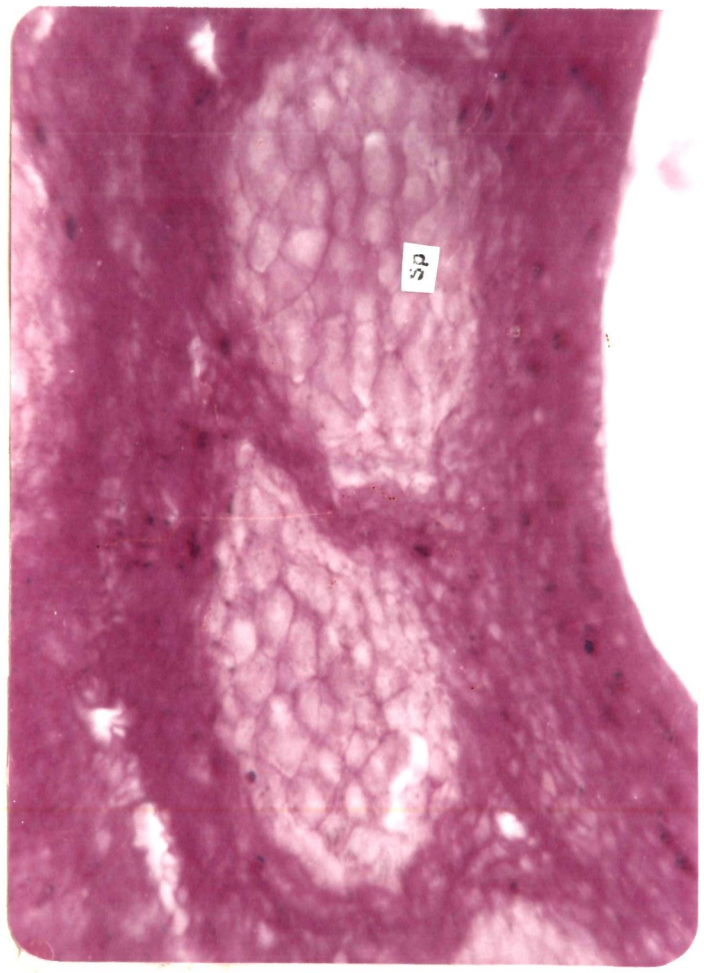
En -- Endothecium

Ep -- Epidermis

P -- Pollen mother cell

Sp -- Sporogenous tissue

T -- Tapetum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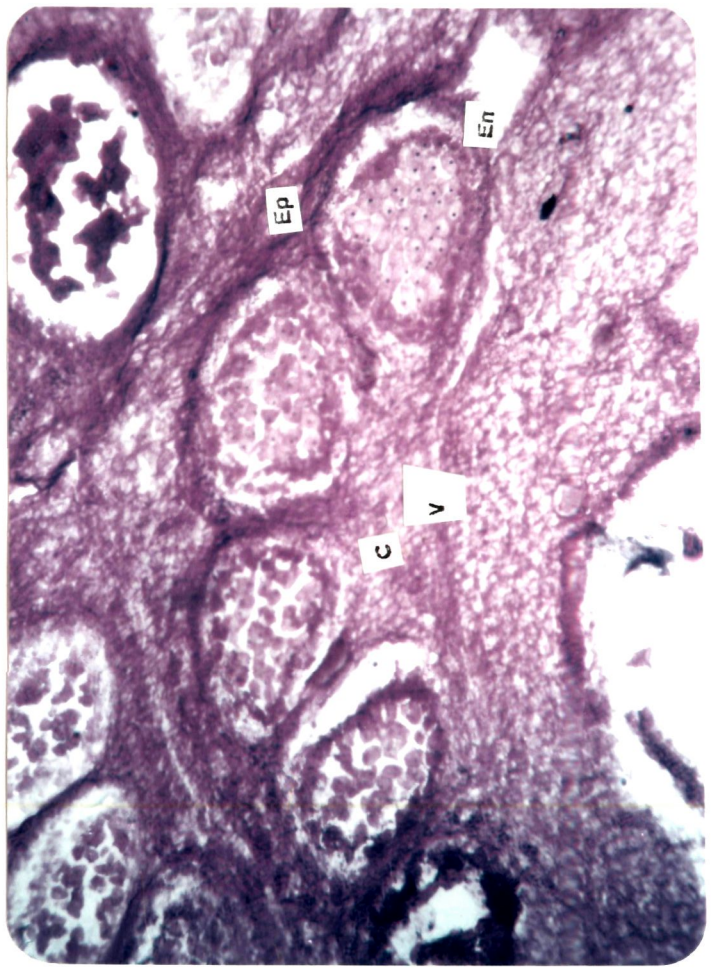
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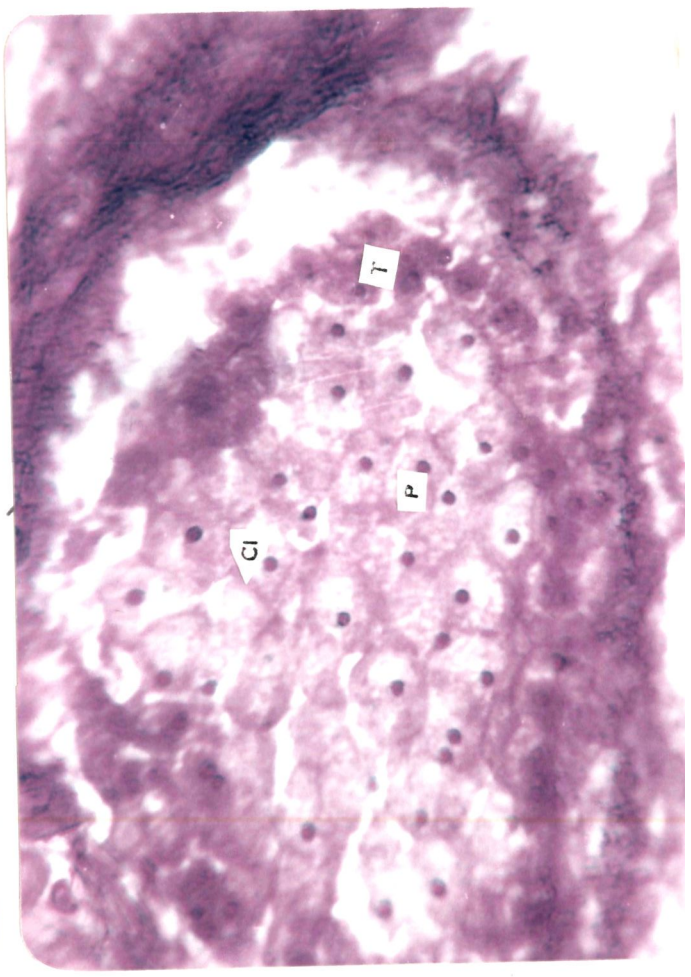

T
Cl
P

PLATE 8

Fig 4.10 : Pollen mother cells low in insoluble polysaccharides surrounded by PAS positive callose deposition, tapetum richly stained for insoluble polysaccharides.

Fig 4.11 : Tetrads and tapetal cells showing rich insoluble polysaccharide content.

Note: Tapetum thick proximal to the connective and thin on the distal portion. x100

Fig 4.12 : Decussate, insobilateral tetrads and tapetal cells showing rich polysaccharide contnet. x400

C -- Connective

cl -- callose

En -- Endothecium

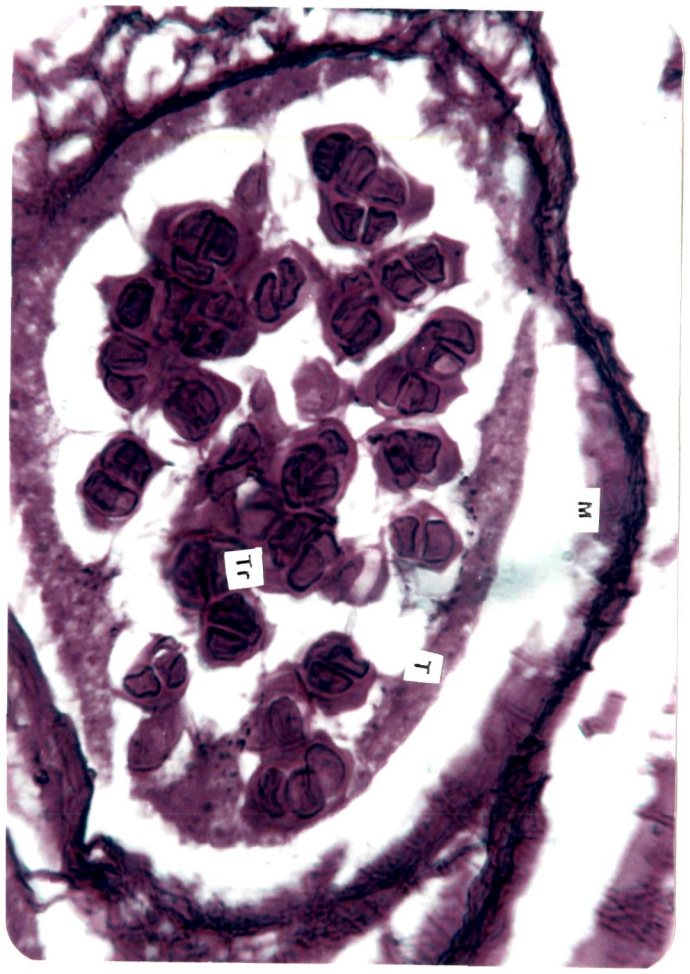
Ep -- Epidernis

M -- Middle wall Layers

T -- Tapetum

Tr-- Tetrad

V -- Vasuclar strand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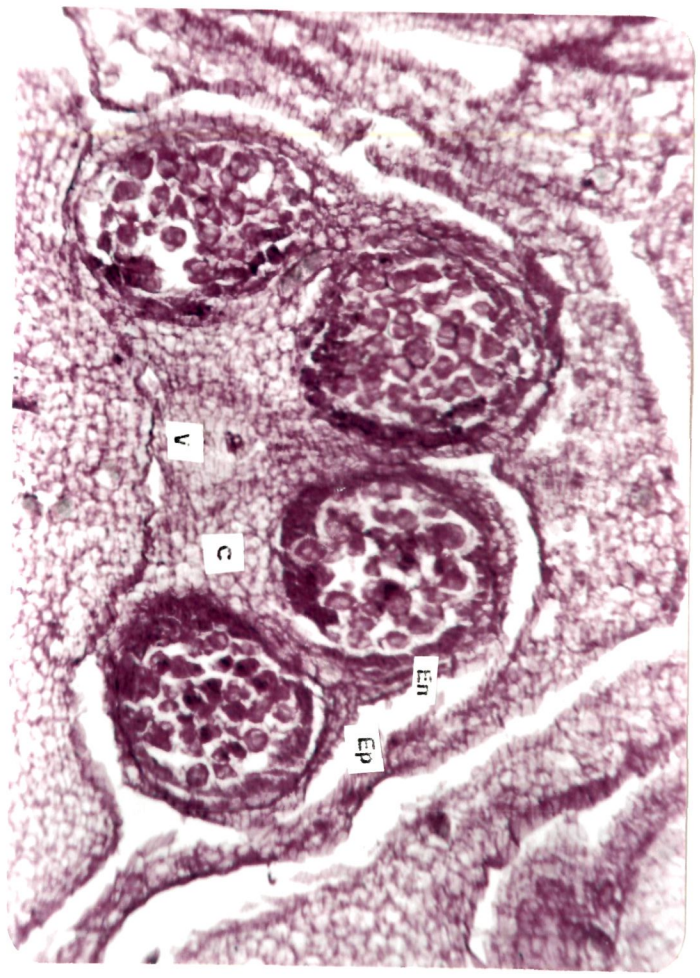
M
T


V
C
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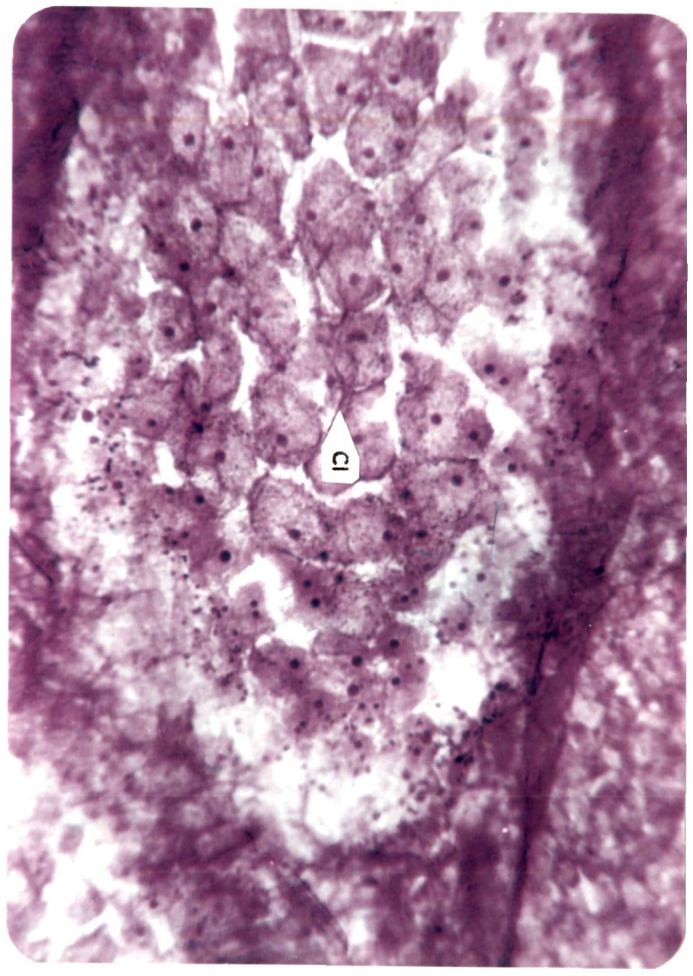

Cl

PLATE 9

Fig 4.13 : Microspores, tetrads and tapetum showing rich insoluble polysaccharide content. x100

Fig 4.14 : Microspores rich in insoluble polysaccharide getting seperated from tetrads x400

Note: Initiation of the diffusion of tapetal contents rich in insoluble polysaccharides.

Fig 4.15 : Microspores just separated from the tetrads rich in polysaccharides and tapetal contents rich in insoluble polysaccharides diffusing into the anther locule.

C -- Connective

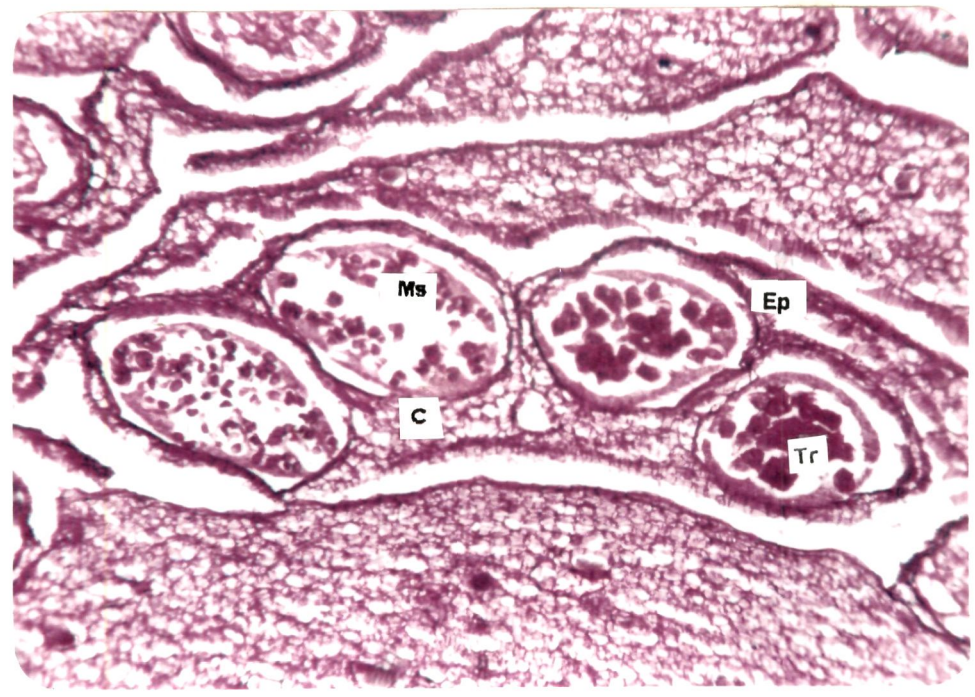
En -- Endothecium

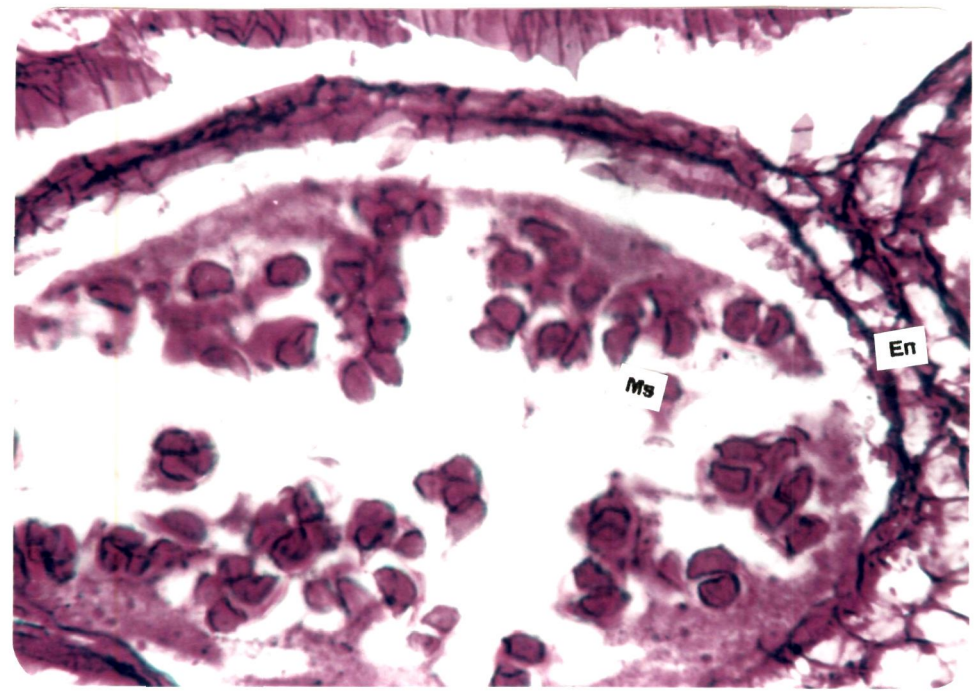
Ep -- Epidermis

Ms -- Microspores

T -- Tapetum

Tr -- Tetrad


Ms
Ep
C
Tr


En
Ms

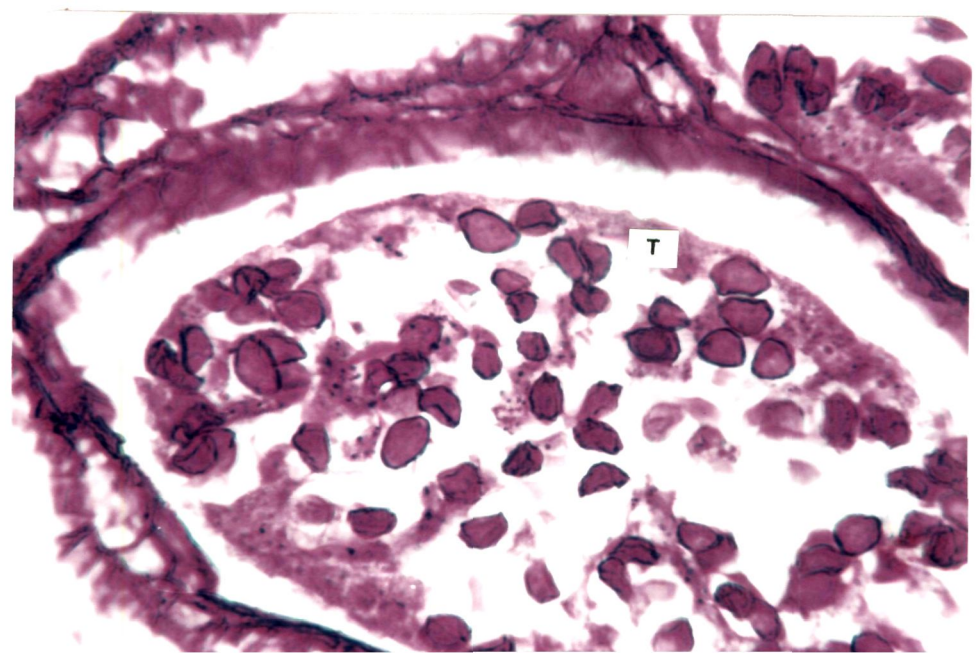

T

PLATE 10

Fig 4.16 : Microspores rich in insoluble polysaccharides . x400

Note: The tapetal contents at the background of the microspores forming the "Periplasmodium."

Fig 4.17 : Microspores rich in insoluble pollysaccharides and the "Periplasmodium." x400

Fig 4.18 : Pollen grains intensely stained for insoluble polysaccharides, endothecium rich in insoluble polysaccharides. x100

Note: The absence of diffused tapetal contents.

C -- Connective

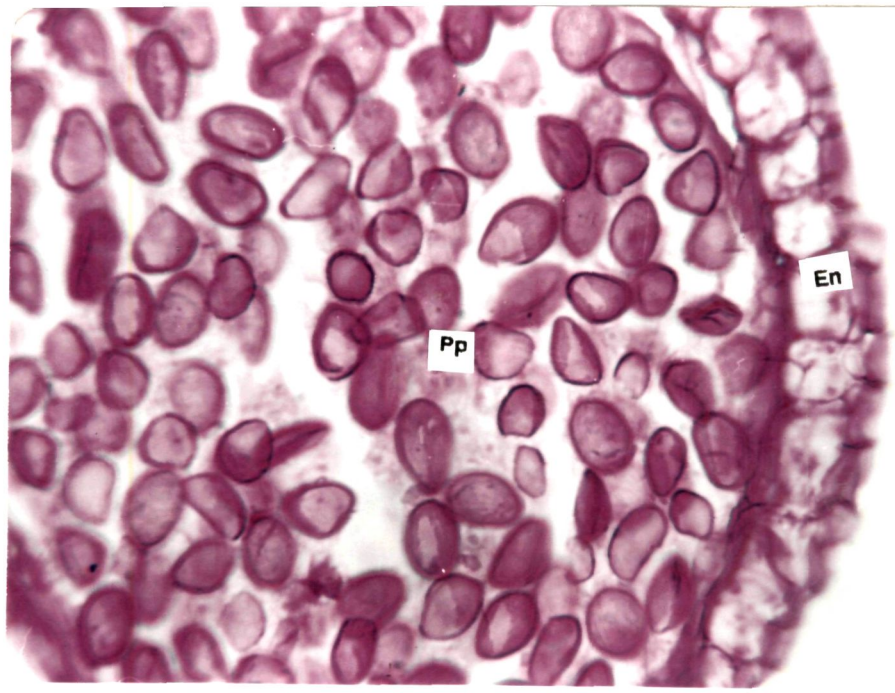
En -- Endothecium

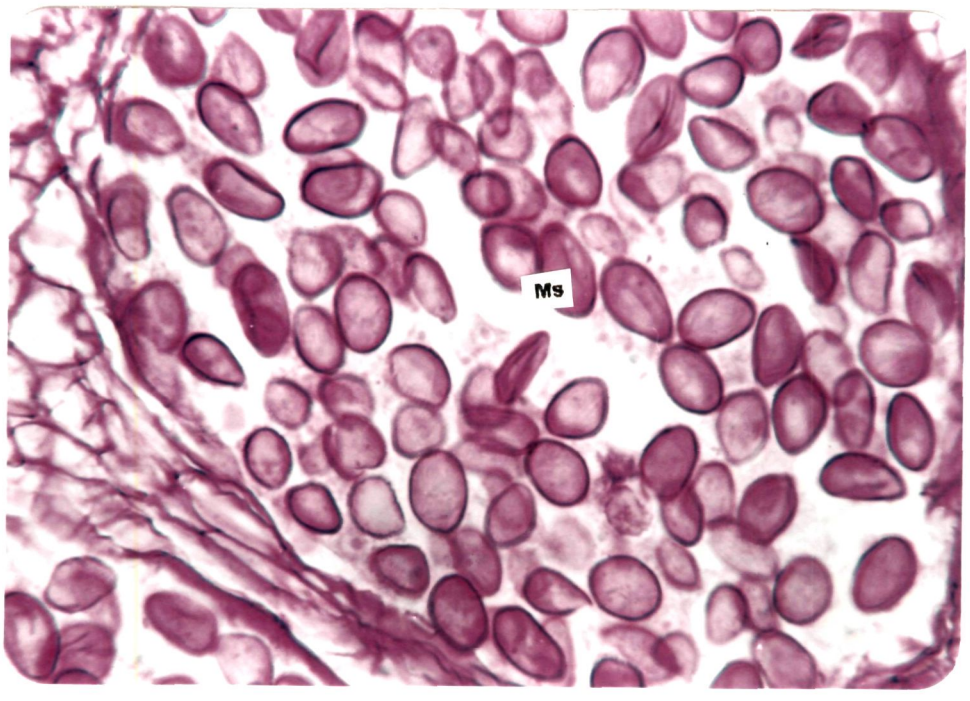
Ms -- Microspores

Pg -- Pollen grains

Pp -- Periplasmodium

V -- Vascular strand


En
Pp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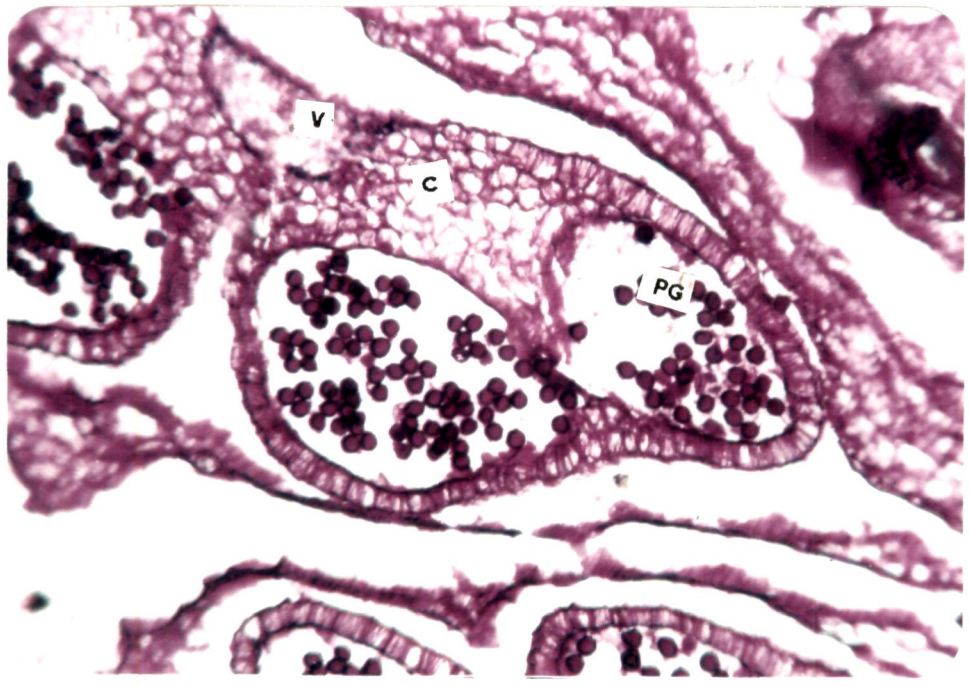

V
C
PG

PLATE 11

Fig 4.19 : Pollen grains intensely stained for insoluble polysaccharides with starch accumulation. x400

Fig 4.20 : Pollen grains intensely stained for insoluble polysaccharides at the stage of anther dehiscence, endothecium with fibrous wall thickenings x100

Fig 4.21 : Endothecium with fibrous wall thickenings, pollen grains with intense polysacchairde content.

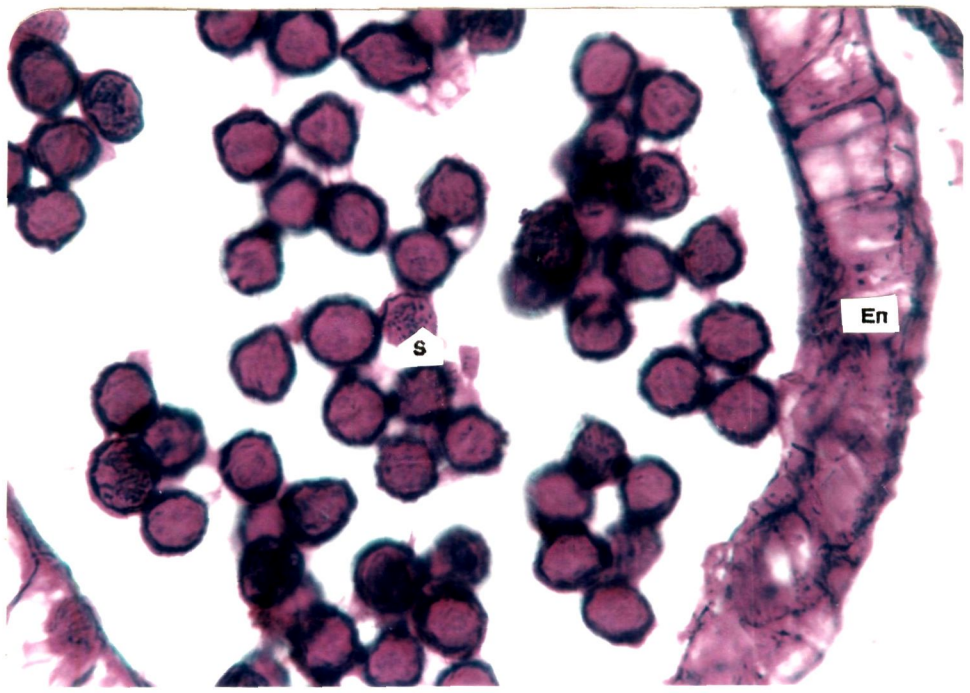
C -- Connective

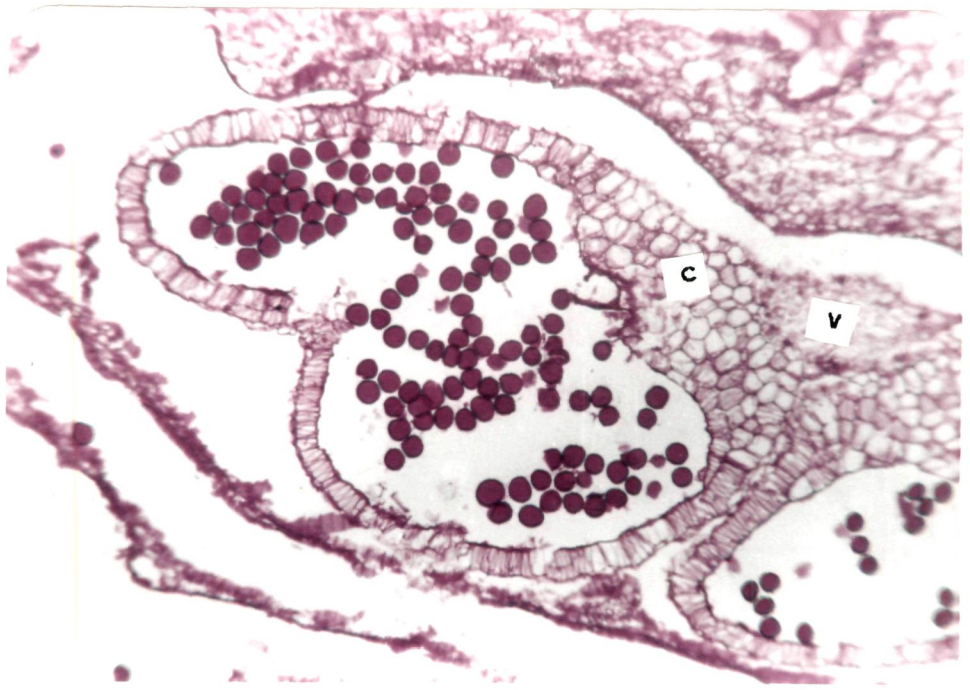
En - Endothecium

PG - Pollen grains

S -- Starch grains

V -- Vascular strand


S
En


C
V

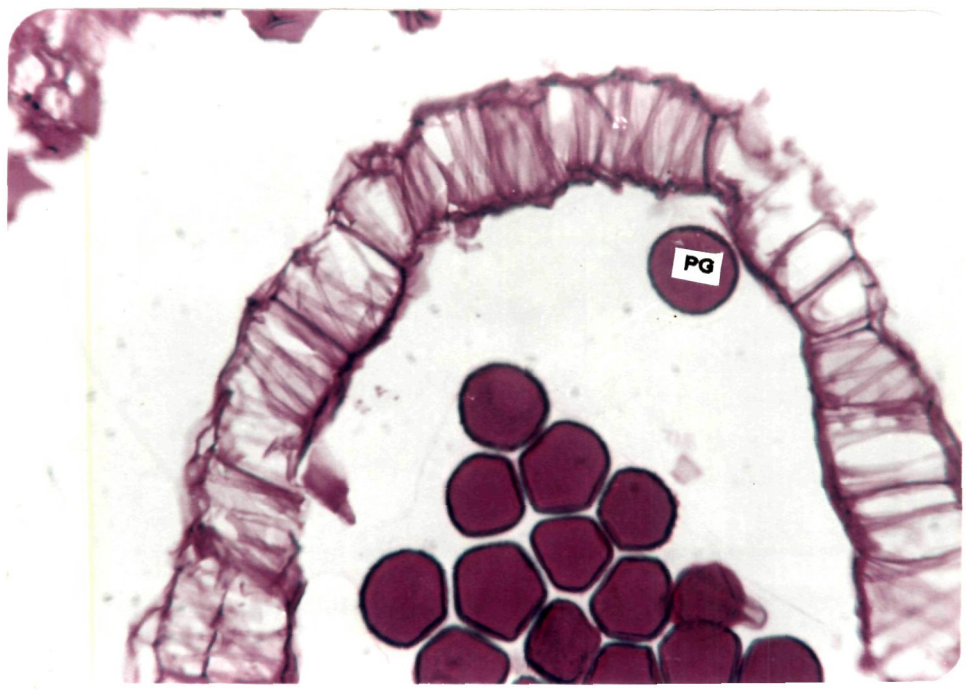

PG

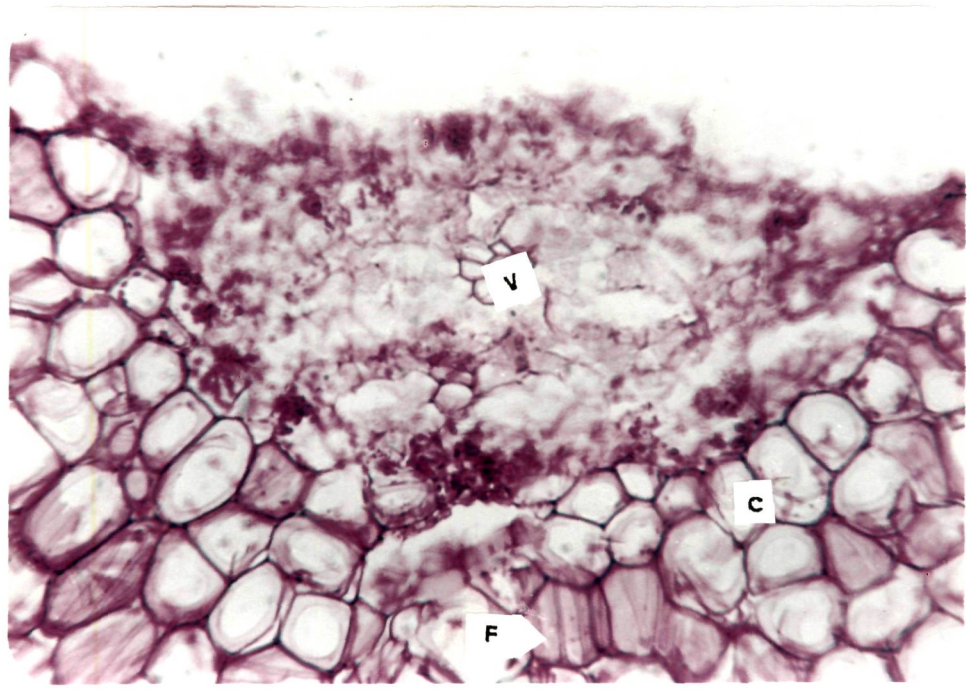
PLATE 12

Fig 4.22 : The cells of the connective contiguous to the endothecium with fibrous wall thickenings that are similar to endothecial thickenings.

C -- Connective

F -- Fibrous wall thickenings

V -- Vascular Strand


V
C
F

The pollen grains in mature anthers before anther dehiscence were intense in polysaccharide content, starch accumulation was observed in them (Figs.4.18,4.19). Even at anther dehiscence, the pollen grains were intense in insoluble polysaccharides but no starch grains were present. The endothecium, before and at anther dehiscence was found to be rich in insoluble polysaccharides (Figs.4.20,4.21).

4.3.2. **RNA**

Toluidine Blue method was used for histochemical localisation of RNA. The changes in RNA content during microsporogenesis and male gametophyte development are presented in Table 7 :The sporogenous cells and the cells of the tapetal primordium were poor in RNA. The cells of the connective had rich RNA content at this stage (Fig.4.23).

When the PMCs differentiated from the sporogenous cells, the tapetal cells showed rich RNA content. The PMCs however, remained poor in RNA content. The middle wall layers, endothecium and epidermis were found to be poor in RNA content. The connective cells were rich in RNA (Figs.4.24,4.25).

At tetrad stage, the tetrads as well as the tapetal cells showed intense RNA content while the middle wall layers, endothecium and epidermis were poor in RNA content. (Fig.4.26). The cells of the connective were also poor in RNA while the vascular tissues showed rich RNA content.

At the time of separation of microspores from the tetrads, the tapetal cells with

Table 7. Changes in RNA content during Microsporogenesis and Male gametophyte development in *S.wallisii*

Stages	Sporogenous tissue	Tapetum	Middle wall layers	Endothecium	Epidermis	Connective tissue	Vascular strand
Sporogenous tissue	+	+	+	+	+ +	+ +	+
Pollen Mother cells	+	+ +	+	+	+	+ +	+ +
Tetrads	+ + +	+ + +	+	+	+	+	+ +
Microspores	+ +	+ + + (diffused)	**crushed**	+		+	+ +
Pollen grains	+ + +	**diffused**	**crushed**	+		+	+ +
Anther Dehiscence	+ + +	diffused	crushed	+		+	+ +

- => Absent
+ => Poor
+ + => Rich
+ + + => Intense

PLATE 13

Fig 23 to 34 : Sections of Anthers tested with toluidine blue mehtod for localising RNA at successive stages of microsporgenesis and male gametophyte development in *S.wallisii*.

Fig 4.23 : Sporogenous tissue of anther with low RNA content in sporocytes. x400

Fig 4.24 : PMC's having low RNA content and tapetum with rich RNA content. x100

Fig 4.25 : PMC's showing divisional stages. x400

C -- Connective

Cl - Callose

En - Endothecium

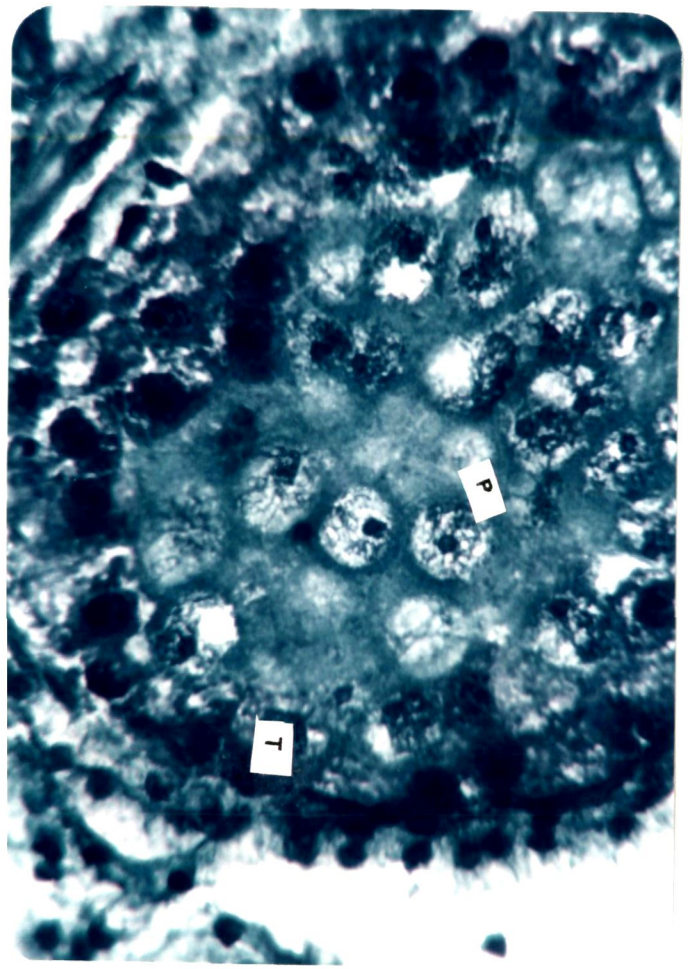
Ep - Epidermis

M -- Middle wall layers

T -- Tapetum

Tr - Tetrad

V --Vascular strand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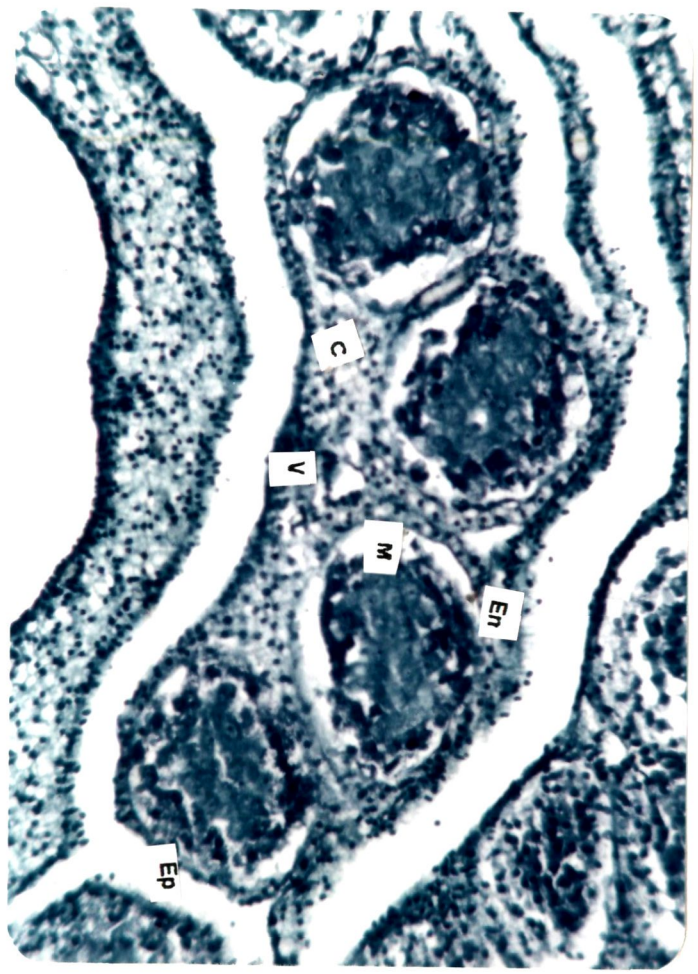

C
V
M
En
Ep

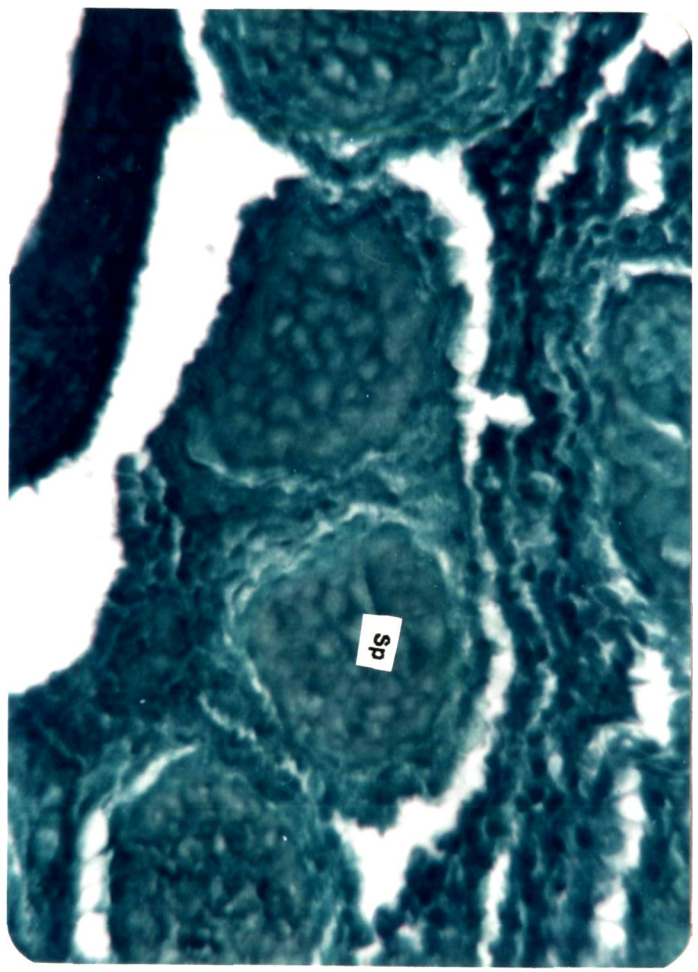

Sp

PLATE 14

Fig 4.26 : Decussate and isobilateral tetrads with rich RNA content and the intact tapetum intensely stained for RNA. x400

Fig 4.27 : Microspores with rich RNA content and tapetal cells intensely stained for RNA. x400

Note: Initial Stages of diffuson of the tapetal contents

Fig 4.28 : Tapetal cells completely diffused forming 'Periplasmodium' at the back ground of the microspores. x400

C -- Connective

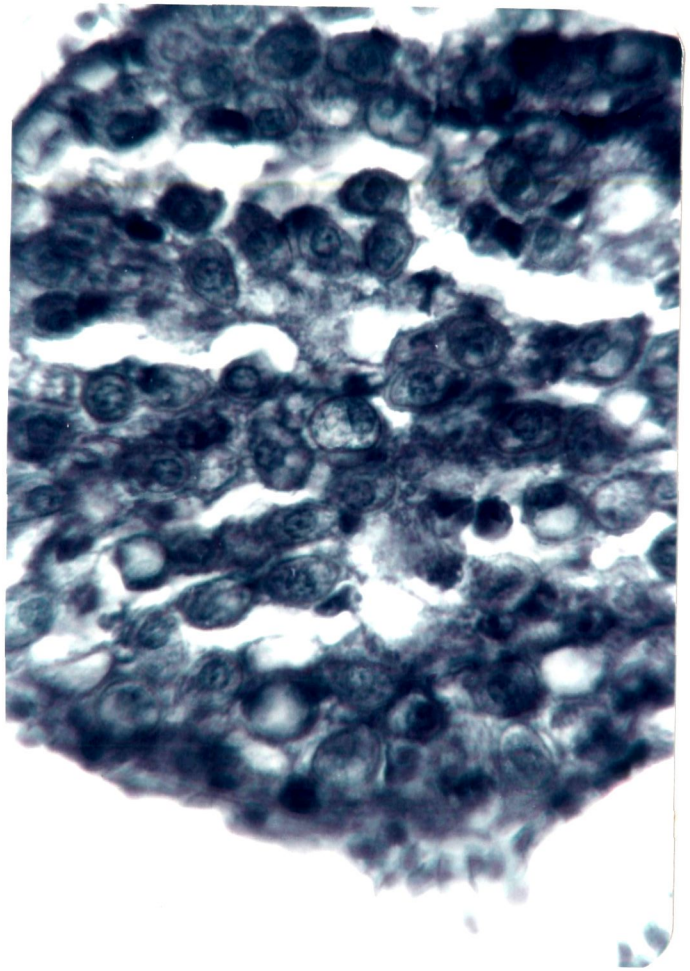
En - Endothecium

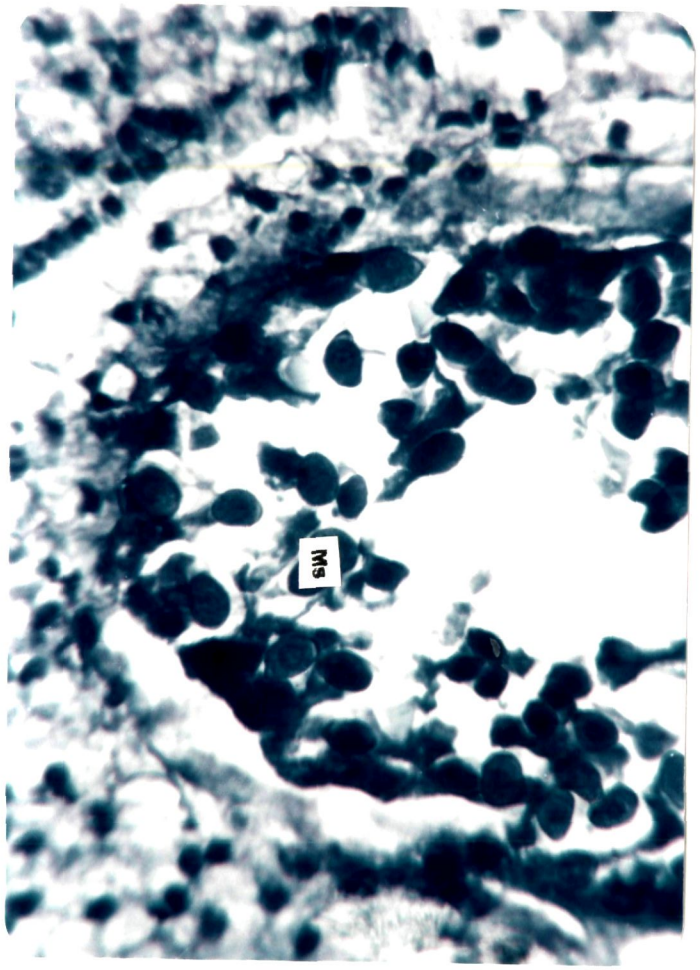
Ep - Epidermis

Ms - Microspores

T -- Tapetum

Tr - Tetrad

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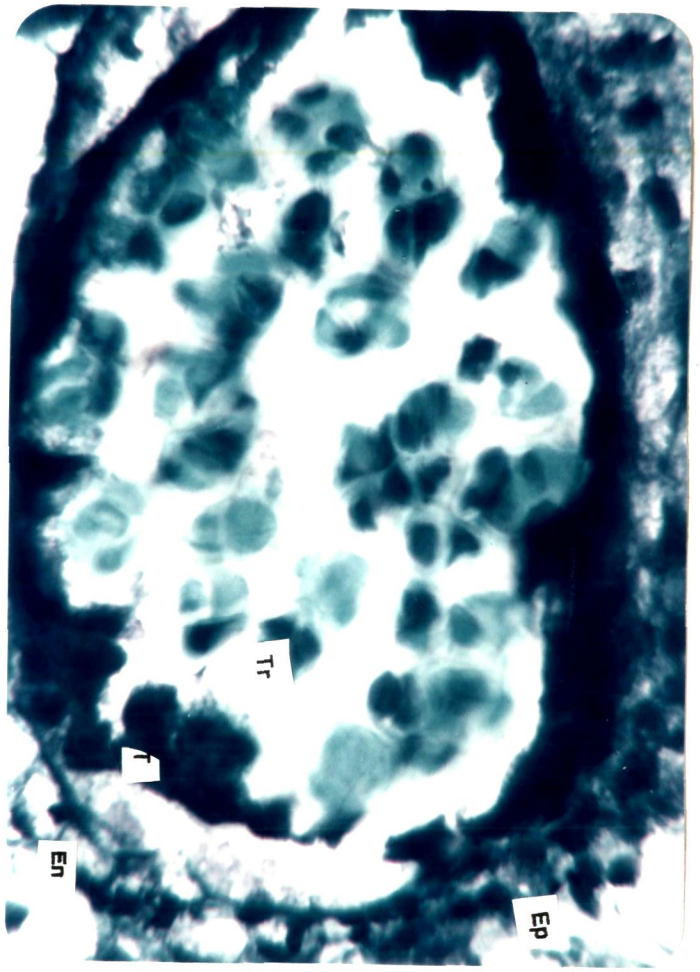

Tr
T
En
Ep

PLATE 16

Fig 4.29 : Microspores with rich RNA content and tapetum with intense RNA content forming 'Periplasmodium' seen at the back ground of the spores. x400

Fig 4.30 : Mature pollen grains intensely stained for RNA and endothecium with rich RNA content.
Note: The cells of connective contiguous to the endothecium with fibrous wall thickenings. x100

Fig 4.31 : Pollen grains intensely stained for RNA and endothecium with rich RNA content. x400

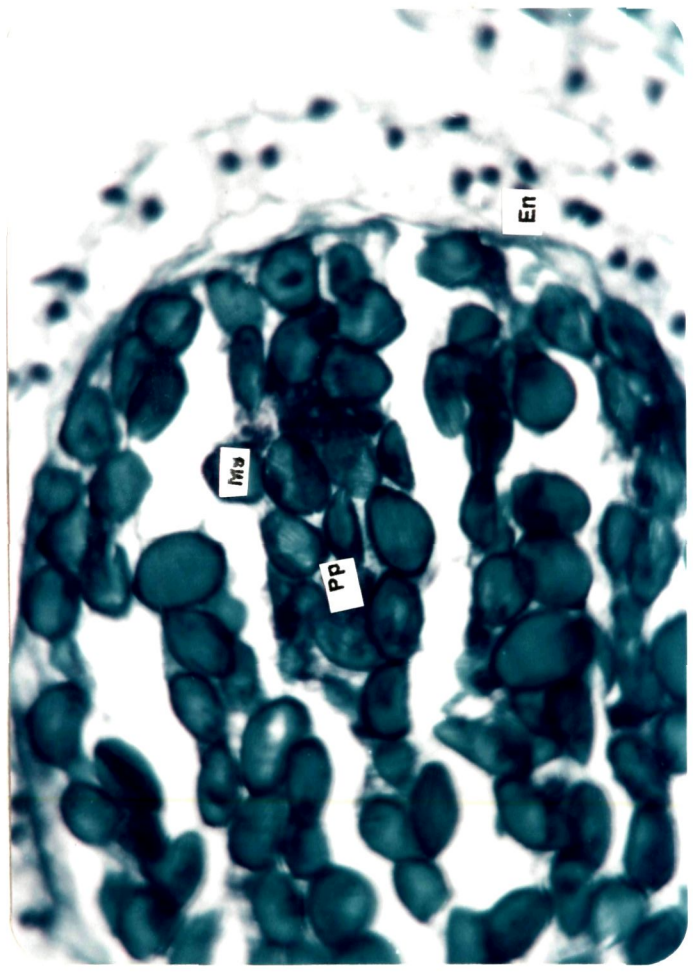
C -- Connective

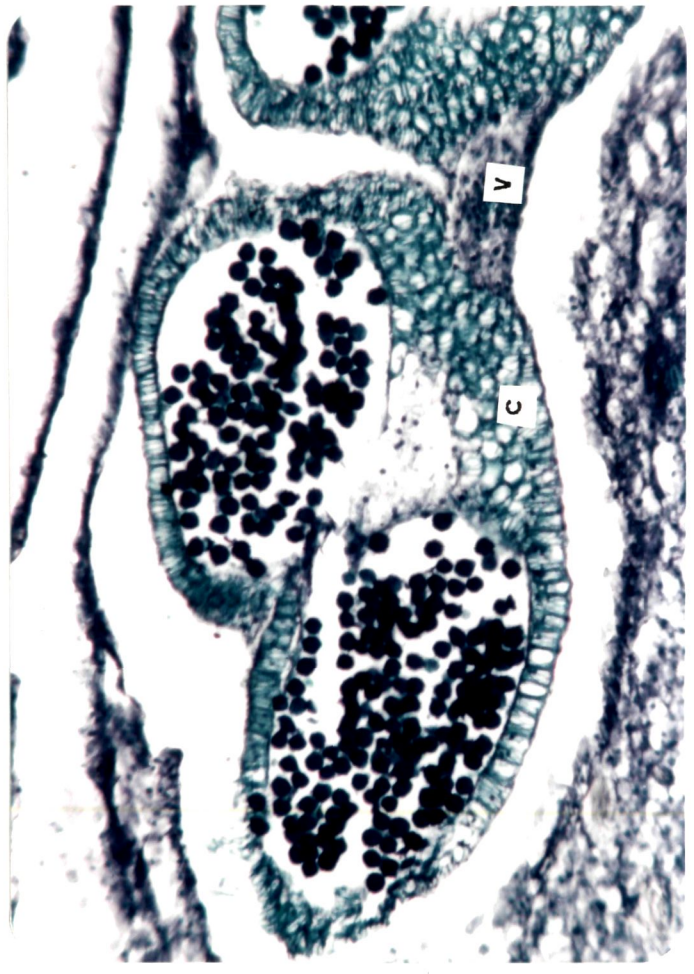
En - Endothecium

Ms - Microspores

PG - Pollen grains

Pp - Periplasmodium


En
Ms
Pp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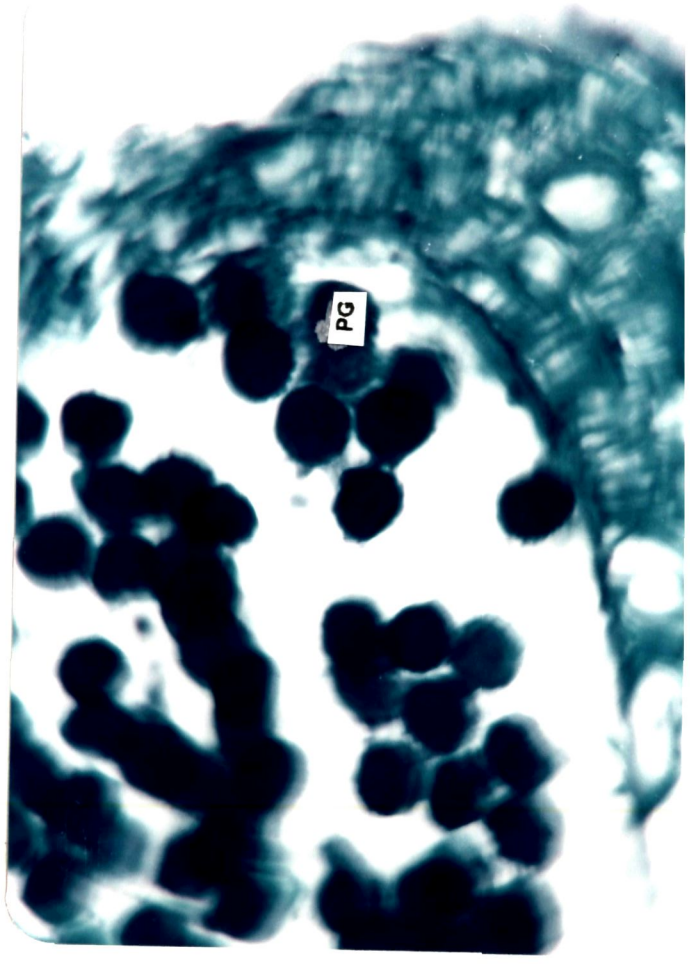

PG

PLATE 16

Fig 4.32 : Pollen grains intensely stained for RNA at anther dehiscence stage. x100

Fig 4.33 : Pollen grains with intense RNA content and endothecium with rich RNA content at Anther dehiscence.

Fig 4.34 : Connective cells contiguous to the endothecium with fibrous wall thickenings.

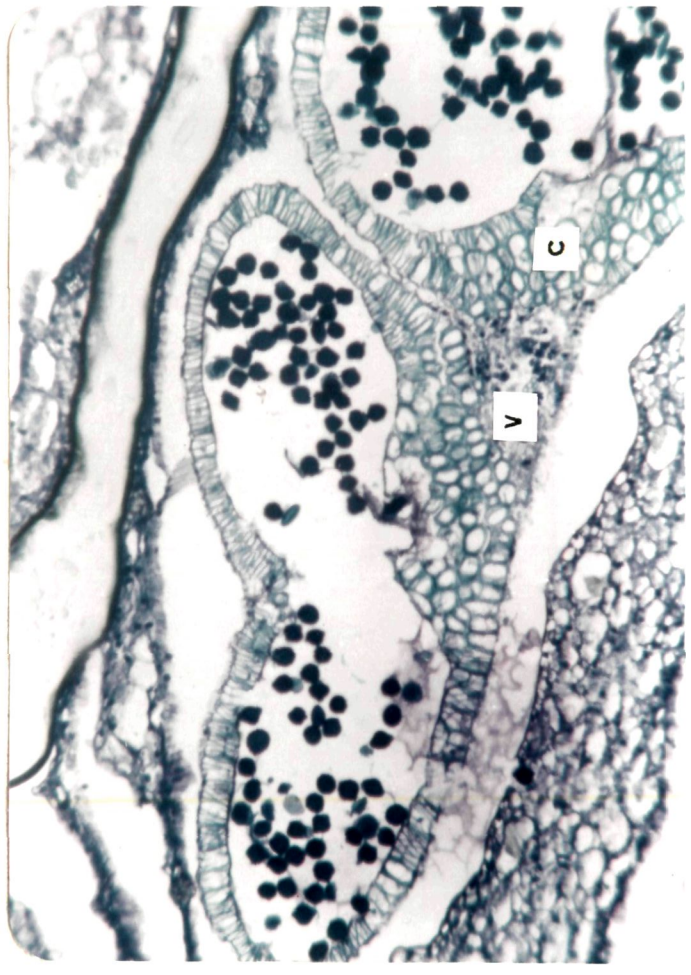
C -- Connective

En - Endothecium

F -- Fibrous wall thickenings

PG - Pollen grains

V - Vascular strand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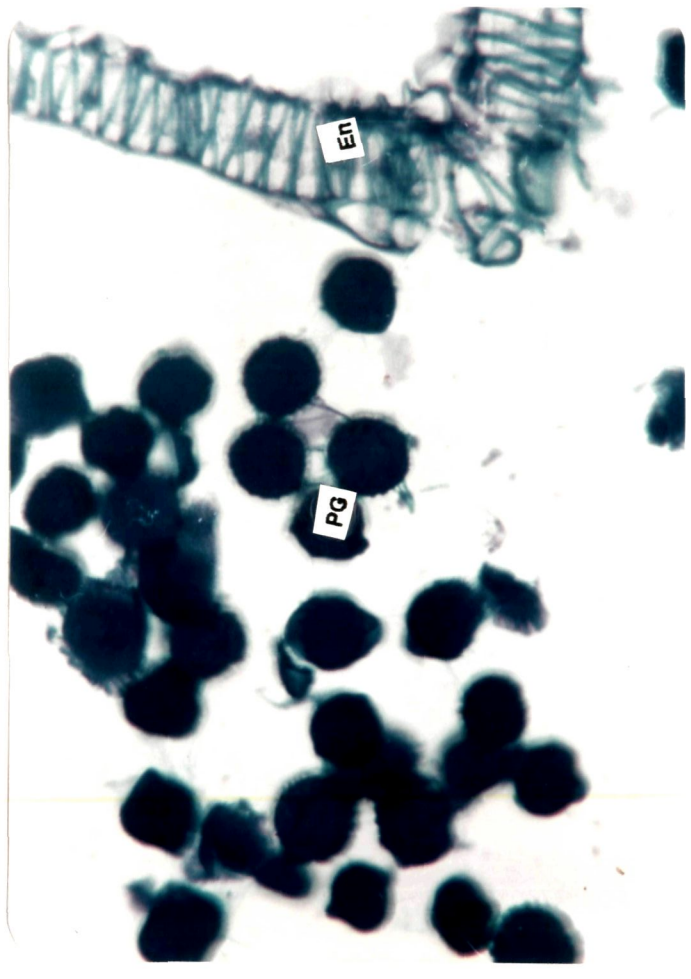
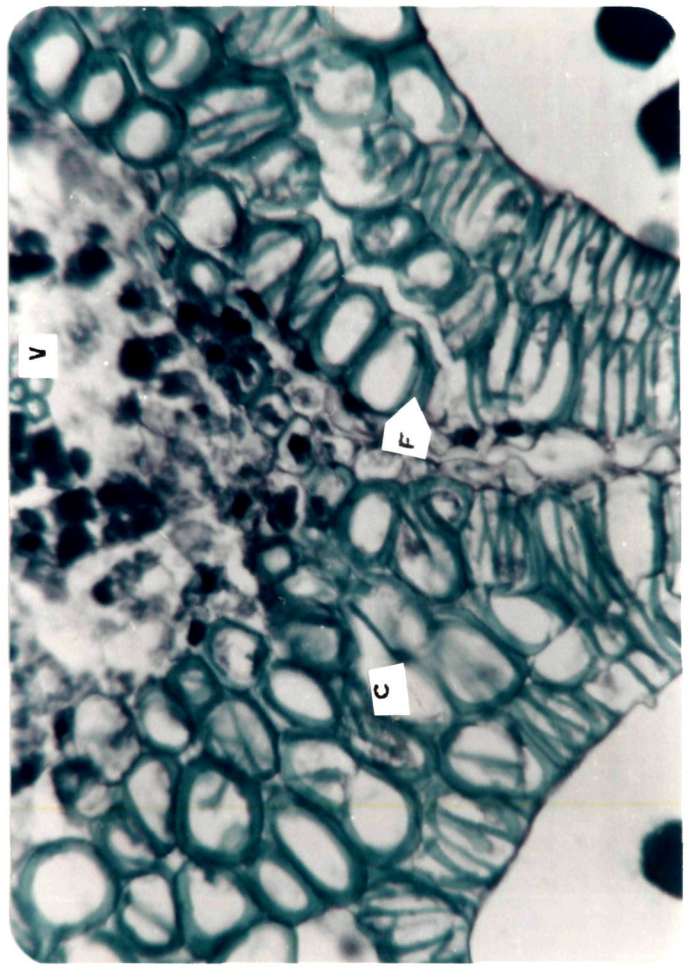
V


En
PG


V
F
C

intense RNA content started getting diffused into the anther locule (Fig 4.27). The microspores were rich in RNA and the tapetal cells showed intense RNA content. The endothecium and the cells of the connective were poor in RNA while the vascular tissue showed rich RNA content (Figs.4.27,4.28,4.29)

At pollen grain stage, the tapetal contents got completely diffused into the anther locule. The cells of the endothecium and the connective were poor in RNA while the vascular cells were rich in RNA content. The pollen grains were intensely stained for RNA (Figs.4.30,4.31,4.32,4.33).

4.3.3. **Proteins**

Mercuric Bromophenol Blue method was employed to localise the proteins in the plant material. The histochemical changes observed at various stages of microsporogenesis and male gametophyte development are presented in Table 8 and described as follows :

At very early stages of development of the anther, the sporogenous cells were poor in proteins. The tapetal primordium middle wall layers, endothecium and epidermis also were poor in protein content (Fig.4.35).

As PMCs differentiated, the tapetum showed rich protein content, while the PMCs were poor in proteins . The middle wall layers, endothecium, epidermis and the

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Table 8. Changes in Protein content during Microsporogenesis and Male gametophyte development in *S.wallisii.*

Stages	Sporogenous tissue	Tapetum	Middle wall layers	Endothecium	Epidermis	Connective tissue	Vascular strand
Sporogenous tissue	+	+	+	+	+	+	
Pollen Mother cells	+	++	+	+	+	+	+
Tetrads	+++	++	+	+	+	+	+
Microspores	+++	++ (diffused)	crushed	+		+	++
Pollen grains	+	diffused	crushed	+		+	++
Anther Dehiscence	+++	diffused	crushed	+		+	++

- => Absent
+ => Poor
++ => Rich
+++ => Intense

PLATE 17

Fig 35 to 45 :Sections of the anthers tested with Mercuric Bromophenol Blue method the localise proteins at successive stages of microsporogenesis and male gemetophyte development in *S.wallisii.*

Fig 4.35 : Sporogenous tisssue showing low protein content of sporocytes x400

Fig 4.36 : PMC's with low protein content x100

Fig 4.37 : Tapetum with richly stained for proteins and PMC's low in protein content. x400

C -- Connective

En - endothecium

Ep - epidermis

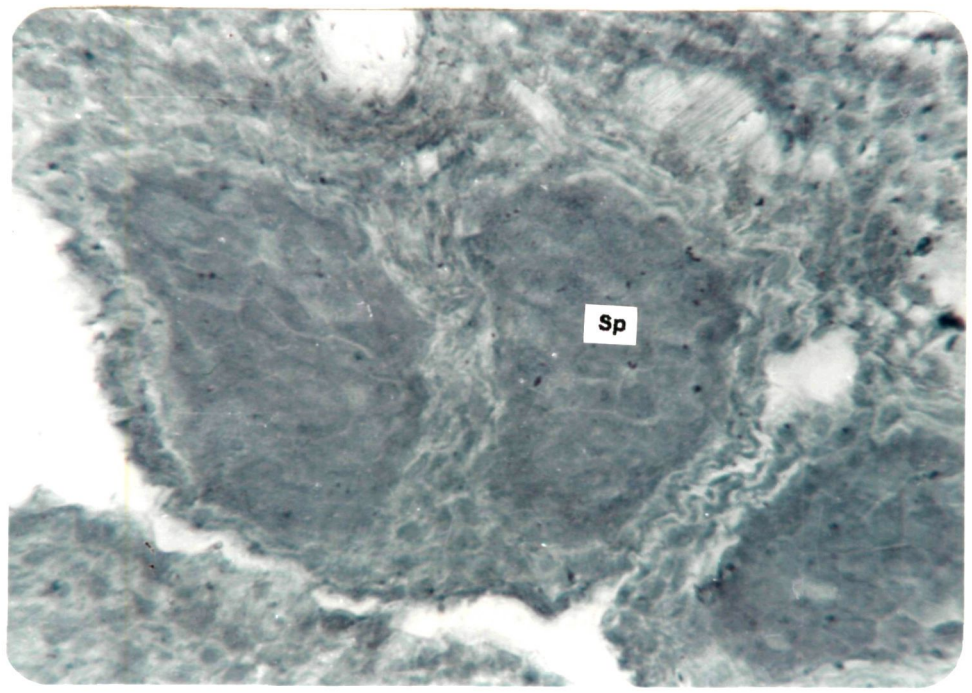
M -- Middle wall layers

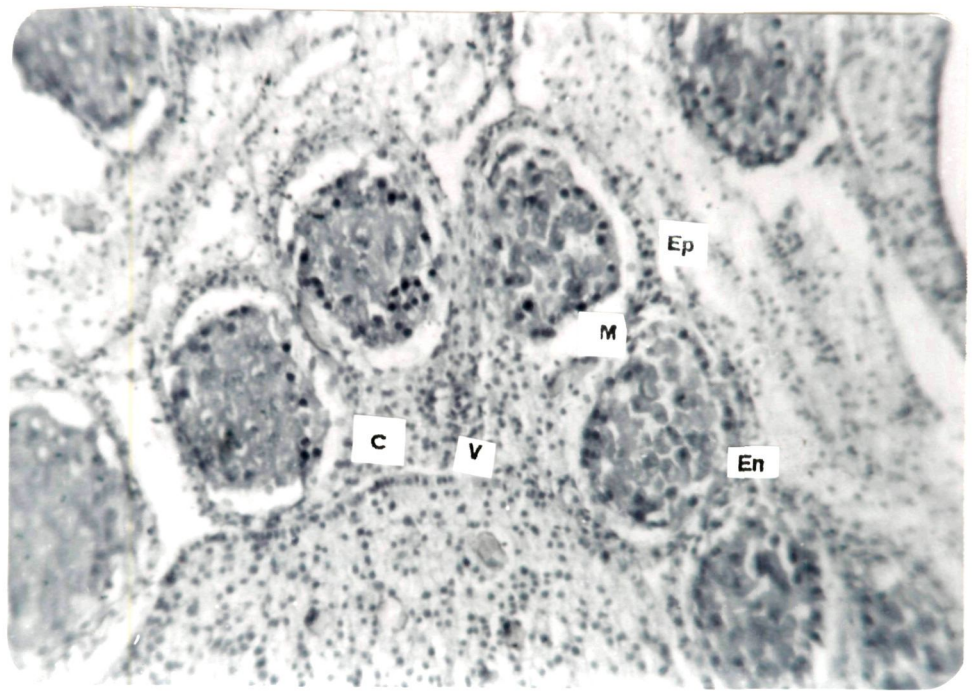
P -- Pollen mother cell

Sp - Sporogenous tissue

T -- Tapetum

V -- Vascular Strand


Sp


Ep
M
C
V
En

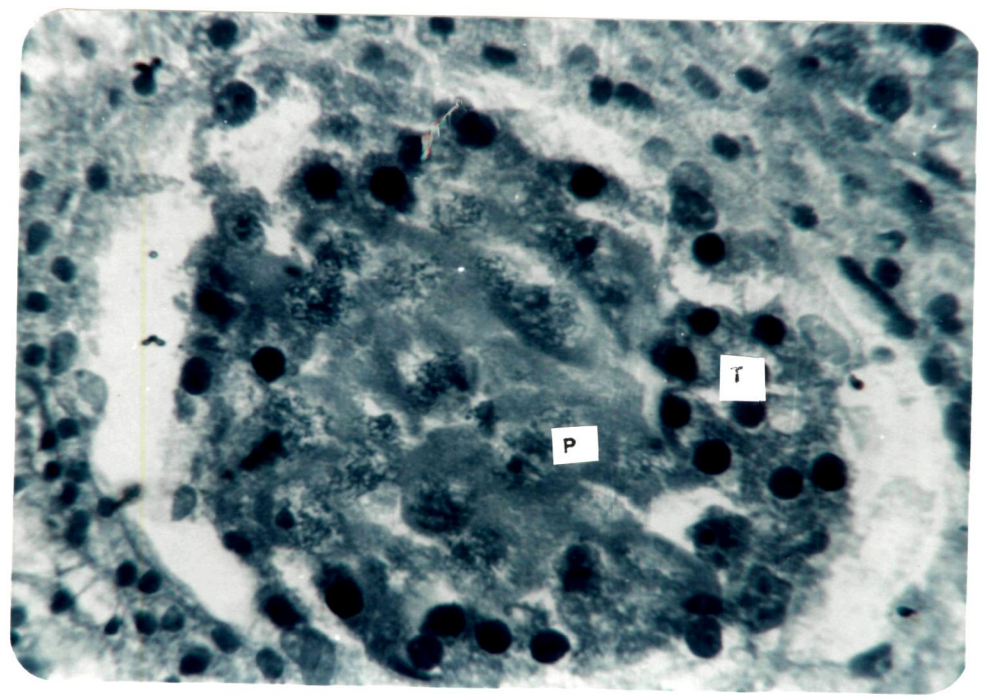

T
P

PLATE 18

Fig 4.38 : Tetrads intensely stained for proteins and intact tapetum with rich protein content. x400

Fig 4.39 : Microspores with intense protein content. x400

Note:Initiation of the diffusion of tapetal contents rich in proteins into the anther locule.

Fig 4.40 : Pollen grains with poor protein content and endothecium also poor in proteins. x100

Note : The fibrous wall thickenings in endothecial cells and the cells of connective next to the endothecium.

C -- Connective

En - Endothecium

Ms - Microspores

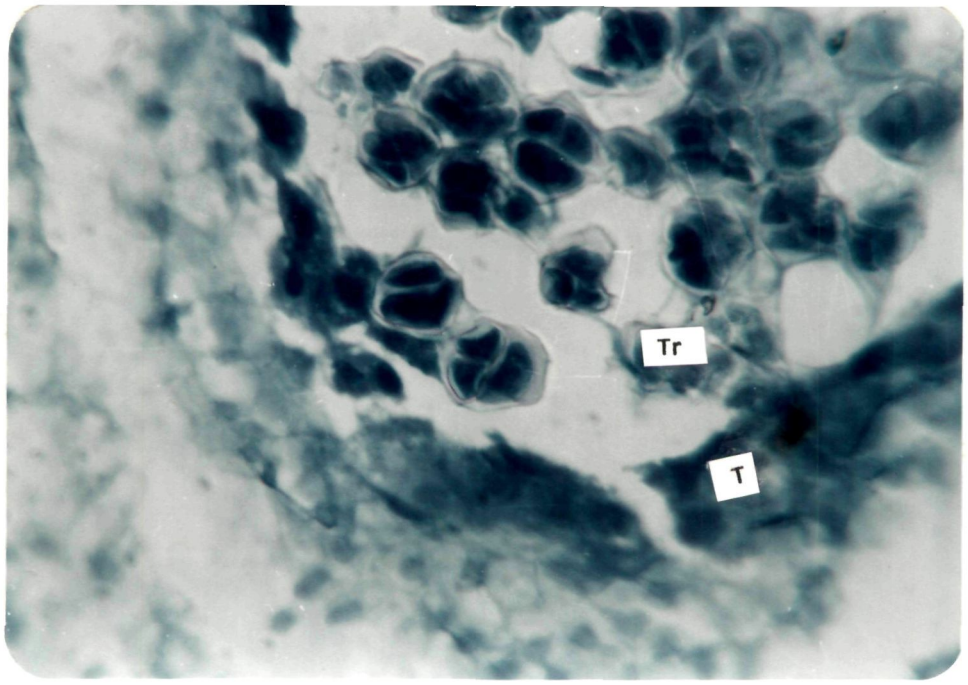
PG - Pollen grains

Pp - Perplasmodium

T -- Tapetum

Tr - Tetrad

V -- Vascular Strand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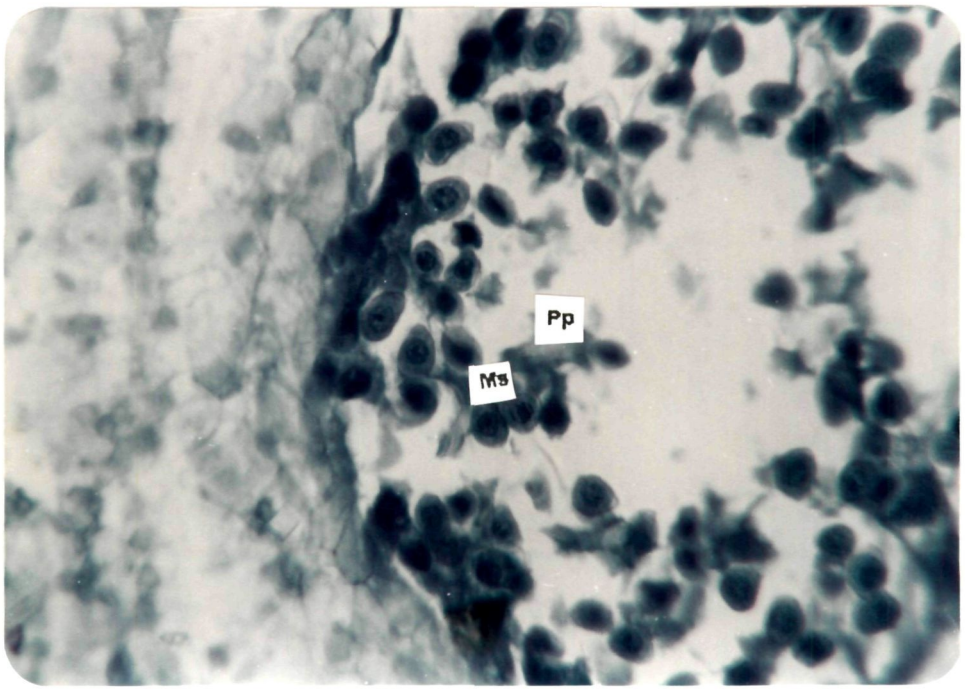

Pp
Ms

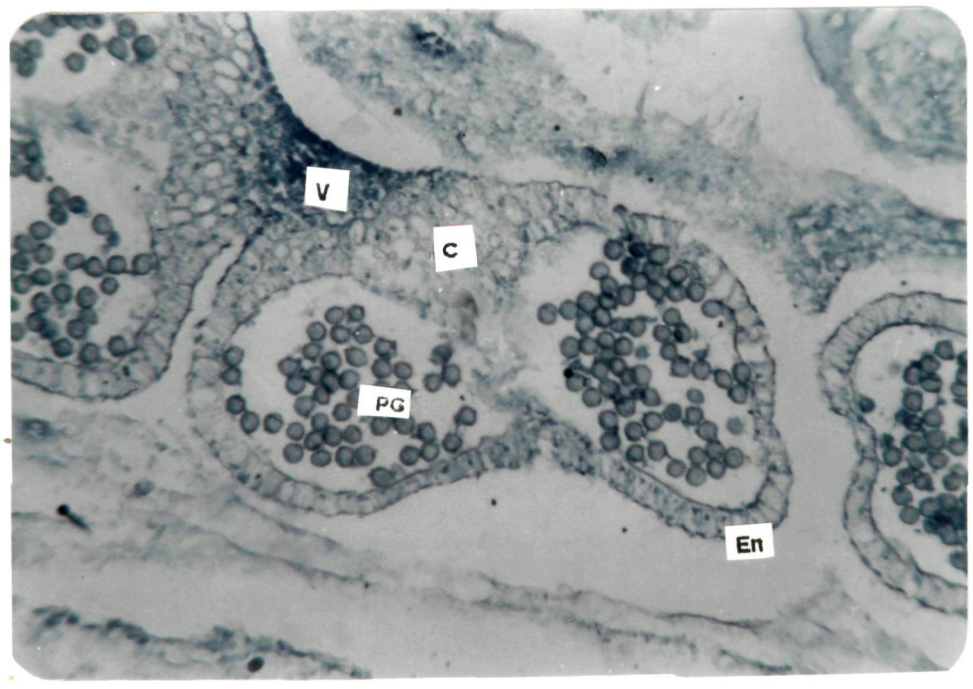

V
C
PG
En

PLATE 19

Fig 4.41 : Younger pollen grains with rich protein content and older pollen grains with poor protein content. x400

Fig 4.42 : Pollen grains nd endothecium with poor protein content, x400

Fig 4.43 : Mature pollen grains intensely stained for proteins at the stage of anther dehiscence.

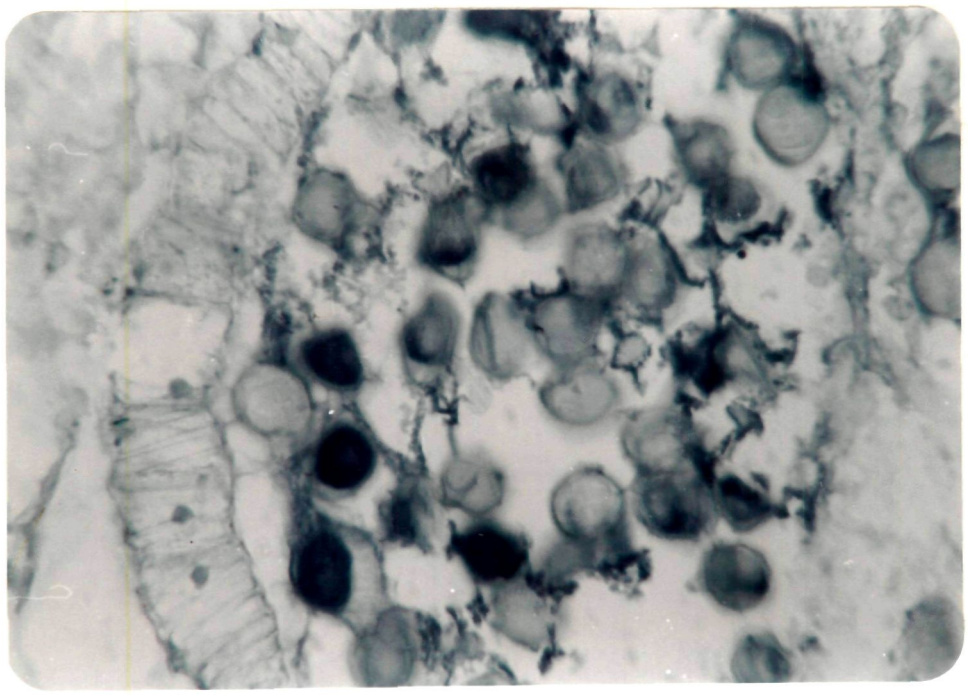
Note : Fibrous wall thickenings on the endothecial cells and cells of connective next to endothecium.

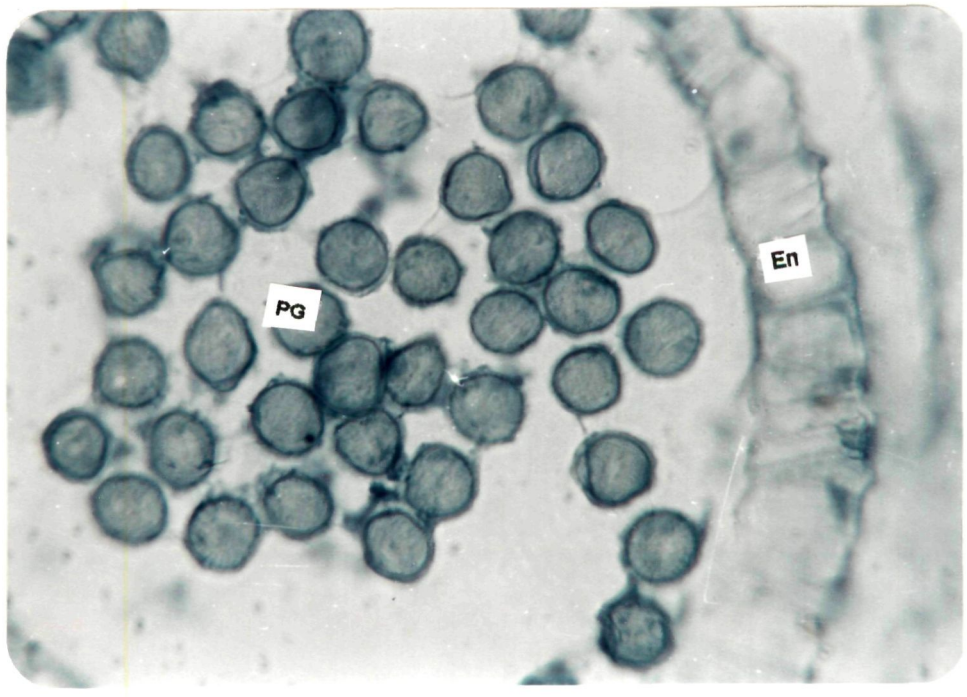
C -- Connective

En - Endothecium

PG - Pollen grains

V -- Vascular strand




En
PG

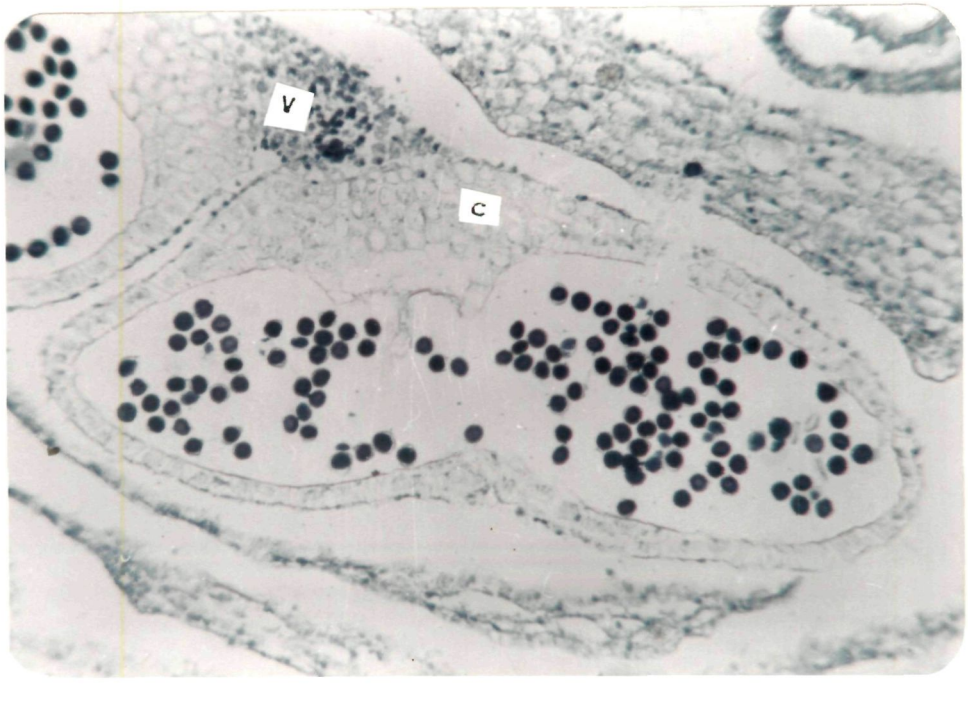

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PLATE 20

Fig 4.44 : Mature pollen grains intensely stained for proteins and endothecium with poor protein content.

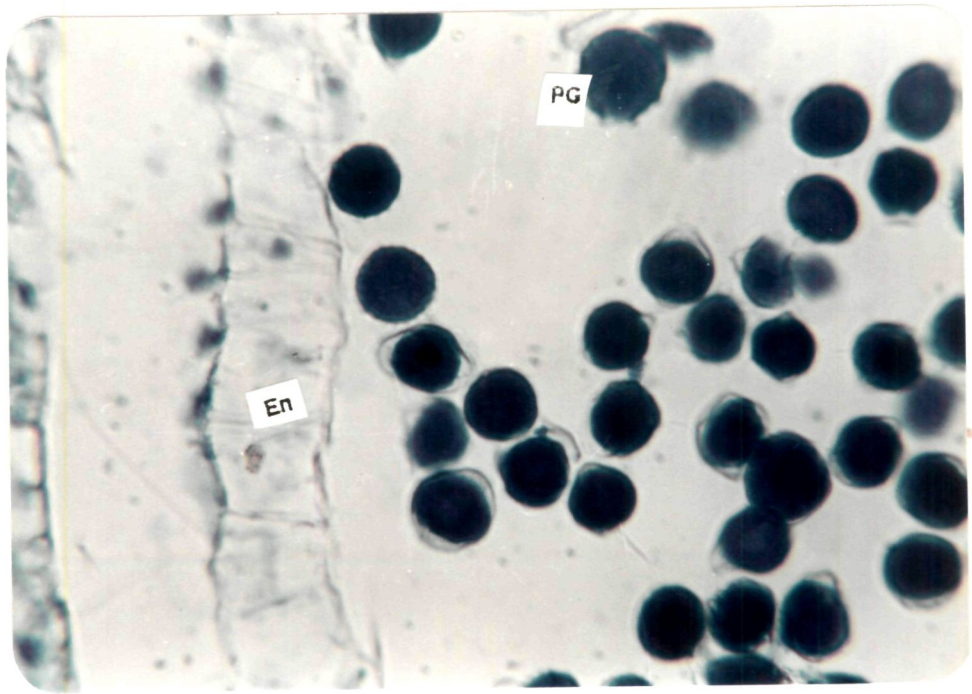
Fig 4.45 : Cells of connective next to th endothecium with fibrous wall thickenings that are similar to those in the endothecium.

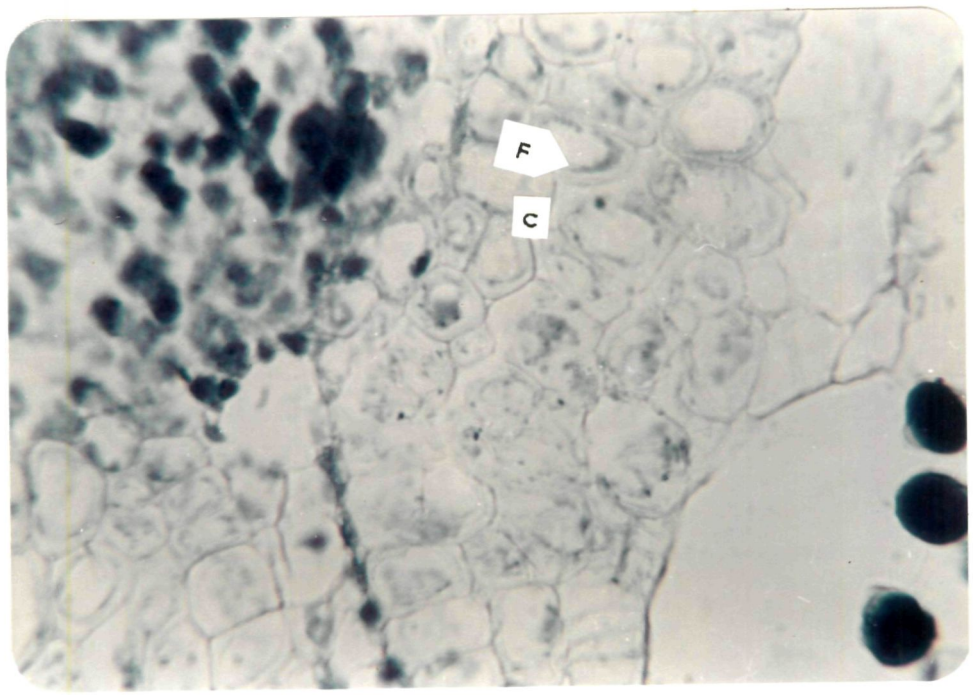
C -- Connective

En - Endothecium

F -- Fibrous wall thickenings

Ps - Pollen grains


PG
En


F
C

connective tissue were poor in proteins (Fig.4.36,4.37).

The tetrads formed after meiosis of the PMCs, were intensely stained for proteins while the tapetum showed rich protein content. The other components of anther were poor in protein content (Fig.4.38).

The microspores separated from tetrads were intensely stained for proteins. The tapetum rich in proteins, started getting diffused into the anther locule. The endothecium and connective cells were poor in protein content (fig.4.39).

Before anther dehiscence, the pollen grains were found to be poor in proteins, so also the endothecium and connective cells. Only the vascular cells showed rich protein content at this stage (Figs.4.40,4.41,4.42). However, at the stage of anther dehiscence, the pollen grains were intensely stained for proteins (Figs.4.43,4.44).

4.4. HISTOLOGICAL CHANGES DURING MEGASPOROGENESIS AND FEMALE GAMETOPHYTE DEVELOPMENT.

The histological changes during megasporogenesis and female gametophyte development and micrometric observations atsuccessive stages of ovule development in *S.wallisii* are presented in Table 9.

The vertical section of the ovule in the early stages of development showed a mass of isodiametric cells from which the archesporium differentiated hypodermally.At this stage the ovule was 60.00 µm in length and 72.00 µm in width. The archesporium divided periclinally and gave a parietal cell to the exterior and a megaspore mother cell (MMC) to the interior following "crassinucellate" pattern of development. At this stage,

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Table 9. Micrometric Observations during Megasporogenesis and Female gametophyte development in *S.wallisii*

Stage	Ovule (m)	Embryo sac (m)	Individual cell size(m)	Nucellus (µm)	Hypostase (µm)	Integuments
Archesporium	L 60.00			36.00		outer 7.2
	W 72.00			31.20		inner 4.8
MMC	L 156.00		L 14.40	72.00		outer 9.6
	W 91.20		W 12.00	38.40		inner 7.2
FMS	L 127.20		L 21.60	81.60		outer 9.6
	W 38.40		W 14.40	48.00		inner 7.2
Organised Embryosac	L 132.00	48.00		Nucellar		outer 19.2
	W 96.00	21.60	Egg : 4.80	cap	24.00	inner 9.6
			Antipodals : 6.00	L : 25.44		
			Synergids : 4.80	W : 19.20		

the ovule was 156.00 µm in length and 91.20 µm in width. The MMC was 14.40µm in length and 12.00 µm in width. The inner and outer integuments were 9.60 and 7.20µm in thickness respectively. The MMC underwent meiosis I and II resulting in the formation of a megaspore tetrad. Out of four, the chalazal megaspore was functional and developed into a mature embryo sac. Three mitotic divisions of the nucleus in this cell, gave rise to an 8 nucleate female gametophyte which organised into a 7 celled, 8 nucleate "polygonum" type embryo sac. The nucellar cells on the sides of the embryo sac disintegrated at early stages of development and this resulted in the direct contact between the inner integument and the embryo sac. The nucellar cells at the apex persisted and developed into a "Nucellar Cap".The cells of the nucellar cap looked hypertrophied at the mature embryo sac stage. The largest cells of the nucellar cap were 25.44 µm long and 19.20 µm broad. At this stage, the ovule was 132.00 µm in length, 96.00 µm in width. The embryo sac was 48.00 µm in length and 21.60 µm in width. The inner and outer integuments were 19.20 and 9.60 µm in thickness respectively. The egg cell had a diameter of 4.8 µm, the antipodals were 6.0µm and synergids were 4.8 µm in diameter.

4.5. HISTOCHEMICAL CHANGES DURING MEGASPOROGENESIS AND FEMALE GAMETOPHYTE DEVELOPMENT IN *S.wallisii.*

At early stages of development, in the ovule primordium, the cells of the nucellus and integuments were poorly stained for insoluble polysaccharides. The megaspore mother cell was poor in insoluble polysaccharides while the nucellar cells were richly stained for insoluble polysaccharides and the integuments were intensely stained for insoluble polysaccharides (Table 10). The functional megaspore was poor in

..... 48

polysaccharide content. At this stage, the cells of the nucellus were richly stained for insoluble polysaccharides, these cells contained starch grains. The hypostase also was rich while the integuments were poor in insoluble polysaccha- rides (Fig 4.46). In the organised embryo sac, the central cell cytoplasm had traces of insoluble polysaccharides; the egg cell and the synergids were poor in polysaccharides. The integuments were found to be rich in insoluble polysaccharides (Fig.4.47). The nucellar cap also was richly stained for insoluble polysaccharides, the cells of which contained starch grains (Fig.4.47). The hypostase was intense in insoluble polysaccharides(Fig.4.48).

At early stages of development, the nucellar cells and the integuments were richly stained for RNA (Fig.4.49) but the megaspore mother cell (MMC) was found to be poor in RNA content. The nucellar cells and integuments were rich in RNA at this stage (Fig.4.50). The functional megaspore was poor in RNA while the integuments and nucellus showed rich RNA content (Table 11). In the organised embryo sac, the cyto- plasm of the central cell had traces of RNA, while the egg cell and synergids were intensely stained for RNA. The integuments were found to be rich in RNA. The hypostase was intensely stained for RNA while the cells of the nucellar cap were poor in RNA content (Fig. 4.51,4.52).

The nucellar cells in the ovule primordium were found to be rich in proteins, while the integuments were intensely stained for proteins (Fig.4.53). The megaspore mother cell, differentiated from the archesporial cell, was poor in protein content while the nucellus and the integuments were rich in proteins. Even at the functional megaspore stage, the nucellus and the integuments were rich in proteins while the functional megaspore was poor in protein content (Table 12). In the mature embryo sac, the central cell cytoplasm had traces of proteins while the egg, synergids were rich in proteins

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Table 11. Changes in RNA content during Megasporogenesis and Female gametophyte development in *S.Wallisii*

Stage	Nucellus	Individual cell	Integuments	Hypostase
Ovule primordium	+ +		+ +	
MMC	+ +	+	+ +	
Functional Megaspore	+ +	+	+ +	
Organized embryosac	+ (nucellar cap)	Egg : + + + Synergid : + + +	+ +	+ + +

- => Absent
+ => Poor
+ + => Rich
+ + + => Intense

Table12. Changes in Protein content during Megasporogenesis and Female gametophyte development in *S.wallisii*

Stage	Nucellus	Individual cell	Integuments	Hypostase
Ovule primordium	+ +		+ + +	
MMC	+ +	+	+ +	
Functional Megaspore	+ +	+	+ +	
Organized embryosac	+ (nucellar cap)	Egg : + + Synergid : + + Polar nuclei : + + Antipodals : + + +	+ +	+ + +

- => Absent
+ => Poor
+ + => Rich
+ + + => Intense

PLATE 21

Fig 4.46 to 4.48 : Longitadinal Sections of ovules tested with periodic acid Schiff's (PAS) reagent to localise insoluble polysccharides at successive stages of megasporogenesis and female gametophyte development in *S.wallisii.*

Fig 4.46 : Functional megaspore poor in insoluble polysaccharides, the nucellar cells rich in insoluble polysaccharides with starch accumulation.

Fig 4.47 : Micropylar end of the organised embryo sac showing egg cell poor in insoluble polysaccharides and the nucellar cap richly stained for insoluble polysaccharides.

Fig 4.48 : Chalazal end of the orgainised embryo sac with hypostase intensely stained for insoluble polysaccharides.

C -- Chalazal end

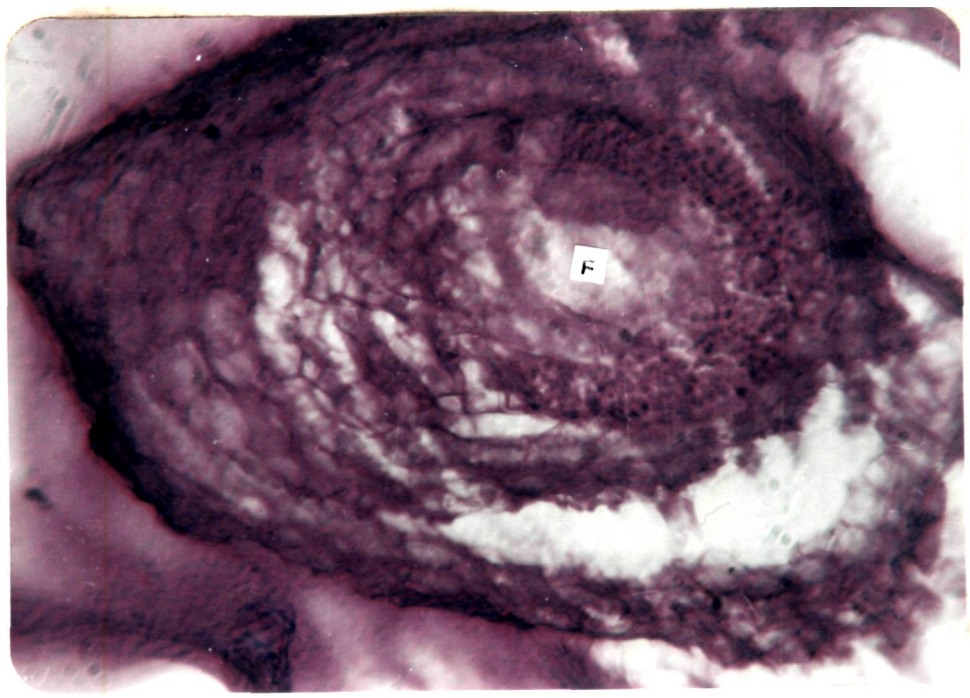
E -- Egg cell

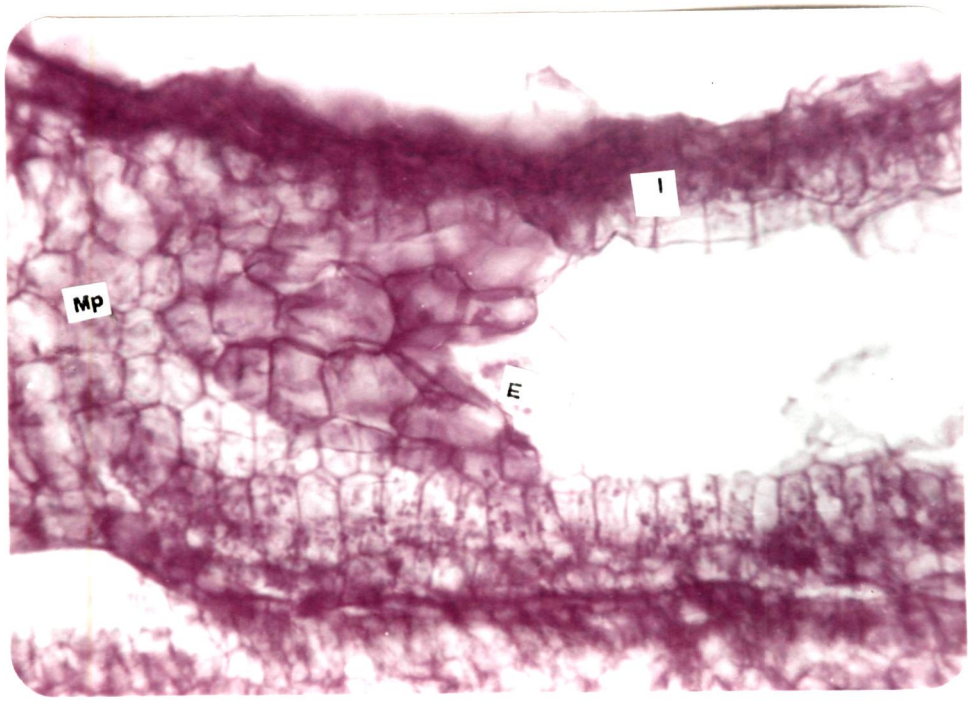
F -- Functional megaspore

H -- Hypostase

I -- Integuments

Mp - Micropylar end


F


I
MP
E

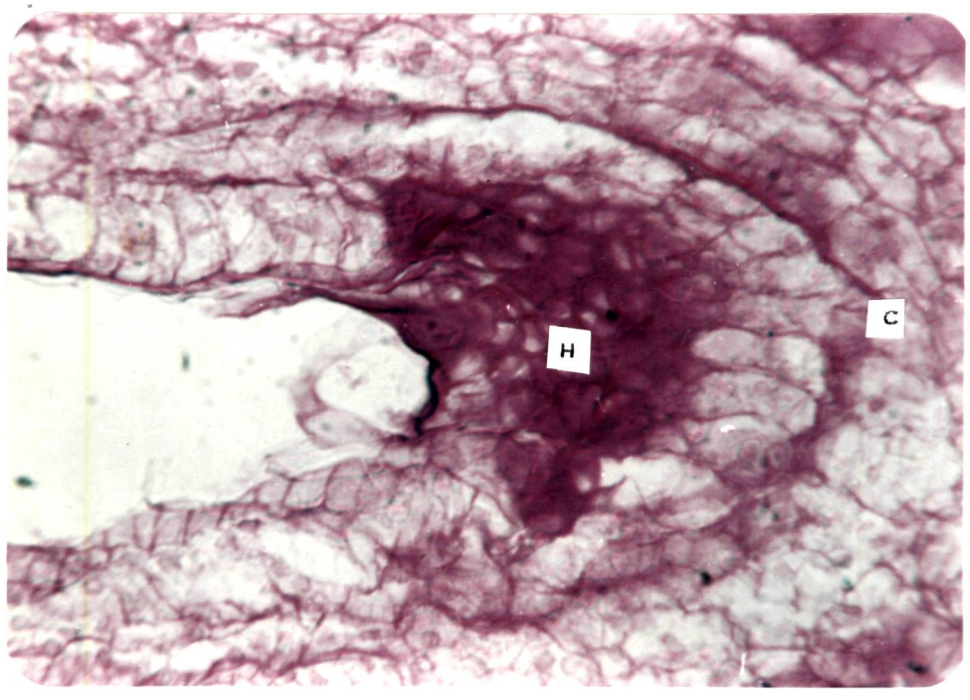

H
C

PLATE 22

Fig 4.49 to 4.52 : Longitudinal Sections of the ovule Stained with Toluidine blue to localise RNA at successive stages of megasporgenesis and female gametophyte development in *S.wallisii.*

Fig 4.49 : Ovule primordium showing nucellus and integuments rich in RNA

Fig 4.50 : Megaspore mother cell poor in RNA, nucellus and integuments richly stained for RNA.

Fig 4.51 : Organised embryo sac showing integuments with rich and cells of the nucellar cap with poor RNA content.

C – Chalazal end

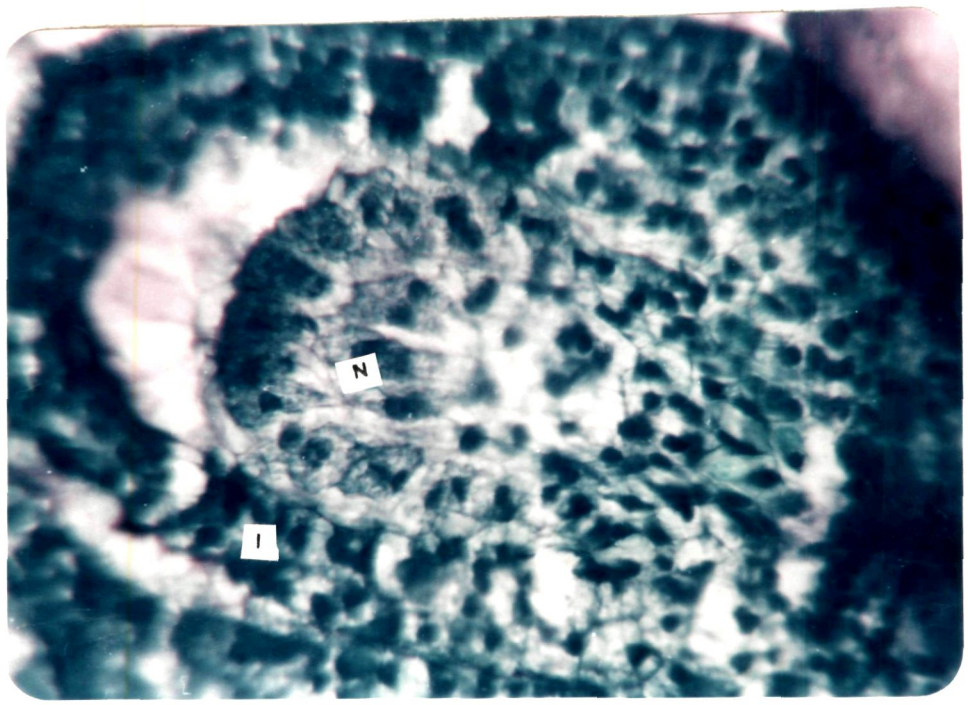
I -- Integuments

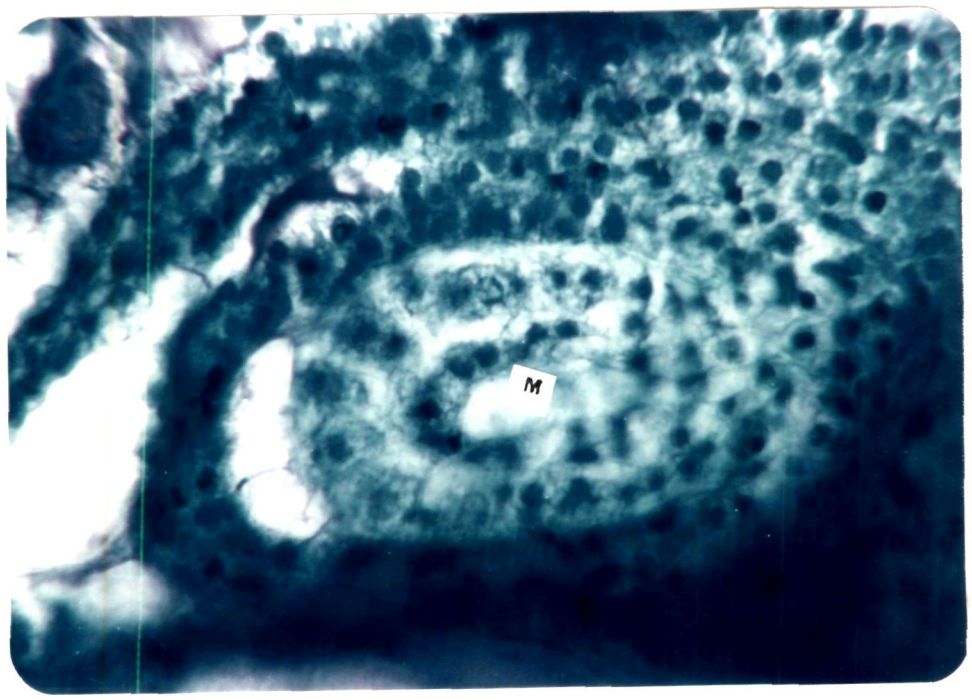
N -- Nucellar

Nc - Nucellar cap

M -- Megaspore mother cell

Mp - Micropylar end


N
I


M

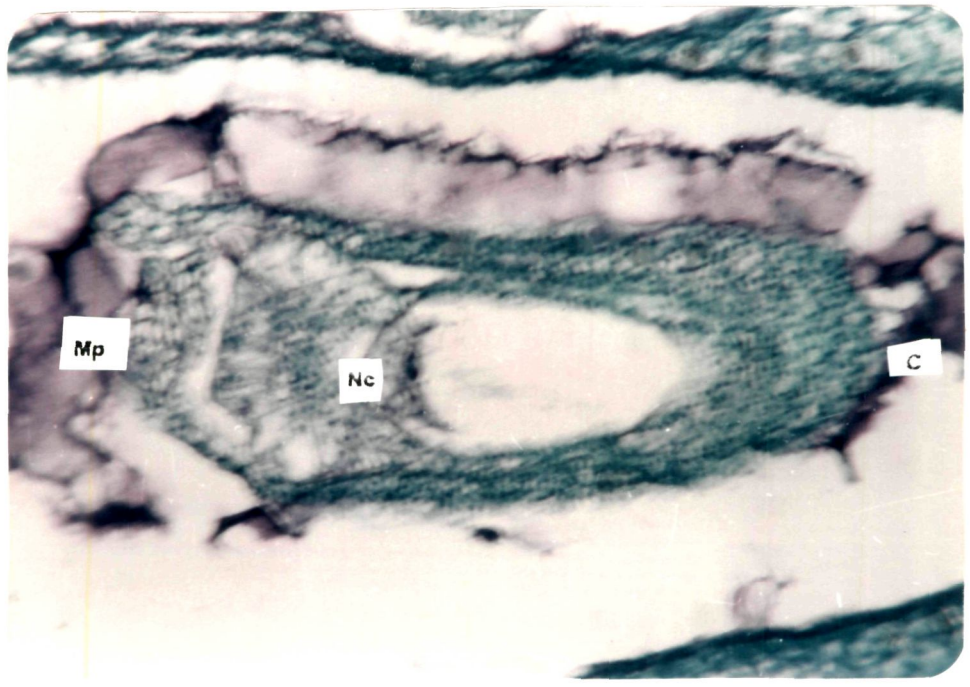

Mp
Nc
C

PLATE 23

Fig 4.52 : Micropylar end of the organised embryo sac, showing the egg cell and synergids intensely stained for RNA.

E -- Egg cell

Nc - Nucellar cap

S -- Synergids

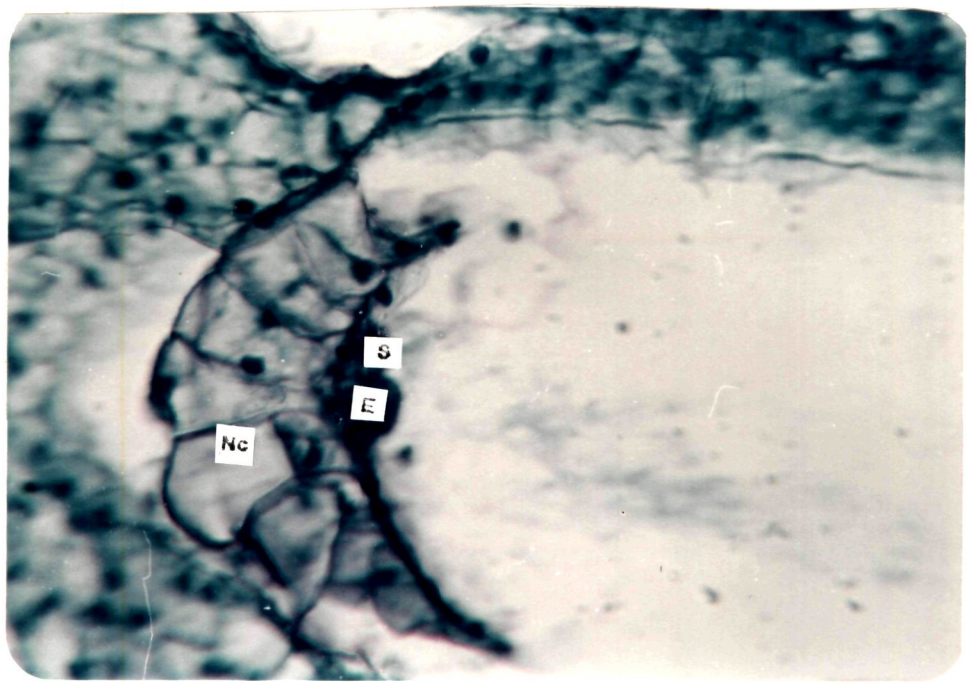

S
E
Nc

PLATE 24

Fig 4.53 to 4.57 : Longitudinal Sections of the ovule tested with Mercuric Bromophenol Blue to localise proteins at successive stages of megasporogenesis and female gametophyte Development in *S.wallisii*.

Fig 4.53 : Ovule primordium showing nucellus richly stained for proteins and integuments intense in Protein content.

Fig 4.54 : Micropylar end of the organised embryo sac showing egg cell richly stained for proteins and cells of the nucellar cap poor in protein content.

Fig 4.55 : Micropylar end of organised embryo sac showing egg cell and synergid rich in protein content.

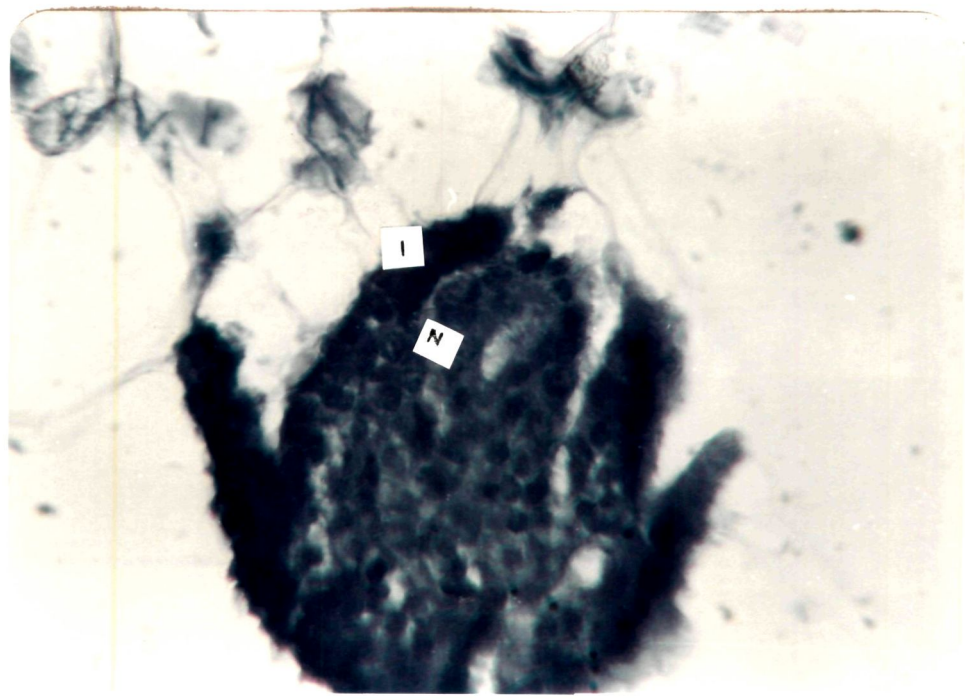
E -- Egg

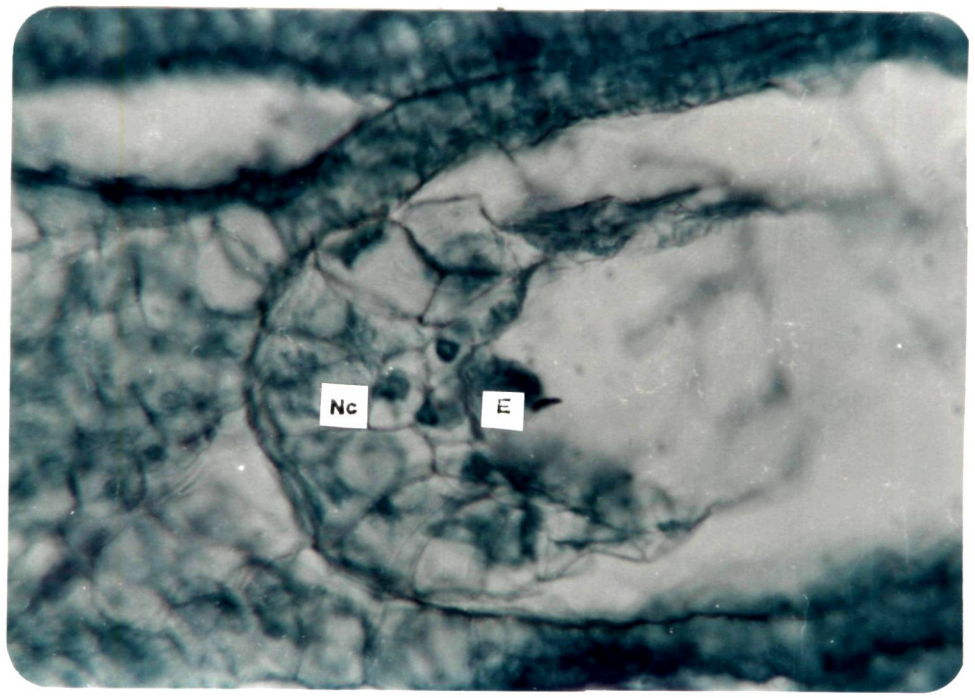
I -- Integuments

N -- Nucellar

Nc - Nucellar cap

S -- Synergids.


I
N


Nc
E

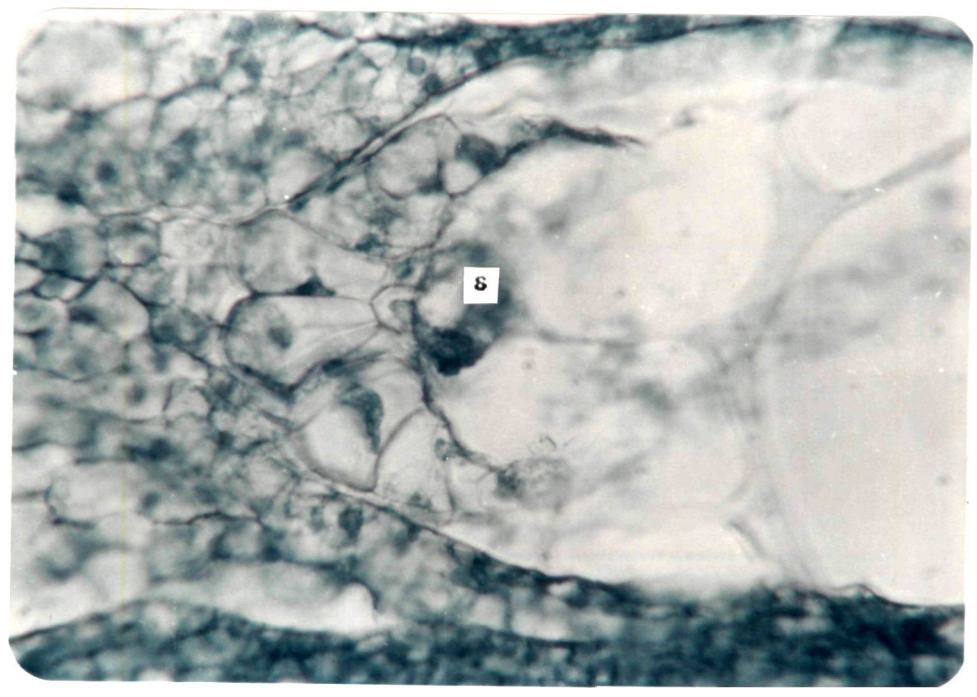

S

PLATE 25

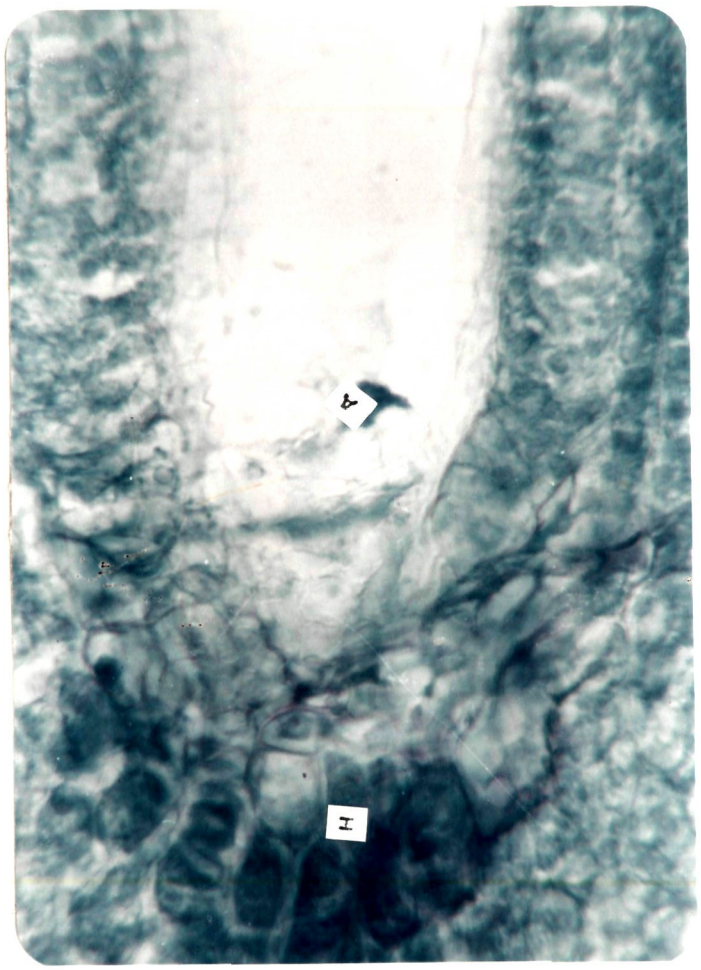
Fig 4.56 : Chalazal end of the organised embryo sac showing hypostase and one of the antipodal cells intensely stained for proteins.

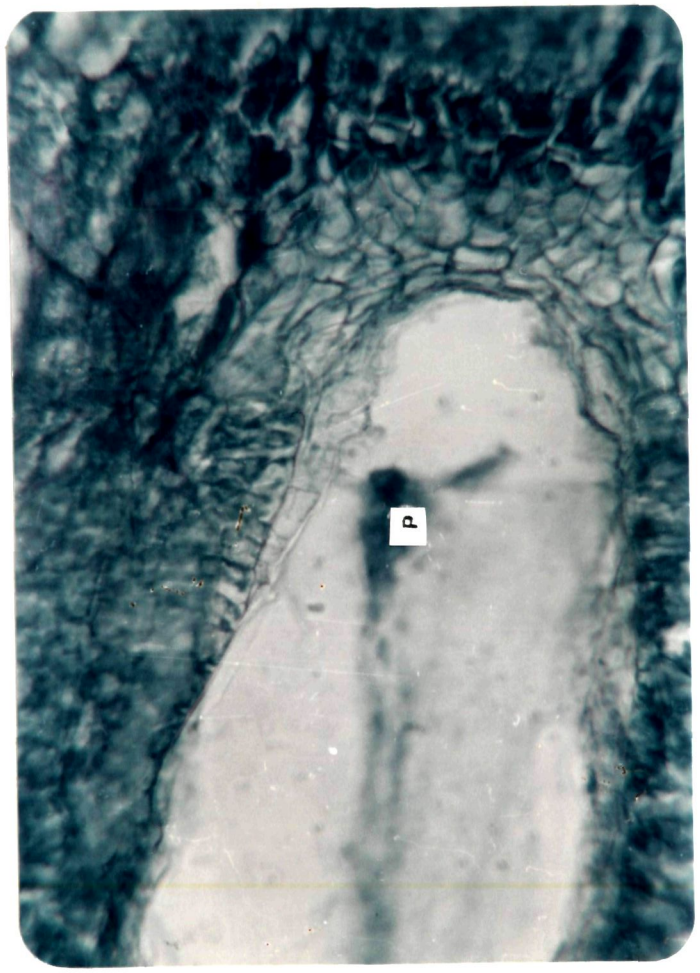
Fig 4.57 : Chalazal end of the organised embryo sac with the polar nuclei richly stained for proteins.

A -- Antipodal cell

H -- Hypostase

P -- Polar nuclei


A
H


P

(Figs.4.54,4.55). The antipodals were intensely stained for proteins (Fig.4.56) and the polar nuclei were rich in proteins (Fig.4.57). The integuments were rich while the cells of the nucellar cap were poor in proteins (Figs.4.54,4.55) and the hypostase was intensely stained for proteins (Figs.4.56,4.57).

4.6. HISTOCHEMISTRY OF THE SEED

The contents of various macromolecular substances in the seeds of *S.wallisii* are presented in Table 13.

In the seeds of *S.wallisii*, the embryo was rich in insoluble polysaccharides (Figs.4.58,4.59). The endosperm was of cellular type and was poor in polysaccharide content (Fig.4.59). There were no storage polysaccharides. The nucellus was poor in cytoplasmic polysaccharide content. The seed coat was devoid of storage polysaccharides (Fig.4.59) and the fruit wall was rich in diffused cytoplasmic polysaccharides.

The embryo was intensely stained for RNA (Fig.4.60). The endosperm was poor in RNA content. The peripheral layer of endosperm was rich in RNA. The endosperm nucleoli were intensely stained with Toludine Blue staining. The nucellus contained traces of RNA (Fig.4.61,4.62). The seed coat stained greenish with toludine blue staining, while its cells were devoid of RNA content (Fig.4.62).

The embryo was rich in cytoplasmic proteins (Fig.4.63) while the endosperm was intense in storage proteins (Fig.4.64). Crystalloid and spherical protein bodies

Table 13. The Content of various Macromolecular substances in the seed of *S.wallisii*

	Insoluble polysaccharides	RNA	Proteins
Embryo	++	+++	++
Endosperm	+	+	+++ (storage)
Seed coat	-	-	+
Fruit wall	++	+++	+

- => Absent
+ => Poor
++ => Rich
+++ => Intense

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PLATE 26

Fig 4.58 to 4.59 : Sections of the seed tested with Periodic Acid Schiff's (PAS) reagent to localise insoluble polysaccharides

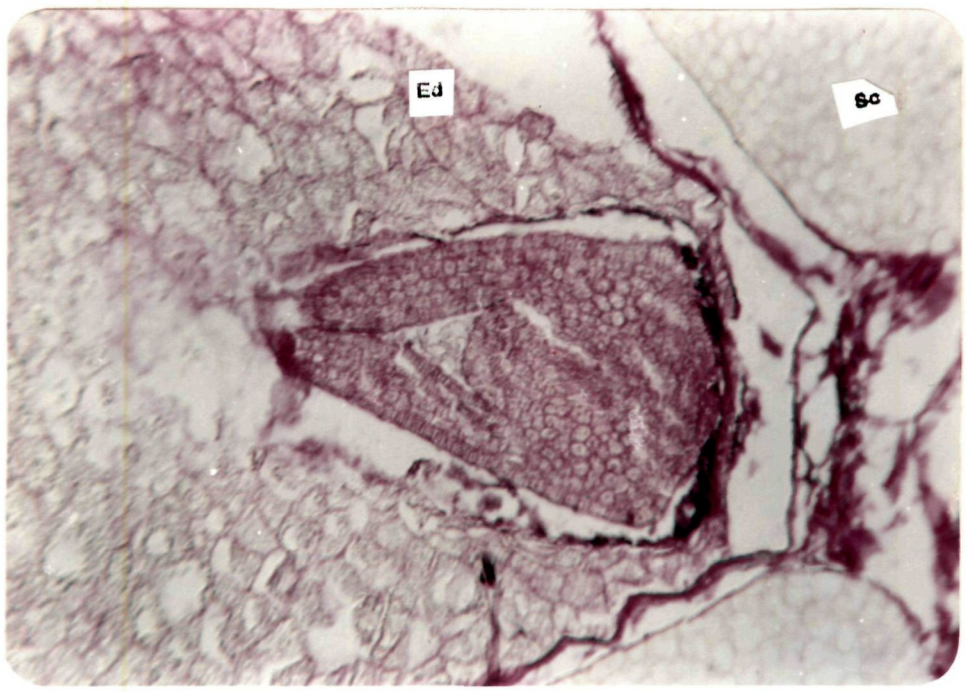
Fig 4.58 : Endosperm poorl and the embryo rich in insoluble polysaccharide content x100

Fig 4.59 : The embryo richly stained for insoluble polysaccharides x400

Eb - Embryo

Ed - Endosperm

Sc - Seed coat


Ed
Sc

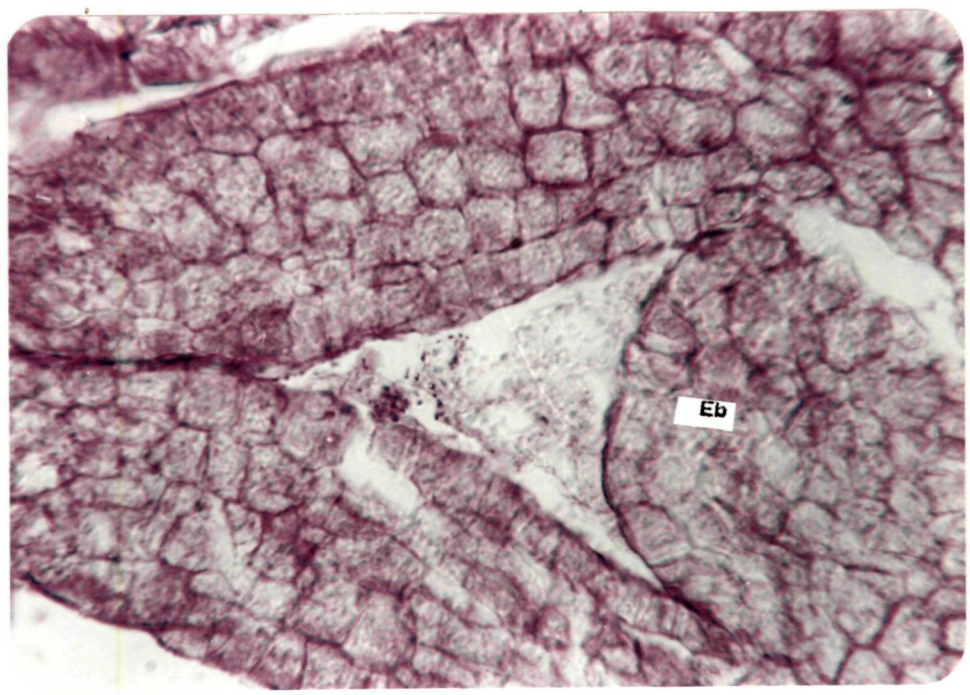

Eb

PLATE 27

Fig 4.60 to 4.62 : Sections of the seed tested with Toluidine blue method to localise RNA.

Fig 4.60 : The embryo intensely stained for RNA x100

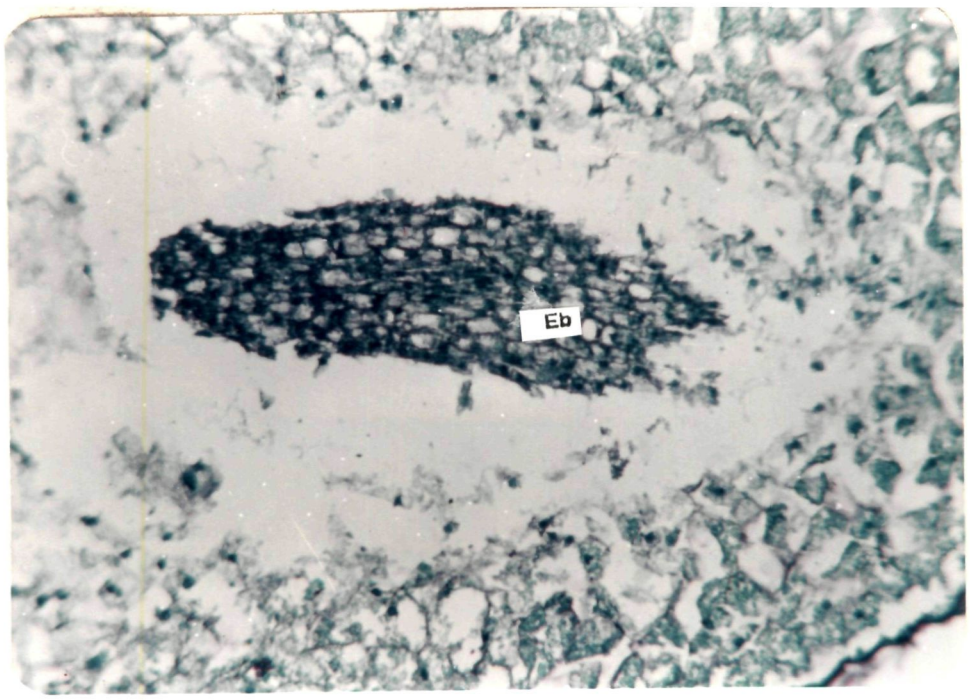
Fig 4.61 : The endosperm poor in RNA content and the seed coat devoid of RNA x100

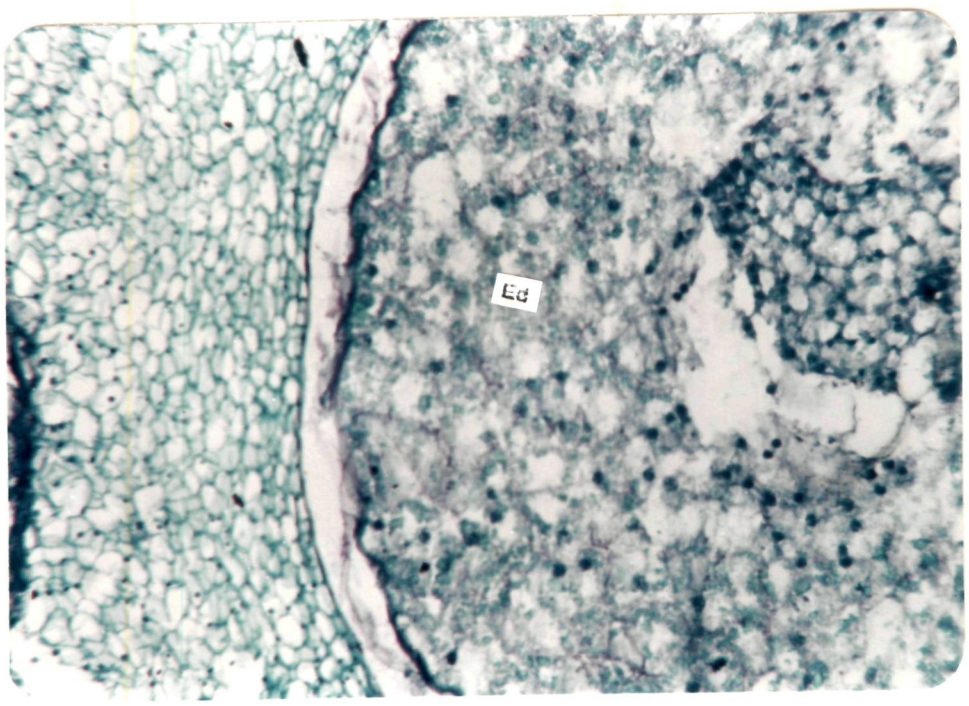
Fig 4.62 : The endosperm poor in RNA and the seed coat devoid of RNA x400

Eb - Embryo

Ed - Endosperm

Sc - Seed coat


Eb


Ed

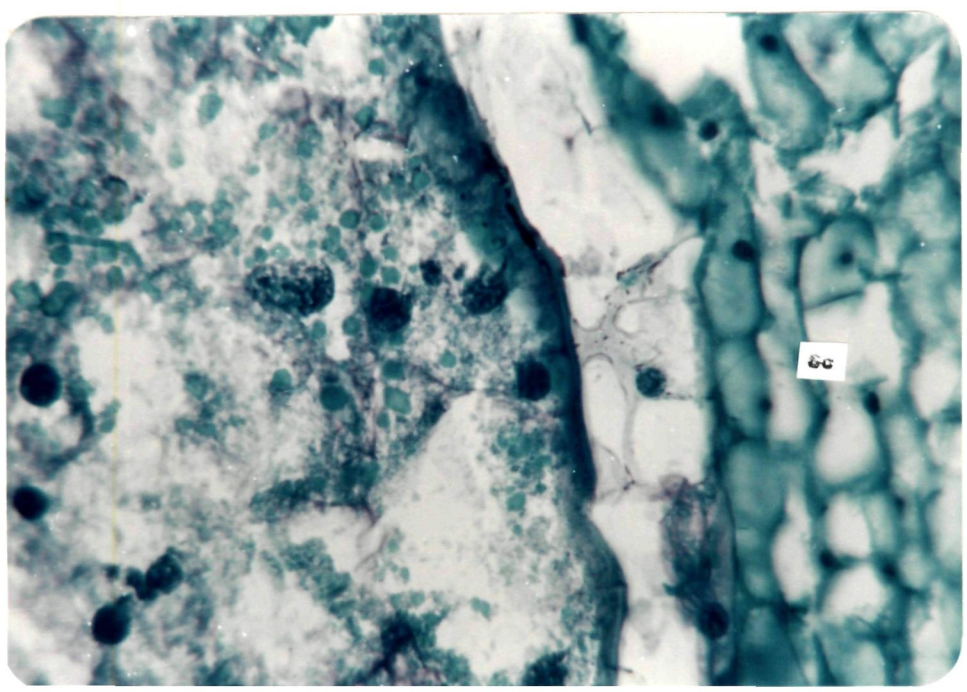

Ec

PLATE 28

Fig 4.63 to 4.64 : Sections of the seed tested with Mercuric Bromophenol Blue to localise proteins.

Fig 4.63 : The embryo, seed coat poor in protein content and the endosperm intense in protein content. x100

Fig 4.64 : Endosperm intensly stained for proteins and seed coat poor in protein content. x100

Fig 4.65 : Endosperm showing the protein bodies x400

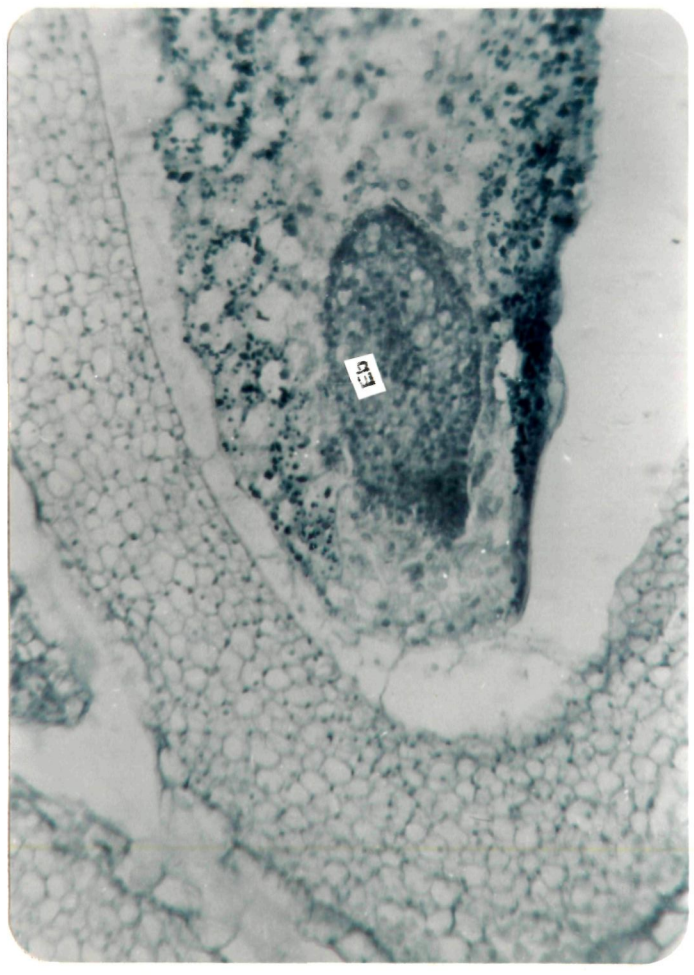
Cp - Crystal like protein bodies

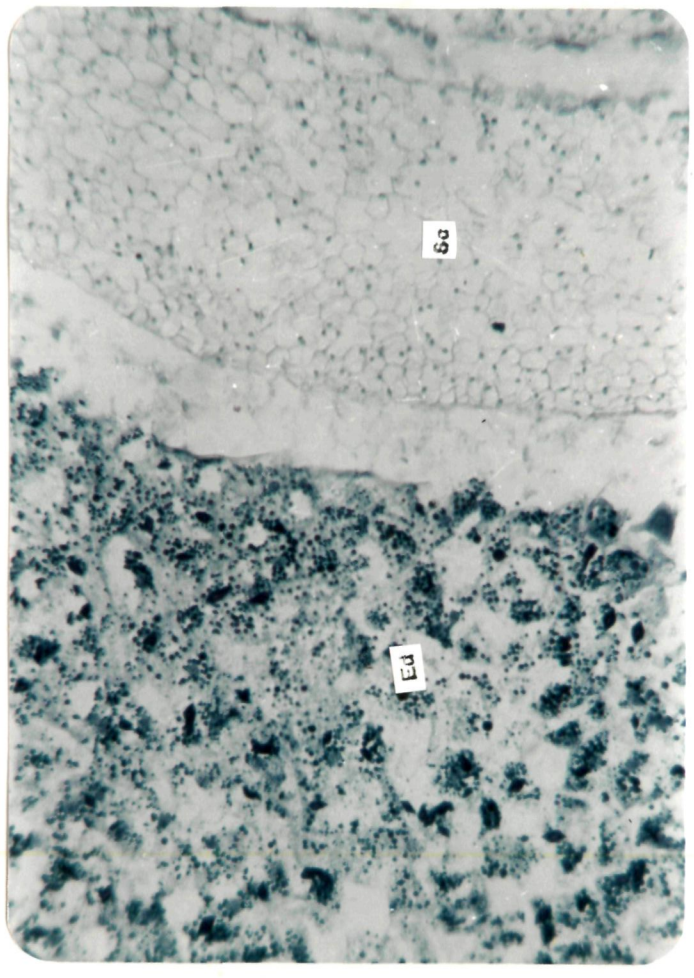
Eb - Embryo

Ed - Endosperm

Sc - Seed coat

Sp - Spherical protein bodies


Ep


Ga
Ed

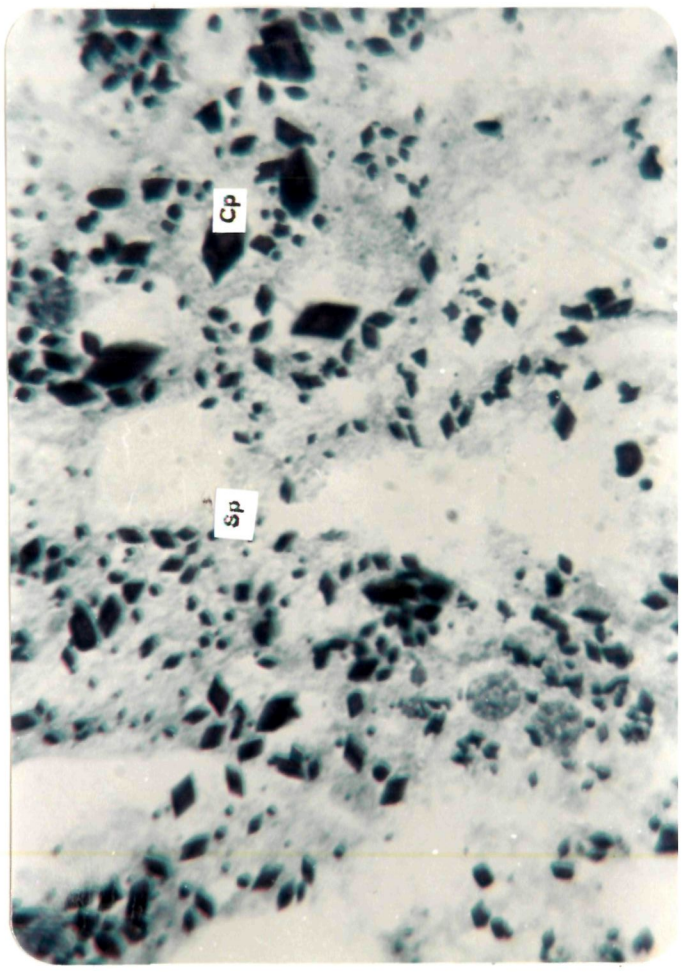

Cp
Sp

PLATE 27

Fig 4.60 to 4.62 : Sections of the seed tested with Toluidine blue method to localise RNA.

Fig 4.60 : The embryo intensely stained for RNA x100

Fig 4.61 : The endosperm poor in RNA content and the seed coat devoid of RNA x100

Fig 4.62 : The endosperm poor in RNA and the seed coat devoid of RNA x400

Eb - Embryo

Ed - Endosperm

Sc - Seed coat

were found in abundance in the endosperm (Fig.4.65). The deposition of storage substances i.e. protein bodies was centripetal (from the periphery towards the centre). The seed coat and fruitwall were found to be poor in proteins (Figs.4.63 and 4.64).

4.7. VASE LIFE STUDIES

The results of the experiment to study the effect of different concentrations of sucrose and aluminium sulphate on vase life of cut Spathiphyllum flowers are presented here:

4.7.1. Effect of Sucrose and Aluminium sulphate on vase life of cut Spathiphyllum flowers.

The effect of different concentrations of sucrose and aluminium sulphate on vase life of cut Spathiphyllum flowers is presented in Table 14.

There were significant differences observed between different treatments. Maximum vase life of 25.166 days was recorded with flowers kept in 0.5 per cent sucrose solution. Minimum vase life of 7.166 days was observed in control (tap water), with 5 per cent sucrose, there was 251.1 per cent increase in vase life over control. All the treatments tried were observed to significantly increase the vase life when compared to control (tap water). Significant differences were observed between 0.5 per cent sucrose solution and 2.0 per cent sucrose and 25,50 and 100 ppm aluminium sulphate vase solutions with 0.5 per cent sucrose being superior over others in extending the vase life. Rest of the differences observed were not significant.

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Table 14. The effect of different concentrations of Sucrose and *Aluminium sulphate on Vase life, Solution uptake and Weight* loss in cut *S.wallisii* flowers.

Tr.	Vaselife (Days)	Solution uptake (ml)	Weight loss (g)
control	07.166	22.540	1.515
0.5% Sucrose	25.166	33.330	2.166
1.0% Sucrose	18.166	14.830	2.116
1.5% Sucrose	18.833	28.330	1.866
2.0% Sucrose	14.000	30.000	1.783
25 ppm Al2(SO4)3	18.000	27.500	1.616
50 ppm Al2(SO4)3	15.330	17.330	1.133
100 ppm Al2(SO4)3	15.330	14.166	1.166
F-Test	*	N S	N S
SEM	3.148	-	-
CD at 5%	6.674	-	-

* Significant at P = 0.05

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4.7.2. Effect of Sucrose and Aluminium Sulphate on solution uptake by cut Spathiphyllum flowers.

Maximum uptake of 33.33 ml was recorded with flowers kept in 0.5 per cent sucrose solution and minimum uptake of 14.166 ml with those in 100 ppm aluminium sulphate solution (Table 14). However, the differences observed were not significant.

4.7.3. Effect of Sucrose and Aluminium Sulphate on weight loss in cut *Spathiphyllum* flowers.

Minimum weight loss (1.133 g) was observed in the flowers kept in 50 ppm aluminium sulphate solution. Weight loss was maximum (2.1669) in flowers treated with 0.5 per cent Sucrose solution (Table 14) but the differences observed were not significant.

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DISCUSSION

V. DISCUSSION

The present investigations were carried out to study the floral biology, the histology and histochemistry of anther and ovule development in *S. wallisii* and vase life of cut *Spathiphyllum* flowers.

5.1 FLORAL BIOLOGY

5.1.1 Flower Development

The number of days required for the complete opening of the Spathe (anthesis) was 30.10 days. The flowers were protogynous, they exhibited pistillate phase first. Croat (1980) reported that the separation of sexes in many species of *Anthurium* was accomplished by marked protogyny.

This protogynous nature effectively prevents cross pollination. Stigma receptivity discerned by the presence of white sticky mass at the apex of the pistil, (Figs 4.4, 4.4a , 4.4b) was noticed after 30.50 days of visible initiation . The pistillate phase almost coincided with anthesis. This is in agreement with the findings of Thangaraj and Muthuswamy (1973) who observed maximum receptivity of stigma at anthesis in *Crossandra undulaefolia*.The protracted phase of Stigmatic receptivity facilitates the hybridisation programme selecting parents with desirable traits. As flowers are protogynous the self pollination is effectively prevented in nature.From anthesis to anther dehiscence, 5.5 days were required on an average. The spathe of the inflorescences turned green after 36.90 days of anthesis, Fruit set required 57.90 days and fruit ripening required 125.4 days from anthesis. There is no published literature available on these aspects of flower development, and the present findings are first of their kind.

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5.1.2 Stigma Receptivity

The average number of days required for stigmas to become receptive was 30.50 which almost coincided with anthesis. Once receptive the stigmas remained so for 3.30 days on an average. A similar observation was made by Croat (1980) in different species of *Anthurium*. He reported that the pistillate phase discerned by the presence of stigmatic droplets or glistening stigmas, lasted for 3 days in *A .fragrantissimun*, 2 - 3 days in *A.schlechtendalii* and 3 - 5 days in *A .binervia*. The protracted phase of stigmatic receptivity can facilitate the hybridisation programme that involves this plant as one of the parents.

5.1.3 Anther dehiscence

The first anther to dehisce required 5.5 days from anthesis. Anther dehiscence started in the basal 1/3 rd of the inflorescence and proceeded in the direction of the apex. A similar pattern of anther dehiscence was observed by Croat (1980) in various species of *Anthurium* which is another importantt member of the "Araceae", as *A.fragrantissimum* , *A.standleyi* , *A.salvadorense*, *A.seibertii*, and *A.brownii*. More than one such cycle was observed in an inflorescence as each flower had anthers at different stages of development. Completion of anther dehiscence in an inflorescence required 7.80 days on an average. Maximum anther dehiscence was noticed between 8.00 a.m and 11.00 a.m. In *Anthurium*, maximum anther dehiscence takes place between 8.00 a.m and 10.00 a.m as reported by Mercy and Dale (1994). So, in *S.wallisii* pollen collection and hand pollination can be easily attended to, in early morning hours.

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5.1.4 Pollen Viability

Pollen viability estimated immediately after extraction of pollen from the inflorescence was found to be hundred per cent. There is no literature available on this aspect among the members of this family and this is the first information on this aspect.

5.1.5 Pollen germination

Among the different media tried, maximum pollen germination (98 per cent) was recorded with a medium containing 20 per cent sucrose and 200 ppm boric acid. There is no published literature available on such pollen germination studies among the members of the family.

5.1.6 Pollination and Friutset

The average number of fruits set in a naturally pollinated inflorescence was 4.00 . In *Anthurium*, a well fertilised inflorescence is reported to give 100 - 200 fruits (Mercy and Dale, 1994) . The average number of fruits set in an artificially pollinated inflorescence was 29.20. Henny (1989) reported that the members of the genera *Anthurium* and *Spathiphyllum* readily set fruits if pollinated when stigmas are glistening. This indicates that, there is no abnormality in the development of floral parts and the seeds. So this plant can conveniently be used in a hybridisation programme.

5.2 HISTOLOGICAL AND HISTOCHEMICAL STUDIES

The growth and differentiation in any organism is a result of the physiological and biochemical changes taking place at cellular level. The study of histological and histochemical changes in a structure helps in understanding these phenomena.

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5.2.1 **Histological and Histochemical changes during microsporogenesis and male gametophyte development**

In the anther primordium, the archesporial cells differentiated at four corners. These divided periclinally to give primary parietal cell to the exterior and primary sporogenous cell to the interior.The former by further anticlinal and periclinal divisions, differentiated into endothecium, middle wall layers and the tapetal primordium. The primary sporogenous cell by mitotic divisions gave rise to the sporogenous tissue. At this stage, the anther had four microsporangia, one at each corner connected by a central connective, which was traversed by a vascular strand. The pollen mother cells, differentiated from the cells of the sporogenous tissue. A similar developmental process was observed in *Theriophonum minutum* (Parameswaran, 1959) and *Arisaema wallichianum* (Maheshwari and Khanna, 1956). As the pollen mother cells differentiated from the sporogenous tissue, the cells of the tapetal primordium increased in size, their cytoplasm became denser and got differentiated into "Tapetum". The tapetum was thicker towards the connective and thinner towards the distal end. The pollen mother cells underwent meiosis I and II. The cytokinesis was successive which resulted in the formation of either isobilateral or decussate microspore tetrads. Successive cytokinesis and the formation of isobilateral, decussate or tetrahedral tetrads was observed also in *Arisaema wallichianum* which is a member of Araceae (Maheshwari and Khanna,1956). There was increase in the thickness of the tapetum at tetrad stage. However Vittala Raya Kini (1981) observed a decrease in the thickness of the plasmodial tapetum at tetrad stage in normal, male fertile lines of *Helianthus annuus*, while the thickness of the tapetum

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further increased in the male sterile lines. The tapetum was of amoeboid, invasive or plasmodial type. As the microspores got released from the tetrads, the tangential and radial walls of the tapetal cells began disintegrating and tapetal protoplasm diffused into the anther locule forming a "Periplasmodium" (Figs 4.16, 4.28) . The presence of periplasmodium has been observed in other representatives of the family like , *Dieffenbachia seguine* (Campbell,1990), *Symplocarpus foetidus, Peltandra undulata* (Duggar , 1900), *Aglaonema versicolor* (Gow, 1980 b) Peltandra verginica (Goldberg ,1941) *Typhonium trilobatum* (Banerji,1947), *Arisaema wallichianum* (Maheshwari and Khanna,1956) and *Theriophonum minutum* (Parameswaran, 1959). As the pollen grains developed, there was increase in the size of individual cell and also the endothecium. At this stage, the tapetal protoplasm that formed the periplasmodium completely disappeared. Disappearance of the tapetal contents with concomitant increase in the size of the pollen grains and thickness of the endothecium indicates that the former is perhaps utilised by the latter for their development. The pollen grains were shed at two celled stage. A similar observation was made by Kapil (1967) in a few other representatives of the family, namely *Arisaema, Dieffenbachia, Spathyema, Symplocarpus, Synandrospadix,* and *Theriophonum.*

During the early stages of development the sporogenous cells were poor in insoluble polysaccharide content (Fig 4.7) which is in agreement with the findings of Aswath et al (1989) in *Pyrostegia venusta* and Panchaksharappa and Syamasunder (1974) in *Iphigenia pallida.* The sporogenous cells were poor in RNA content in the present study (Fig 4.23) which is in conformity with the findings of Aswath et al (!989) in *P. venusta.* However on the contrary, Panchaksharppa and Syamasunder (1974) observed

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rich RNA content in sporogenous cells of *I.pallida.* The sporogenous tissue was found to be poor in protein content (Fig 4.35) which is in agreement with the findings of Panchaksharappa and Syamasunder (1974) who reported poor protein content in the sporogenous tissue of *I. pallida.* Aswath et al (1989) reported that the sporogenous cells were rich in proteins which is not in agreement with the present work.

As the PMC's differentiated from the cells of the sporogenous tissue, there was deposition of callose around them, seperating the sister meiocytes. (Fig 4.9, 4.10) The cells of the tapetal primordium increased in thickness , became densely cytoplasmic and differentiated into "Tapetum". At this stage, the PMC's were poor in insoluble polysaccharides, RNA and proteins (Fig 4.8, 4.9, 4.25, 4.36, and 4.37) where as the tapetal cells showed rich content of insoluble polysaccharides RNA and .proteins which indicates high metabolic activity in them. Panchaksharappa and Syamasunder (1974) observed poor insoluble polysaccharide content in PMC's , which is in conformity with the present study but they reported rich nucleolar and cytoplasmic RNA in the PMC's which is not in agreement with the present study. The tapetal cells were intense in insoluble polysaccharides ,RNA and protein content in *Mangifera indica* as reported by Anitha Karun (1989) . This is in conformity with the present study where tapetal cells were found to be rich in these macromolecular substances.

The tetrads formed after successive cytokinesis following meiosis I and II were decussate or isobilateral. The tapetal thickness at this stage increased further. At this stage, the microspore tetrads were rich in insoluble polysaccharides, intense in RNA and proteins. (Figs 4.12, 4.26, 4.38) This is in conformity with the findings of Aswath et al

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(1989) in *P. venusta*. The tapetal cells at this stage showed rich insoluble polysaccharides and protein content (Figs 4.11, 4.12, 4.13) while they were intense in RNA content (Fig 4.26) . This is conformity with the findings of Taylor (1959) who reported that, proteins accumulated in tapetum after meiosis in *Lilium longifolium* and also with the findings of Moss and Heslop Harrison (1967) who observed a similar trend in maize anther.

With the disappearance of callose, the microspores were released from the tetrads. At this stage, the tapetal contents started getting diffused into the anther locule. The microspores were rich in insoluble polysaccharides , RNA (Figs 4.14, 4.5, 4.27) and were intense on protein content (Fig 4.39). This is in conformity with the findings of Aswath et al (1989) in *P. venusta* and Panchaksharappa and Syamasunder (1974) in *I. pallida*.

The microspores differentiated into pollen grains after the development of pollen wall. Before anther dehiscence, the pollen grains were intense in insoluble polysaccharides and starch accumulation was noticed in them (Fig 4.19). At this stage the pollen grains were intense in RNA and low in protein content (Fig 4.31,4.42). At anther dehiscence the pollen grains were intense in polysaccharides but devoid of starch grains (Fig 4.21). At this stage the pollen grains were intense in RNA and protein content (Fig 4.44). This indicates that protein synthesis in the pollen grains takes place at later stages. Panchaksharappa and Rudramuniappa (1972) observed rich polysaccharide and protein contents in the pollen of *Pennisetum typhodium*. By the time the pollen grains were formed the tapetal contents had completely disappeared.

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5.2.2 **Histological and Histochemical changes during Megasporogenesis and female gametophyte development.**

The ovules in the ovary of *S. wallisii* were anatropous. The archesporium differentiated hypodermally, underwent periclinal division to give a pareital cell to the exterior and megaspore mother cell to the interior following the 'Crassinucellate' pattern of development. A similar observation was made in *Arisaema wallichianum* by Maheshwari and Khanna (1956). The megaspore mother cell underwent meiosis I and II to give rise to megaspore tetrad. Out of the four, the chalazal megaspore was functional. This functional megaspore, by mitotic divisions of its nucleus gave rise to an eight nucleate female gametophyte which organised into a seven celled, eight nucleate "Polygonum" type embryosac. The occurence of 'Polygonum' type embryosac has also been observed in *Theriophonum minutum* and other members of the family (Parameswaran, 1959). The cells of the nucellus towards the sides of the gametophyte disintegrated at early stages of development and those at the apex persisted. These cells of the nucellus became hypertrophied and formed a 'Nucellar cap'. The presence of nucellar cap has been reported in *Arisaema wallichianum* (Maheshwari and Khanna, 1956) and *Theriophonum minutum* (Parameswaran, 1959) which also belong to the same family.

At early stages of development, the nucellus and integuments in the ovule primordium were poor in insoluble polysaccharides. The megaspore mother cell and functional megaspore were also poor in polysaccharide content (Fig 4.46) . This observation is in agreement with the findings of Panchaksharappa and Syamasunder (1975) who reported low cytoplasmic polysaccharide in archesporium, MMC, megaspore

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tetrad, 2 and 4 nucleate embyo sacs in the developing ovules of *Dipcadi montanum.* Aswath et al (1989) also observed low cytoplasmic polysaccharide content in archesporium and MMC of P. venusta. On the contrary, PAS negative cytoplasm was observed at archesporium stage to 2 and 4 nucleate embryo sac stages in *Stellaria media* and *vanda* by Pritchard (1964) and Alvarez and Sagawa (1965) respectively.

At early stages, the nucellus and the integuments were rich in RNA content (Fig 4.49). The MMC was poor in RNA (Fig 4.50) which is in conformity with findings Alvarez and Sagawa (1965) who reported low RNA content in MMC of *vanda.* The fuctional megaspore also was found to be poor in RNA. This is not in agreement with observations of Alvarez and Sagawa (1965) and Panchaksharappa and Syamasunder who reported rich RNA content in the megaspores of *vanda* and *Dipcadi montanum* respectively.

In the ovule primordium, the nucellus was rich and integuments were intense in protein content (Fig 4.53) . The MMC was poor in proteins. This is in conformity with the findings of Pritchard (1964) who observed low protein content in MMC of *S. media.* The functional megaspore also was found to be poor in proteins. On the contrary, rich protein content was observed in the megaspores of *vanda* (Alvarez and Sagawa, 1965) and *Dipcadi monatum.* (Panchaksharappa and Syamasunder, 1975) .

In the organised embryo sac, the central cell had traces of cytoplasmic polysaccharides, RNA and proteins (Fig 4.47, 4.48, 4.51, 4.52, 4.55, 4.56, 4.57). This is in conformity with the findings of Panchaksharappa and Syamasunder (1975) who reported

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low contents of polysaccharides, RNA and proteins in the central cell of *D .montanum.* The egg cell had low polysaccharide content (Fig 4.47) and rich protein content (Fig 4.54, 4.55). This is in conformity with the findings of Panchaksharappa and Syamasunder (1975) in *D. montanum.* The egg cell was found to be intense in RNA content (Fig 4.51, 4.52) . The egg cell in cotton was reported to be rich in RNA (Jensen, 1965 a,b) also in *vanda,* a similar observation was made by Alvarez and Sagawa (1965). The synergrids were low in insoluble polysaccharides, intense in RNA content (Fig 4.52) and rich in proteins (Fig 4.55). In cotton, the synergrids were found to be rich in RNA and intense in protein (Jensen,1965,a,b) and in *vanda,* the synergrids were reported to be rich in RNA and proteins (Alvarez and Sagawa,1965) . The antipodals were found to be intense in protein content (Fig 4.56) Panchaksharappa and Syamasunder (1975) reported that the antipodals were rich in proteins in *D. montanum.* The cells of the hypostase were found to be intense in polysaccahride, RNA and proteins (Fig 4.48,4.56,4.57). A similar observation was made in *D. montanum* by Panchaksharappa and Syamasunder (1975).

5.2.3 **Histochemistry of Seed**

In the seeds of *S. wallisii* , the embryo was found to be rich in cytoplasmic polysaccharides, proteins (fig 4.58,4.59,4.62) and intense in RNA content (fig 4.60). The endosperm was of cellular type. Such endosperm has been observed in other members of the family also (Johri et al , 1993). The endosperm was poor in cytoplasmic and storage polysaccharides (fig 4.59). It was poor in RNA content also (fig 4.61,4.62) but intense in crystalloid and spherical protein bodies (figs 4.62, 4.63, 4.64). The seed coat was devoid of storage polysaccharides, RNA and was poor in protein content (ifg 4.59, 4.61, 4.63). The fruit wall was found to be rich in diffused cytoplasmic polysaccharides, intense in RNA

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and poor in proteins. Well differentiated embryo in the seeds with storage protein in the surrounding endosperm indicate the normal germination of seeds. There is no published literature available on the histochemistry of seeds among the members of this family.

5.3 VASE LIFE STUDIES

5.3.1 Effect of Sucrose and Aluminium Sulphate on vase life of cut Spathiphyllum flowers

Sucrose and Aluminium Sulphate tried at different concentrations were found to significatly increase the vase life of cut Spathiphyllum flowers. Maximum vase life of 25.166 days was recorded with flowers kept in 0.5 per cent Sucrose solution. A simlilar observation was made by Gowda and Gowda (1990) in *Gladiolus* . They observed significant increase in the vase life when flowers were kept in sucrose (2 and 3 per cent) and aluminium sulphate (0.5, 1.0 mm) when compared to control (Disttilled water). The increase in vase life due to sugar was mainly attributed to decreased moisture stress and improved water balance, osmotic potentials (Acock and Nichols, 1979). The increase in vase life due to aluminium sulphate could be due to its role in lowering the petal pH, stabilising the anthocyanins, and acidifying the holding solution, thus reducing the bacterial growth, as reported by Gowda and Gowda (1990). Nagarajaiah and Reddy (1991) also observed a significant increase in vase life of Queen Elizabeth roses when flowers were treated with 2 and 4 per cent sucrose, when compared to control. Rath et al (1991) also observed a significant increase in vase life of cut roses Cv Laura and Love, when kept in a vase solution containing 20mg/lt of sucrose.

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In the present study there were significant difference observed between 0.5 per cent sucrose and different concentrations (25,50 and 100 ppm) of aluminium sulphate. The vase life in sucrose solution was significantly greater than the vase life of flowers in 25, 50 and 100 ppm aluminium sulphate. Gowda and Gowda (1990) also observed a significant difference in the vase life of Gladiolus flowers kept in 2 per cent sucrose and 1.0mm Aluminium Sulphate. But they observed longer vase life in case of aluminium sulphate than sucrose.It may be that mere reduction of pH is not of much help, but carbohydrate supply and assoiated changes in osmotic concentrations may be contributing to promotion of vase life to a greater extent ,atleast in this case.

5.3.2 **Effect of sucrose and aluminium sulphate on solution uptake by cut Spathiphyllum flowers.**

Sucrose (0.5 ,1.0,1.5 and 2.0 per cent) and aluminium sulphate (25,50,100 ppm) were found to have no significant effect on the uptake of vase soultion by cut Spathiphyllum flower. Maximum solution uptake of 33.33 ml was recorded in flowers held in 0.5 per cent sucrose solution and minimum uptake of 14.199 ml in those held in 100ppm aluminium sulphate.

These further indicate that with sucrose there were associated changes in water relations. There was lower uptake of water with aluminium sulphate compared to sucrose.

5.3.3 **Effect of sucrose and aluminium sulphate on weight loss in cut Spathiphuyllum flowers**

Maximum weight loss of 2.166g was recorded in case of flowers kept in 0.5 per

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cent sucrose solution and minimum weight loss (1.133g) was recorded with 50ppm aluminium sulphate solution. However, the differences observed were not significant.

The phenomenon of vase life and prolongation with sucrose and aluminium sulphate when examined in the light of the changes in solution uptake and weight loss it appears that sucrose uptake favours greater lenth of vase life inspite of higher weight losses. The weight loss in aluminium sulphate was lower, at the same time the vase life was relatively shorter. These indicate that sucrose promotes vase life with the associated changes in water relations and higher metabolic rather than the aluminium sulphate. The aluminium sulphate seems to reduce solution uptake and also weight loss, which indirectly suggests a retardation in metabolism, at least at higher concentrations which may also be an effect of changes in pH. However these aspects need to be looked into in greater detail.

SUMMARY

VI SUMMARY

The floral biology, histological and histochemical changes in the developing anther and ovule and vase life of cut Spathiphyllum flowers were studied in the present investigation. The salient findings of these studies are as follows:

6.1 FLORAL BIOLOGY

In *S. wallisii,* an average of 30.10 days were required for anthesis from the stage of visible initiation and 30.50 days for stigma receptivity that almost coincided with anthesis. The pistillate phase lasted for 3.30 days on an average in an inflorescence. The anther dehiscence started 5.50 days after anthesis. Maximum anther dehiscence was observed between 8.00 and 11.00 am. The protracted phase of stigma receptivity and anther dehiscence in the early morning hours make it very convenient to take up hybridisation programme. The pollen viability immedietly after extraction was hundred per cent and maximum pollen germination (98%) was obtained in a medium with 20 per cent sucrose and 200ppm Boric acid. The percentage of fruit set in an inflorescence artificially pollinated was much higher (23.81%) compared to that in a naturally pollinated inflorescence (5.71%). This hopefully indicates the success of a hybridisation programme.

6.2 HISTOLOGY AND HISTOCHEMISTRY

6.2.1 Anther

A study of the histological and histochemical changes in anthers of *S wallisii*

revealed that, the sporogenous cells and PMC's were poor in insoluble polysaccharides, RNA and proteins. The PMC's had PAS positive callose deposition around them. The tapetum was of amaeboid, invasive or plasmodial type. At this stage it was rich in insoluble polysaccharides, RNA and proteins. The microspore tetrads formed were either decussate or isobilateral. The microspores were rich in insoluble polysaccharides and intense in RNA and proteins. The tapetum was rich in insoluble polysaccharides, proteins and intense in RNA content. These observations indicate high rate of metabolism in these cells. The microspores were released from tetrads after the disappearance of callose. They were rich in insoluble polysaccharides, RNA and intense in protein content. At this stage, the tapetal contents got diffused into the anther locule forming "Perliplasmodium."

Just before and at anther dehiscence the pollen grains were intense in polysaccharide and RNA content. However the pollen grains were low in proteins at early stages but were intense in protein content at anther dehiscence. This indicates that the pollen grain store various macromolecular substances that are required for the growth and differentiation of the male gametophyte.

These observations indicate that the processes of microsporogeneis and male gametophyte development are normal.

6.2.2 **Ovule**

The ovules were found to be anatropous, crassinucellate. The archesporium differentiated hypodermally which developed into MMC. This underwent meiosis to give a megaspore tetrad. The chalazal megaspore was functional. The MMC and the functional

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megaspore were found to be poor in insoluble polysaccharides, RNA and proteins. The mitotic divisions of the nucleus in the functional megaspore gave an eight nucleate female gemetophyte which organised into a "Polygonum" type embryo sac. The nucellar remians formed a "Nucellar cap" at the micropylar end of the embryo sac. The egg cell and synergids were poor in insoluble polysaccharides, intense in RNA and rich in proteins which indicates a high rate of metabolism in them.

The embryo had rich insoluble polysaccharide, protein and intense RNA content. The cellular endosperm was poor in storage polysaccharides and RNA but intense in storage proteins. The protein bodies were crystalloid and spherical. The mode of deposition of these storage substances was centripetal.

6.3 VASE LIFE STUDIES

The vase life of cut spathiphyllum flowers was observed to be maximum (25.16 days) when kept in a vase solution with 0.5 per cent sucrose. Among the different concentrations of aluminium sulphate tried, maximum vase life (18 days) was recorded with 25 ppm aluminium sulphate solution.

Spathiphyllum wallisii is a very hardy plant with attractive foliage. It flowers through out the year. It is free from any serious disease or pest, and drought tolerent. It is very easy to maintain and propagate. This plant shows the normal development of male and female gametophytes. The fruit and seed set can be improved by artificial pollination.

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Hence this plant can be used conveniently in a hybridisation programme with the aim of incorporating its desriable traits into other plants.

The cut *Spathiphyllum* flowers keep well in Vase. If harvested at right stage and maintained properly, these flowers last at least for a period of two weeks. So this plant can be popularised as a source of cut flowers.

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