BLOSSOM BIOLOGY AND POLLINATION STUDIES IN *Anthurium andreanum* Lind.

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BLOSSOM BIOLOGY AND POLLINATION STUDIES IN *Anthurium andreanum* Lind.

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Thesis submitted to the University of Agricultural Sciences, Bangalore in partial fulfillment of the requirements for the award of the degree of

MASTER OF SCIENCE (HORTICULTURE)

in

FLORICULTURE

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Dedicated to

my beloved parents

Thiru A. Pattel & Thirumathi Lakshmi

and

affectionate Sister

Mrs. Shanthi

DEPARTMENT OF HORTICULTURE
UNIVERSITY OF AGRICULTURAL SCIENCES, BANGALORE

CERTIFICATE

This is to certify that the thesis entitled **"BLOSSOM BIOLOGY AND POLLINATION STUDIES IN *Anthurium andreanum* Lind"** submitted by **Mr. P. BALAKUMAR** for the degree of **MASTER OF SCIENCE (HORTICULTURE) IN FLORICULTURE** of the University of Agricultural Sciences, Bangalore is a record of research work done by him during the period of his 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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Dec. 1999

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INTRODUCTION

I INTRODUCTION

Anthurium andreanum Lind. is also called as 'Painter's palette' flower. Anthurium is one of the largest genus of the monocot family 'Araceae'. The word anthurium refers to the tail like spadix present in the center of the spathe, derived from the Greek words 'anthos' meaning flower, and 'ura' being tail. It is the 'National flower of Mauritius'.

The genus *Anthurium* contains nearly, 600-800 species (Croat, 1979) and it is one of the most morphologically diverse and taxonomically complex genus. Among all 'Araceae' members *Anthurium andreanum* is the most important cut flower crop, gaining importance in Indian cut flower industry, which fetched nearly 100 crores as foreign exchange (Vikram reddy, 1999).

Besides this, other important species are *A. scherzerianum* (as a potted flowering plant) and foliage species viz., *A. veitchii* (King anthurium), *A. warocqueanum, A. panduratum, A. ornatum, A. crystallinum* (Crystal anthurium), *A. clarinervium, A. grande, A. watermaliense* (coppery black spathe), *A. cordatum, A. negrito* (Black anthurium) etc.

Aroids are mostly tropical herbs with fleshy woody stems, while others are root climbers with variable leaf forms (Graf, 1982).

The most distinguishing feature of the ornamental aroids is their floral structure, which consists of a slender floral axis (candle) bearing sessile flowers, subtended by a modified bract or leaf called 'Spathe', which is often brightly coloured and highly ornamental. It

may be narrow and elongated or broad and heart shaped with pointed tips. It may be smooth, thick and glossy without prominent veins or it may be thinner, deeply veined and blistered. Spathe colour may be white, pink, coral red, orange, brown, red, crimson and liver red. The candle also have varying colours viz., red, pink, green, yellow, white, pink, red or combinations.

The basic chromosome number of *Anthurium* genus varies from 24 to 124, but somatic chromosome number of *A. andreanum* is 2n=30 with two B chromosomes. The present day cultivars are derived from complex polyploids showing higher chromosomal irregularities (Kaneko and Kamemoto 1978).

Commercial cultivation of anthurium cut flower being new to India, only very few attempts have been made in our country to domesticate the breeding of a variety with novel qualities which is of utmost importance for successful cultivation. Though there are reports about floral biology from elsewhere, floral biological studies under Bangalore conditions are much needed because, climatic parameters have a dramatic influence on all aspects of flower growth. Hence to breed a variety under Bangalore conditions, the basic information on floral biology, pollen storage, histological and histochemical changes during flower development is required. With this background the present study was undertaken in cv. 'Crinkle Red' with the following objectives.

To study

(i) Morphological characters and floral biology
(ii) Microsporogenesis and male gametophyte development and
(iii) Megasporogenesis and female gametophyte development.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Literature on the reproductive biology of 'Araceae' are available but there is no report incase of *Anthurium*. Studies on floral biological aspects of *Anthurium* was done elsewhere, and no work has been reported for Bangalore conditions. A brief review of the work done with respect to floral biology, pollen storage and histological and histochemical reports of microsporogenesis and megasporogenesis of aroids and a few other crops has been presented here.

2.1 Floral biology

2.1.1 Anthurium

The earliest report available in anthurium was by Campbell(1900). He reported that all the flowers in anthurium were similar in structure and densely crowded on the spadix. He also observed the protogynous nature of flower and presence of small ant, probably a pollinating agent.

Croat(1980), also described anthurium as protogynous and studied 31 species. Flower consisted of four tepals which were thin at the base where they are in contact with the pistil and much thicker and truncate at the apex, which formed rhombic, square or four lobed outline. Stamens were opposite to tepals with flattened filaments squeezed between the pistil and the tepals. In all the species studied, lateral pair of stamens emerged first, followed by anterior and then the posterior stamen of the alternate pair with considerable time gap between these stages. This process usually started from base to apex, but few exceptions were also observed, wherein it started from the middle or apex (*A. Lentii*). This flowering process continued from few days to several weeks.

He also observed two kinds of stigma viz., slitlike and brush like. Stigmatic receptivity was often expressed by stigmatic droplets or glistening of the stigmatic surface. Duration of this stigmatic phase ranged from few hours to 28 days. Time gap between female and male phases usually ranged for several days but, maximum of 10-20 days in *A. pittieri.*

Pollen color was generally orange, yellow, white, and purplish, usually turned white within a day. Variations in the degree of exertion of stamen, disposition, degree of retraction, flower aroma, stigmatic droplets were also reported. Both fly and bee pollination syndrome were noticed.

Henny (1989), reported that anthurium produced seeds readily, if pollinated when the stigmatic surface appears to glisten.

A. andreanum was an out-breeding species with protogynous flowers, since the stigma became receptive in about 7-10 days, before the pollen was shed(Singh, 1992)

Mercy and Dale (1994) observed 150 to 300 flowers per inflorescence in anthurium and were protogynous. Anthesis occurred between 8-10 A.M. on a fairly sunny day. On cloudy and rainy days, dehiscence was delayed. They also observed 7 to 10 days gap between female and male phase. The whole spadix took 3 to 4 weeks for complete flowering. They also reported about systematics, morphology, cytogenetics, floral biology, pollination, fertilization, diseases and pest control.

2.1.2 Other crops

In *Spathiphyllum wallisii* (Araceae) tiny bisexual flower were arranged on cylindrical spikes, with 13.8 flowers per cm^2. The flowers were sessile with six tepals, six stamens and pistil had trifid sessile stigma. The emergence of first anther from the perianth whorl was only after the stigma lost its receptivity. Peak anthesis was observed during 8-11 a.m. (Savitha, 1995).

2.1.3 Flower development

Vonk noordegraaf(1973) reported that light has a major influence in anthurium flower development. He also observed the deleterious effect of shading which reduced the flower development due to bud abortion, leading to reduction in yield.

The growing period of a bud (flower bud emergence to harvest) in anthuriums, varied between 48 (July and August) to 72 days (January and February), which had a positive relationship with light intensity. Leaf production was essential for flower production as each and every leaf axis usually have a flower bud but the abortion rate was nearly 50 per cent. (Klapwijk and Vander spek, 1988).

Dai and Paull (1990) studied the role of subtending leaf on anthurium flower growth. Removal of young growing subtending leaf resulted in 18 days earlier emergence of flower. Since the leaves were negative in net photosynthetic rate, but matured subtending leaf was the source of nutrients to the flower and its removal had less effect on flower development. Peduncle growth was sigmoidal, but spathe growth was double sigmoidal.

Mercy and Dale (1994) reported that spadix took 3 to 4 weeks to open from the day of its visibility from the leaf base and became receptive 4 to 7 days after the spathe opening. Male phase followed the female phase after 7 to 10 days. Fruits matured by 4 to 7.5 months and their number ranged from 100 to 200.

In *Spathiphyllum wallisii*, spadix took 25.50 days to open from the date of visible initiation. After 5 days of spathe opening, stigmas become receptive followed by male phase with a gap of 5 days. The whole spadix took 155.50 days from the date of visible initiation to fruit maturity. The number of fruits in an artificially pollinated inflorescence was 29.20, while it was only 4 in naturally pollinated ones (Savitha, 1995).

2.2 Artificial pollination and breeding

2.2.1 Artificial pollination

Sheffer and Kamemoto(1976) studied the cross compatibility of 56 species of anthurium by 1952 cross and self pollination. They classified *Anthurium* species into 6 groups based on morphological characters. Presence of B chromosome affected the viability of the seeds produced, but there was no difference between selfed and crossed progenies, and *A. scherzerianum* failed to form seeds even by crossing. However *A. andreanum* cv.'Uniwai' was more compatible with atleast closely related species.

The time required from pollination to seed maturity was about 180-200 days (Swaminathan, 1986).

Mercy and Dale(1994) described a method for artificial pollination. They have used fine moist brush to collect the pollens and

artificial pollination was effected by passing the tip of the brush with the collected pollen over the receptive candle for few times. They also recommended repeated pollination of 7 to 10 days to fertilize all the flowers in a candle.

2.2.2 Breeding of anthurium

Breeding work was initiated well early and several hybrids were released. Details are as follows

Name	Source	Authors
New species hybrid	*A. scherzerianum* *xA.wendlingerii*	Kamemoto and Sheffer (1978)
Rose Opal Green, Coral Green, Avenue, Chameleon		Kamemoto and Nakasone (1963)
Uniwai, Marian Seeforth, White Green		Kamemoto *et al.* (1969)
Anthurium X Southern Blush	*A. andreanum X* *A. amnicola*	Henny *et al.* (1988)
Southern Blush	-	Henny (1989)
IIHR 26, IIHR 139, IIHR 243, IIHR 51		Singh (1995)
Ruth Morat or Lady Ruth		Anon. (1996)
Champion		Anthura (1997)

2.3 Pollen storage

Calcium was found to have a great influence on germination of pollen indicating its viability. *Anthurium* pollens gave highest germination (82.7%) and pollen tube length (1250 μ) at higher concentrations of calcium (Kwack, 1965). He also described the media for germination of pollen.

Khosh- khui et. al. (1976), reported that rose pollen grains when stored at 0°C and 50 or 70 per cent relative humidity showed longest survivability compared to 25°C. They also observed that pollen viability increased by 2nd or 3rd week of storage and then started to decline then onwards.

In spathiphyllum, pollen stored at 7°C and 65 per cent relative humidity showed highest germination even after 24 weeks, where as pollen stored at 23°C lost its germinability rapidly by 4 weeks. (Henny, 1978a).

Henny (1978b) reported the effect of storage temperature and vacuum drying on *in vitro* germination of spathiphyllum pollen. Optimum storage condition was 7°C with 53 per cent germination, even for 16 weeks. Vacuum drying at -50°C gave 78 per cent germination. They also found that pollen was sensitive to low humidity (below 10%).

Savitha(1995) observed 98 per cent pollen germination in the media containing 10 per cent sucrose + 200 ppm boric acid in *Spathiphyllum wallisii.*

2.4 Histological studies in Araceae

2.4.1 Anther

In *Theriophonum minutum*, the archesporium in young anther primordium differentiated very early in the hypodermal layers consisting of a single vertical file of cells. The cells were larger in size with a prominent nuclei (Parameswaran, 1959). Anthers in *Arisaema wallichianum* exhibited an uncommon disposition of curved nature. When microspores are being formed, the cells of the tapetum lost their lateral contact with one another and cytomixis occured. The *en masse* protoplasts started expanding centripetally and ensheathed the microspores forming a 'Periplasmodium". The presence of periplasmodium has been reported in many aroids such as *Dieffenbachia senguine,* (Campbell, 1900), *Symplocarpus foetidus* (Duggar, 1900), *Peltandra virginica*(Goldberg, 1941), *Typhonium trilobatum* (Banerji, 1947) *Arisaema wallichianum* (Maheswari and Khanna, 1956) *Theriophonum minutum* (Parameswaran, 1959) and *Spathiphyllum wallisii* (Savitha, 1995).

The division of pollen was complete, while the ovules are still very small in *Anthurium senguine*(Campbell, 1905).

The tapetum was amoeboid in *Arisaema wallichianum.* Tetrads were isobilateral, decussate or tetrahedral in arrangement. The pollen grains were spheroidal or echinulate and shed at two celled stage (Maheswari and Khanna, 1956). Exine was relatively thin and had tiny spinescent sculptures. The epidermis of the anther was intact during maturity (Parameswaran, 1959). Dehiscence of anther was longitudinal in *Arisaema* (Maheswari and Khanna, 1956). Two celled pollens were reported in *Arisaema, Dieffenbachia, Spathyema, Symplocarpus, Synandrospadix and Theriophonum.* Pollen grains were

shed at three celled stage in *Aglaonema, Arum, Peltandra and Zantedeschia* (Kapil, 1967).

2.4.2 Ovule

The archesporium in *Arisaema triphyllum* has two to four cells in cross section which are the products of division of a single primary archesporial cell(Campbell, 1903). Similar archesporium was also found in *Aglaonema commutatum.* The primary archesporial cell usually divides once by a transverse wall, the outer and smaller cell represents the tapetum which was destroyed without any divisions and the inner larger cell develops into embryosac (Campbell, 1905).

The archesporial cell was hypodermal in *Arisaema wallichianum,* but subepidermal in *Theriophonum minutum.* These archesporial cells directly acted as megaspore mother cell (Mahaswari and Khanna, 1956; Parameswaran, 1959).

Archesporium in *Spathiphyllum wallisii* was found to be a mass of 150 isodiametric cells, from which archesporium was differentiated and divides periclinally with 'Crassinucleate' pattern of development (Savitha,1995).

In *Arisaema* only one single sporogenous cell functions and the megaspore mother cell underwent usual meiotic divisions resulting in 'T' shaped tetrad. The chalazal megaspore was active and others degenerated (Maheswari and Khanna, 1956). The megaspore mother cell, after meiotic divisions gave rise to a linear tetrad of megaspores (Parameswarn, 1959). 'Polygonum' type of embryosac was found in *Arisaema, Theriophonum and Spathiphyllum.*

The synergids in *Arisaema wallichianum* were pear shaped and lacked the usual basal vacuoles. The polar nuclei usually fused before fertilization. The synergids and antipodal cells usually degenerated before fertilization. The antipodals are ephemeral (Maheswari and Khanna, 1956). But they are persistent upto post fertilization stage in *Theriophonum*. They enlarge in *Arum orientale, Symplocarpus foetidis* and multiply in *Aglaonema, Lysichiton*(Campbell, 1899), *Xanthosoma* (Johri *et. al.*, 1993).

In *Dieffenbachia senguine,* ovule was axial in structure. Ovules were straight with the massive base. Presence of mucilage around the ovule was recorded in *Anthurium* (Campbell, 1900). The young ovules were in pairs and were nearly hemispherical masses of tissue (Campbell, 1905) in *Anthurium violaceum.*

In *Aglaonema,* the ovules from initial stage itself were very massive and integuments were developed at an early period and the chalazal region was large which is a common feature of Araceae (Campbell, 1903).

The ovule is bitegmic and crassinucleate in *Arisaema.* The inner integument formed the micropyle. The laterally situated cells of nucellus degenerated early which forced the embryo sac to come in contact with inner integument. In *Theriophonum minutum,* ovule was tenuinucleate. The inner and outer integuments developed from the nucellus base. Though the inner integument was rapid in growth, the outer integument overgrew the inner integument during late ontogeny (Parameswaran, 1959). But both the integuments were of same size in *Aglaonema versicolor* (Gow, 1908). Presence of nucellar cap was observed in *Theriophonum minutum* and *Spathiphyllum wallichi* (Savitha, 1995). In many aroids, the cells of inner integument that

line the embryosac elongated radially in perpendicular plane to long axis of embryosac and differentiate into 'Endothelium' (Maheswari and Khanna, 1956).

2.4.3 Endosperm and Embryo

In 'Araceae' early filling of embryosac was very characteristic and development of peripheral protoplasmic layer with embedded free nuclei, was observed in *Aglaonema commutatum* (Campbell, 1900). He also reported that in anthurium, embryo sac was filed with solid large celled tissue.

In *Anthurium*, the free endosperm nuclei was absent but this was quite common in other aroids. The primary endosperm was at the base of the embryo sac (Campbell, 1905).

In aroids, the development of endosperm is of cellular type. Primary endosperm nuclear division was followed by wall formation which separates the micropylar chamber from chalazal chamber. The micropylar chamber develops into cellular endosperm. The chalazal chamber remained uninucleate and become haustorial with a hypertrophied nucleus, which is common in Araceae, otherwise known as 'Basal apparatus' (Maheswari and Khanna 1956; Johri *et al.*, 1993).

An *ab initio* cellular endosperm, has been reported in eleven out of twenty four species of 'Araceae'. In remaining species, the endosperm was either nuclear or helobial (Parameswaran, 1959). Among the species with cellular endosperm, presence of a distinct chalazal haustorium was known only in *Peltandra virginica* (Goldberg, 1941), *Typhonium trilobatum* (Banerji, 1947), *Arisaema*

(Maheswari and Khanna, 1956) and *Theriophonum minutum*. Gowda(1980) reported the helobial type of endosperm in *Ariopsis peltata*.

The embryo of *Typhondorum lindleyanum* showed a definite suspensor at the micropylar end which is uncommon in other aroids (Boodle and Hill, 1929). Early filling of endosperm was not seen and endosperm was restricted to upper portion of embryosac for long time.

2.5 Histochemical studies

The review of research works carried out in localization of macromolecular substances at different stages of anther and ovule are presented below.

2.5.1 Anther

Autoradiographic studies in *Lilium longifolium*, showed that sporogenous tissue was rich in total proteins in both nucleus and cytoplasm. Labelled RNA accumulated in tapetum during DNA synthesis and even after late nuclear division. Tapetum showed protein accumulation after nuclear division, as it was necessary for microspore wall formation. (Taylor, 1959).

In the tapetum of maize, early nuclear divisions occur without prior DNA synthesis, subsequently the DNA content increased. Tapetal RNA synthesis also increased during the meiotic prophase and accumulated upto meiotic prophase in tapetum. In the meiocytes, early prophase had shown the principal accumulation but no synthesis during meiotic divisions or tetrad period. Protein accumulation occurred in the tapetum upto mid meiotic prophase. In

the meiocytes protein accumulated in the early prophase and active accumulation occurred in the developing spores (Moss and Heslop-harison, 1967).

In *Pennisetum typhoideum,* pollen was rich in starch, proteins, lipids and polysaccharides, but not in reducing sugars (Panchaksharappa and Rudramuniappa, 1972).

Panchaksharappa and Joshi (1974) reported the presence of rich RNA in both nucleus and cytoplasm of sporogenous tissue while cytoplasmic polysaccharides and protein are at low levels in *Iphigenia pallida..* It suggests the high rate of metabolism for further differentiation. Large number of starch grains were present in young pollen grains which rendered PAS positive cytoplasm.

Pollen was rich in RNA, protein and storage polysaccharides. Tapetum was also found to be rich in polysaccharides, RNA and protein. Fibrous thickenings were also observed in endothecium which was rich in lignin.

The pollen grains in Agaveceae, Liliaceae Amaryllidaceae and Iridaceae (all monocots) were thoroughly starchless (Baker and Baker, 1979).

Grayum (1985), reported that smaller pollengrains tend to store more lipids, while larger pollens (> 26-30 μm) accumulated starch. Storage of starch is widely in vogue in 'Araceae', but pollens of *Acorus, Gymnostachys, Anthurium* and *Spathiphyllum* were starch less.

The sporogenous cells of mango anther had rich polysaccharides, RNA and intense protein content. The tetrads were

rich in polysaccharide, intense in RNA and protein. The differentiation of the glandular, binucleate tapetum was evident at tetrad stage. (Anitha Karun, 1989).

Roopashri *et al.* (1992), observed the presence of intense protein and RNA in sporogenous tissue of *Ipomoea horsefalliae.* PMC's(Pollen mother cells) were surrounded by callose, and tapetum was rich in RNA, cytoplasmic polysaccharides and proteins. Pollens were found to be rich in RNA, protein and polysaccharides.

Protein was abundant in early stages of development of anthers in *Lilium longiflorum,* but polypeptides appeared during meiosis of PMC's, by that time tapetum became vacuolated. During mitosis the protein content of microspores was reduced (Wang, *et al.,* 1992).

Histochemical studies in *Hamerocallis flava* showed that sporogenous tissue was rich in polysaccharides, proteins, RNA and DNA. PMC's, tetrads and microspores, had abundant polysaccharides, proteins and RNA, but DNA is less. Microspores were released after the callose dissolution. pollens were rich in all the three macromolecular substances (Uma *et al.*, 1994).

In *Spathiphyllum wallisii,* sporogenous cells and PMC's were poor in insoluble polysaccharides, RNA and proteins, but PMC's had a positive callose deposition. Tapetum and microspores were rich in insoluble polysaccharides, RNA and proteins while the pollen grains were rich in insoluble polysaccharide and RNA. But at later stage pollens were found to have more protein (Savitha, 1995).

2.5.2 Ovule

Miki-Hirosige (1964) observed that, megaspore mother cells (MMC's) had reducing sugars, lipids, and high RNA content. Rise in protein and RNA content was noticed at the early stages of development of embroyo sac in *Lilium longifolium.*

In *Stellaria media,* the archesporium and MMC's(Megaspore mother cells) had cell wall rich in polysaccharides, while cytoplasm was deficient in polysaccharides but rich in RNA. Archesporium was rich in protein, while MMC's were less in it. Egg cell has PAS positive cytoplasm and rich protein, synergids also rich in polysaccharides but RNA and proteins were low in level, but this was opposite in central cell cytoplasm. Antipodal cells had low RNA and protein. Starch grains were found to be accumulated in 2 and 4 nucleate embryo sac stage (Pritchard, 1964).

In *Vanda,* the archesporial cells had PAS positive cell wall, while cytoplasm was PAS negative. MMC's were poor in both polysaccharides and RNA. Megaspores were rich in RNA and protein but their cytoplasm was PAS negative. 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 in protein. Synergids had PAS positive cytoplasm, but were low in RNA and proteins. The central cell had low PAS positive cytoplasm, but rich in RNA and proteins (Alvarez and Sagawa, 1965).

In *Dipcadi montanum,* the insoluble polysaccharides were at very low level in archesporium, MMC,'s dyads, tetrads and functional megaspore stages, while RNA and protein were rich in all of them. RNA and protein were abundant in degenerating spores. The

cytoplasm of young embryosac and central cells of mature embryosacs had low concentration of polysaccharides, RNA and proteins. In mature embryosac, the egg cells contained low cytoplasmic polysacharides and RNA, while protein content was rich. Synergids had dense PAS positive filiform apparatus and were rich in protein in cytoplasm, but less in RNA. Cytoplasm of antipodal cells were rich in polysaccharides and proteins in *Dipcadi montanum*. Rich RNA and protein were found in hypostase and integuments (Panchaksharappa and Joshi, 1975).

Archesporium with poor insoluble polysaccharides, rich RNA and intense protein content, were reported in *Pyrostegia venusta*. Megaspore displayed starch accumulation, poor RNA and protein content (Aswath *et al.*, 1989).

In *Hemerocallis flava* archesporium, MMC's and megaspore tetrads were rich in polysaccharides, proteins and RNA and DNA (Uma *et al.*, 1994).

In *Spathiphyllum wallisii*, the MMC's and functional megaspore were found to be poor in insoluble polysaccharides, RNA and protein. The egg cell and synergids were poor in insoluble polysaccharides but had intense RNA and proteins. The embryo was rich in polysaccharides, proteins and intense in RNA, while endosperm was poor in polysaccharides and RNA but had good deposits of storage proteins (Savitha, 1995).

MATERIAL AND METHODS

III MATERIAL AND METHODS

The present studies were carried out during the year 1998-99 at the Horticulture Research Station, Division of Horticulture, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore.

Studies conducted were pertaining to floral biology, pollen storage, histological and histochemical changes during micro and megasporogenesis of *Anthurium andreanum* cv. 'Crinkle Red'.

3.1 Morphological characters

The different morphological characters of *Anthurium* cv. 'Crinkle Red', like plant height, number of leaves, length and width of leaves, length of peduncle and root characters were recorded. Besides this, floral characters like length of inflorescence, spathe shape, spathe colour (visual observation) and petiole length were also recorded.

3.2 Floral biology

3.2.1 Flower development

Fifteen inflorescences were tagged at the stage of visible initiation, and the average number of days required for opening of spathe, initiation and duration of male and female phase, greening of spathe and fruit set were recorded.

3.2.2 Stigmatic receptivity and anther dehiscence

Time of anther dehiscence and stigmatic receptivity were recorded from 6 a.m. to 6 p.m. at hourly intervals in fifteen inflorescences. Stigma was considered to be receptive, when the stigmatic fluid was oozing out.

3.1.3 Storage of pollen

Pollen grains were collected in the vials in the morning hours and were stored at three different temperatures viz., 0°C, 4°C and room temperature. Viability of pollen was recorded at weekly intervals using acetocarmine method(Sass, 1985). The constituents of the stain were

Carmine	2g
Acetic acid	45 ml
Distilled water	55 ml

Mixture of acetic acid and distilled water was warmed up and then carmine was added to it. The prepared stain was stored in refrigerated condition.

Pollen was dusted on to 1-2 drops of stain and stirred well with a needle. A cover slip was mounted on it and examined under compound microscope after 4 to 5 minutes. The pollen grains which absorbed the stain were considered viable, and the count was recorded.

3.2.4 Artificial pollination

Pollens were collected from the varietal collections maintained at floriculture unit, Lalbaugh, Bangalore and stored in refrigerated condition. The collected pollen grains were applied to receptive stigmas using a wet camel hair brush. Pollinated inflorescence were covered and number of fruits set was recorded

3.3 Histological and histochemical studies

3.3.1 Materials

The inflorescences were collected from the plants maintained at

PLATE 1

General view of *Anthurium andreanum* cv. 'Crinkle Red'

the floriculture unit, Horticulture Research Station, UAS, GKVK, Bangalore.

3.3.2 Sampling

The inflorescences were grouped into eight different classes subdivided into 3 groups namely, top, middle and bottom. Care was given to include all the representative stages of the development of anther and ovule.

3.3.3 Fixation

Materials were fixed in Carnoy's B fixative (6 parts of ethanol, 3 parts of chloroform and 1 part of acetic acid v/v) for 3 hours.

3.3.4 Dehydration

Dehydration was done using different grades of ethanol. The material was steeped in each grade for atleast 3 hours.

Dehydration using ethanol and n-butanol grades

	Water	Ethanol	n-butanol
01	30%	70%	-
02	20%	80%	-
03	10%	90%	-
04	-	100%	-
05		75%	25%
06		50%	50%
07		25%	75%
08		-	100%
09		-	100%

3.3.5 Infiltration and embedding

The materials in n-butanol media were added with molten paraffin wax and kept in oven at 2-3 °C above the melting point of wax (60 °C to 62 °C). Later 6 changes of molten paraffin wax was given at four hours interval to remove the traces of n-butanol, subsequently the material was embedded in paraffin wax mixture (9:1 paraffin wax : beewax) by using the paper boat technique (Jensen, 1962).

3.3.6 Microtomy

Uniform sections of 8 μm thickness were taken using ERMA rotary microtome.

3.3.7 Affixing the sections

Wax ribbons or microtome sections were fixed on the glass slides using few drops of gelatin (1% of gelatin with pinch of potassium dichromate). Material were kept on gelatin surface and warmed to 45°C to facilitate the stretching of wax ribbon. Excessive adhesive was poured off and stored in dust free environment for 24 hours.

3.3.8 Deparaffinisation and hydrating the sections

The slides with paraffin wax were treated with xylol for removal of wax and then dipped in n-butanol and 100 per cent ethanol to eliminate the traces of xylol. Later a coating of Celloidin 0.5 per cent in 1:1 (ether :alcohol) was given to prevent the washing of sections. Hydration was done if necessary.

Steps for deparaffinisation

Xylol-1	-	2min
Xylol-2	-	2 min
n- Butanol-1	-	2 min
n- Butanol-2	-	2 min
100 % Ethanol	-	2 min

3.3.9 Histochemical staining procedures

For localization of macromolecular substances like, insoluble polysaccharides, protein and ribo nucleic acids, the sections were subjected to different histochemical staining procedures. After staining, sections were subjected to dehydration using n-butanol, cleaned in xylol and mounted in DPX.

3.3.9.1 Total insoluble polysaccharides

The technique used was Per-iodic Acid Schiff's (PAS) method.

Preparation of Schiff's reagent (Longley, 1952)

Basic Fuchsin 1g and 1.8g of potassium metabisulphate mixed with 100 ml of 0.15 N hydrochloric acid in an airtight container and shaken for 24 hours to obtain straw yellow colour solution. After adding 100 mg of activated charcoal, it was shaken and filtered. The colourless filtrate was stored in amber coloured bottles.

Staining procedure was

(a) deparaffinisation and hydration

(b) Per-iodic acid treatment for 15 minutes at room

temperature

(c) washed thoroughly in running water

(d) Schiff's reagent treatment for 15 minutes

(e) rinsed in water for differentiation and bleached with potassium metabisulphate solution (5 ml of 1 N HCl and 50 ml of 10% potassium metabisulphate + 90 ml of distilled water)

(f) washed in running water

(g) dehydration and mounting

3.3.9.2 Ribo Nucleic acid

Toluidine blue was used for RNA localisation (0.5 g Toluidine Blue powder in 100 ml of water)

Staining procedure was

(a) deparaffinisation and hydration

(b) dipped in Toluidine Blue for 90 seconds

(c) shaken in water to remove the excess stain

(d) dehydration and mounting

Presence of RNA was confirmed by characteristic purple colour.

3.3.9.3 Total proteins

The Mercuric Bromophenol Blue method (Mazia *et al.*, 1953) was used. Only the insoluble proteins were stained since all the soluble proteins were assumed to be lost during processing. Stain was prepared by dissolving 10 g of Mercuric Chloride and 100 mg of Bromophenol Blue powder in 100 ml of 100 per cent ethanol.

Staining procedure was

(a) deparaffinisation and dehydration
(b) five minutes treatment with Mercuric Bromophenol Blue
(c) shaken in water
(d) dehydration and mounting

Presence of protein was noted by blue colour.

3.3.10 Cytochemical assessment

Based on visual observations of the degree of cytochemical reactions with specific reagents, the grading was

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

3.3.11 Micrometry

The size of different tissues of the anther 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 standardized using stage micrometer. The ocular micrometer readings were converted into actual values by multiplying with the standard value.

3.3.12 Photomicrography

The desirable events were captured using PENTAX photomicrograph equipment.

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The results of the present investigations on floral biology, pollen storage, histology and histochemistry of microsporogenesis and megasporogenesis are presented in this chapter.

4.1 Floral biology

In *Anthurium andreanum* tiny bisexual flowers were arranged closely on the spadix. The number of true flowers per cm^2 was about 26.80 and the diameter of each flower was 3 x 1.95 mm. The flowers were almost sessile with four tepals, four stamens and single capitate sessile stigma. The tepals were arranged in two whorls with outer tepals being bigger and overlapping the inner two. These four tepals combined to form a rhombic outline. Stamens in the outer whorl emerge first than the inner two anthers. On an average, anther lobes measured 555 x 294 μm. Stamens were squeezed between the tepals and pistil. The pistil was simply exposed and surrounded by stamens. The gynoecium was bicarpellary (Plate 2).

The flowers were protogynous in nature and anther emerges only after complete loss of stigmatic receptivity, which was pronounced by the complete drying of the stigma (Plate 2.3, 2.4). The fruit set was about 19 fruit per spadix, if artificially pollinated (Plate 2.6).

4.1.1 Flower development

From the stage of visible initiation from the leaf base inflorescence took 13.2 days to reach the $1/3^{rd}$ of the full size and by 32.93 days it reached $2/3^{rd}$ of the full size. It reached full maturity at around 61.33 days and spathe started to unfurl and unfurling was completed in 69.93 days. (Table 2).

Table 1 Morphological characters of *Anthurium andreanum*, cv.'Crinkle Red'.

Characters		
Plant height (cm)		60.1
Leaf	Number	5.2
	Length (cm)	29.1
	Width(cm)	18.03
Petiole length (cm)		40.33
Inflorescence length(cm)		55.2
Spathe	Shape	Cordate shaped, with puckered surface, without basal overlapping
	Length(cm)	13.64
	Width (cm)	10.84
	Colour	Coral red
Peduncle length (cm)		55.2
Spadix	Colour	White at the base and orange at the tip
	Length(cm)	6.88
Root	Length (cm)	17.26
	Diameter(cm)	0.54
Aerial root	Length (cm)	3.68
	Number	10.9
Pollen	Size (μm)	19.125
	Shape	Colourless, smooth, oval to spherical, thin walled

Table 2 : Complete cycle of floral biology in 'Crinkle Red'

Stages	Days after visible initiation	Days after unfurling of spathe	Days after pollination
Visible initiation	0		
1/3rd of Spadix maturity	13.2		
2/3rd of Spadix maturity	32.93		
Beginning of unfurling	61.33		
Completion of unfurling	69.33		
Stigmatic receptivity			
Initiation	76.26	6.93	
Completion	82.60	13.27	
Dry period	5.73	5.73	
Anther dehiscence			
Initiation	89.33	20	
Completion	95.66	26.33	
Greening of spathe	181.66		105.4
Initiation of berry development	224.26		148
Fruit ripening	308.26		232
Number of berries (Artificially pollinated)	19		

PLATE 2

Inflorescence

2.1 Developing stages of inflorescence

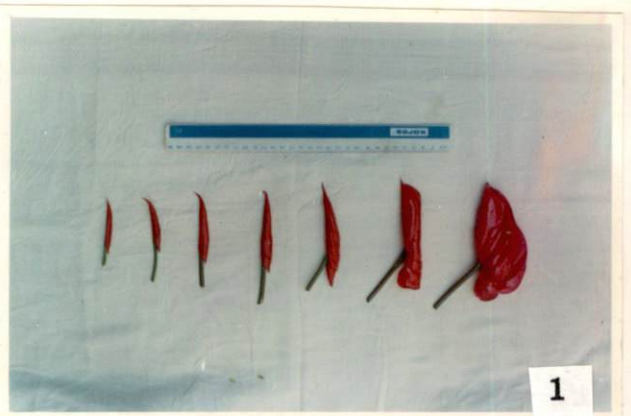
2.2 Inflorescence with fruit set.

2.3 Cut flower

2.4 Inflorescence showing stigmatic exertion

2.5 Transverse section of the inflorescence

2.6 Inflorescence showing developed anthers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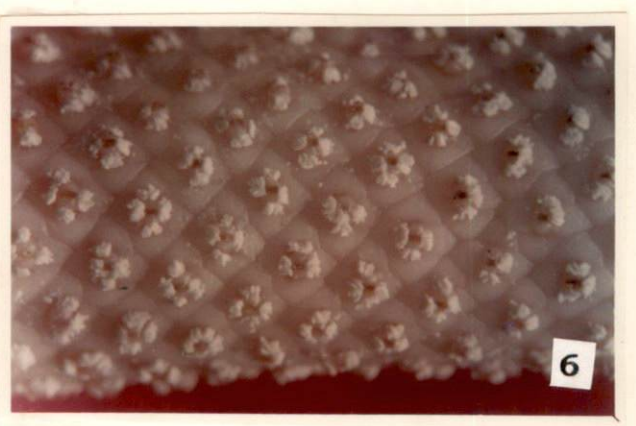

6

4.1.2 Stigmatic receptivity

Stigmas took 6.93 days to become receptive from the date of complete unfurling (pronounced by the presence of stigmatic droplets) and receptivity of the stigmas lasted upto 13.27 days. The average number of days of stigmatic receptivity was 7.13 days, therefore it is clear that anthurium requires daily pollination of 7-8 days for complete fruit set (Table No 2). Stigmatic receptivity started from base and progressed upwards in the inflorescence.

4.1.3 Anther dehiscence

The first dehiscence of anther was observed in basal part of the inflorescence at around 20 days of spathe opening (after a gap of 5.73 days from the date of loss of stigmatic receptivity). Anther dehiscence continued upto 26.33 days with an average period of about 7.33 days(Table No. 2).

4.1.4 Time of stigmatic receptivity and anther dehiscence

Stigmatic receptivity was noticed from 6.00 a.m. in the morning (evidenced by oozing of stigmatic droplets) and continued upto 3.36 p.m. (pronounced by the drying up of the stigmas). Anthers dehiscence started early in the morning (6.00 a.m.) and continued upto 9.00 a.m. with peak being 7.00 a.m. (Table 3).

4.1.5 Pollination and fruit set

The average number of fruits in artificially pollinated spadix was 19 (Table 2;Plate 2.6), but those allowed for natural pollination failed to set fruits

Table 3 : Time of stigmatic receptivity and anther dehiscence

Time	Per cent of inflorescence showing	
	Receptivity	**Anther dehiscence**
6.00 a.m.	40	29.41
7.00 a.m.	70	52.94
8.00 a.m.	90	11.76
9.00 a.m.	100	5.88
10.00 a.m.	100	0
11.00 a.m.	100	0
12.00 a.m.	100	0
1.00 p.m.	100	0
2.00 p.m.	100	0
3.00 p.m.	100	0
4.00 p.m.	50	0
5.00 p.m.	10	0
6.00 p.m.	0	0

4.2 Storage of pollen

The data pertaining to viability of anthurium pollen stored at different temperatures is presented in Table 4.

Storage temperatures had significant influence on viability percentage of pollen. Immediately after extraction, pollen grains were found to have 90.33 per cent viability. Pollen grains stored at 0°C showed a significant reduction in viability(at weekly intervals) as the storage period was advanced with an exception during first and second weeks. Even after storage for 8 weeks, pollen grains remained viable to a considerable extent (8.73%). Similarly pollen grains stored at 4°C showed significant reduction in viability with an increase in the duration of pollen storage. The pollen grains turned non viable from seventh week onwards. Although fresh pollen grains had 88.99 per cent viability, it was reduced to 0 per cent by just one week at room temperature conditions. From 2nd week onwards, there was a significant difference in viability between 0°C and 4°C treatments and both the treatments showed a drastic reduction in viability in their final phases i.e. just before they became non viable.

4.3 Histology of anthers during microsporogenesis and male gametophyte development

The data on micrometric observations at successive stages of anther development are presented in Table 5.

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 68µm. Number of cells were 25 and its size was 8.5 x 10.63 µm. Thickness of the outermost layer was 12.75 µm (Plates 3.1, 4.1, 5.1).

Table 4 : Effects of storage temperatures on the viability (%) of pollen at different weeks

Temperature °C	0	1	2	3	4	5	6	7	8
O	91.15	80.74	76.62	67.71	56.85	46.50	34.50#	6.24#	8.73#
4	90.85	79.65	68.80	58.18	48.35	30.59	8.23#	0#	0#
Room temperature	88.99	0#	0#	0#	0#	0#	0#	0#	#0

not included for analysis

* Significance

SEm ± 1.910

CD (P=0.05) 5.294

CV (%) 7.396

Table 5 : Micrometric observations during microsporogenesis and male gametophyte development in 'Crinkle Red'

Stages	Pollen sac diameter (µm)	Number of cells	Individual cell size (µm)	Tapetum thickness (µm)	Middle wall layers (µm)	Endothecium (µm)	Epidermis (µm)
Sporogeneous tissue	68	25	10.63 x 8.5	ND	ND	ND	12.75
Pollen Mother cells	85	12	12.75 x 12.75	8.5	4.25	ND	4.25
Tetrads	102	15	17 x 12.75	12.75	4.25	ND	4.25
Microspores	170	82	12.75 x 10.63	DG	4.25	4.25	DG
Pollen grains	233.75	73	17 x 12.75	DG	DG	34	DG

ND = Not differentiated

DG = Degenerated

As the pollen mother cells (PMC's) differentiated from sporogenous cells, callose deposited around them separating sister microcytes. The thickness of the tapetum was 8.5 μm and this was formed by the differentiation of cells of innermost wall layer accompanied by increase in size. The pollen sac diameter was, increased to 85 μm and the number of PMC's in a section of microsporangium was 12, whose size was 12.75 x 12.75 μm. The middle wall layers and epidermis where 4.25 μm in thickness (Plates 3.2, 4.2, 5.2).

The PMC's underwent meiosis I and II, and produced four haploid microspores. Upon cytokinesis they formed, isobilateral or tetrahedral tetrads (17 x 12.75μm). The tapetum further increased in thickness to 12.75 μm, the pollen sac diameter also increased to 102 μm in size. The number of tetrads in a section of microsporangium was 15. Both the middle wall layer and epidermis were of equal size(4.25μm) and degeneration of middle wall layers started at this stage (Plates 3.3, 4.3, 5.3).

Callose disappearance released the microspores from tetrads. The tapetum was amoeboid or plasmodial type. As microspores were released, the tangential and radial wall of the tapetal cells began to disintegrate and the tapetum protoplasm diffused into the anther locule forming the periplasmodium. The contents of these protoplasts disappeared as the microspores developed further. The pollensac diameter had increased to 170μm with 82 individual cells in a section of microsporangium, whose size was 12.75 x 10.63 μm. The epidermis degenerated at this stage. The endothecium was 4.25 μm in thickness (Plates 3.4, 4.4, 5.4).

The microspores released from tetrads developed into pollen grains accompanied by an increase in size of pollen sac to 233.75 μm. The average number of pollen grains in a section of microsporangium was reduced to 73 whose size was increased to 17 x 12.75 μm. The thickness of endothecium had enormously increased to 34 μm and the individual cells developed fibrous wall thickenings (Plates 3.5, 4.5, 5.5).

4.4 Histochemical changes during microsporogenesis and male gametoplyte development

4.4.1 Total insoluble polysaccharides

The PAS (Per -iodic Acid schiff's) reaction was used to localize the insoluble polysaccharrides and the changes observed at various stages of microsporogenesis are presented in Table 6.

The sporogenous cells were poor in cytoplasmic polysaccharides but the outermost layer of cells were rich in it (Plate 3.1).

At PMC's stage the tapetal cells showed intense polysaccharide content while the PMC's, middle wall layer and epidermis were rich in polysaccharide, but the connective tissue was poor in polysaccharides (Plate 3.2). The tetrads formed after meiosis I and II showed intense staining for polysaccharides, while the tapetum was rich in it (Plate 3.3).

As the microspores began to separate, the tapetal cell contents rich in polysaccharides started diffusing into the anther locule. The microspores had rich polysaccharide content. The middle wall layer, endothecium and connective tissue were poor in polysaccharides (Plate 3.4).

Table 6 : Changes in insoluble polysaccharides content during microsporogenesis and male gametophyte development in 'Crinkle Red'.

Stages	Sporogenous tissue	Tapetum	Middle wall layers	Endothecium	Epidermis	Connective tissue	Vascular strand
Sporogeneous tissue	+	++	++	++	++	++	++
Pollen Mother cells	++	+++	++	++	++	+	+
Tetrads	+++	++	++	++	++	+	+
Microspores	++	D	+	+	DG	+	+
Pollen grains	+++	D	DG	++	DG	+	+

\+ Poor

++ Rich

+++ Intense

DG Degenerated

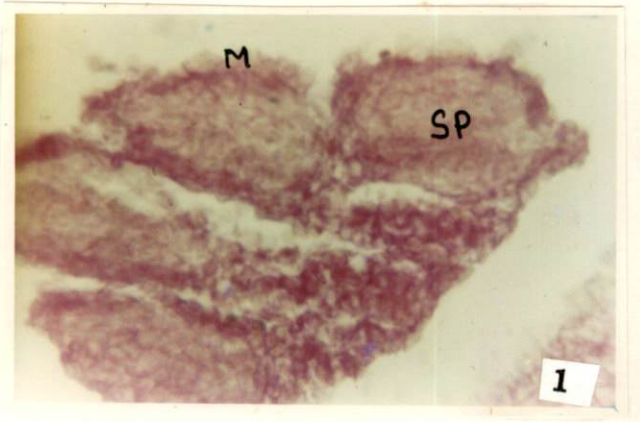
D Diffused

PLATE 3

Transverse sections of anthers tested with per-iodic acid schiff's reagent (PAS) to localize insoluble polysaccharides.

3.1	**Sporogenous tissue**	**x 400**
3.2	**Pollen mother cells (PMC's)**	**x 400**
3.3	**Tetrads**	**x 400**
3.4	**Microspores and periplasmodium**	**x 400**
3.5	**Developing pollen grains**	**x 400**

SP – **Sporogenous tissue**
P – **Pollen mother cells**
T – **Tapetum**
Tr – **Tetrads**
M – **Middle wall layers**
Ms – **Microspores**
C - **connective**
En – **Endothecium**
Ep – **Epidermis**


M
SP
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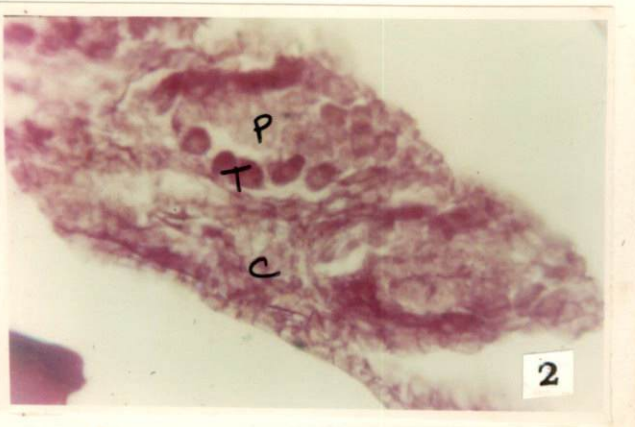
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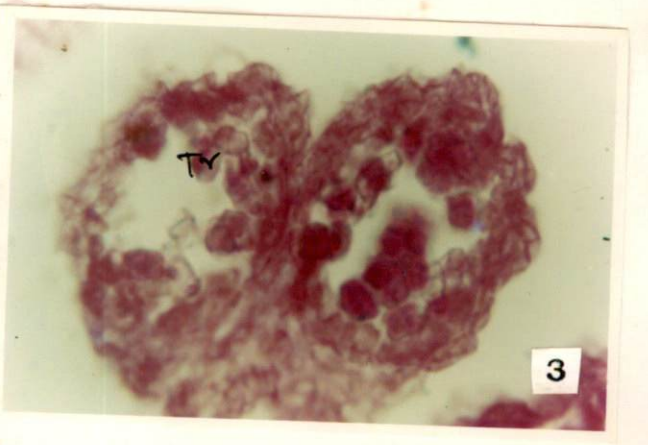
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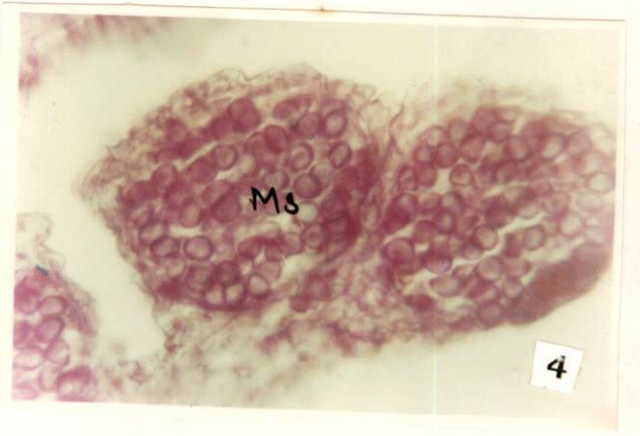
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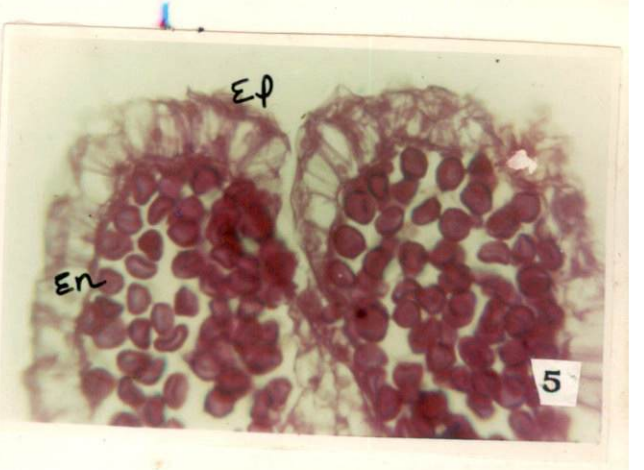

Ep
En
5

Pollen grains stained intense for insoluble polysaccharides. Starch accumulation was observed in them. The endothecium before anther dehiscence was rich in insoluble polysaccharides (Plate 3.5).

4.4.2 Localization of RNA

Toluidine Blue method was used for histochemical localization of RNA. The changes in RNA are presented in Table 7 and described as follows.

The sporogenous tissue had, rich RNA content. All other layers were poor in RNA (Plate 4.1).

At the PMC's stage, tapetum showed intense staining while the PMC's were rich in RNA content. The middle wall layers, endothecium, epidermis, connective and vascular strands were poor in RNA content (Plate 4.2).

The tapetal cells at tetrad stage stained intense for RNA content, while the tetrads, middle wall layers, endothecium and epidermis were rich in RNA (Plate 4.3).

At the time of separation of microspores from the tetrads, the tapetal contents with rich RNA content diffused into anther locule. The microspores had intense RNA, while the middle wall layer and endothecium were poor in RNA (Plate 4.4).

At pollen grain stage, pollen grains were intense and endothecium was rich in RNA content. Whereas the connective and vascular tissues were poor in RNA content (Plate 4.5).

Table 7 : Changes in RNA content during microsporogenesis and male gametophyte development in 'Crinkle Red'.

Stages	Sporogenous tissue	Tapetum	Middle wall layers	Endothecium	Epidermis	Connective tissue	Vascular strand
Sporogeneous tissue	++	+	+	+	+	+	+
Pollen Mother cells	++	+++	+	+	+	+	+
Tetrads	++	+++	++	++	++	+	+
Microspores	+++	D	+	+	DG	+	+
Pollen grains	+++	D	DG	++	DG	+	+

\+ Poor

++ Rich

+++ Intense

DG Degenerated

D Diffused

PLATE 4

Sections of Anthers tested with toluidine blue for localising RNA

4.1 Sporogeneous tissue x 400

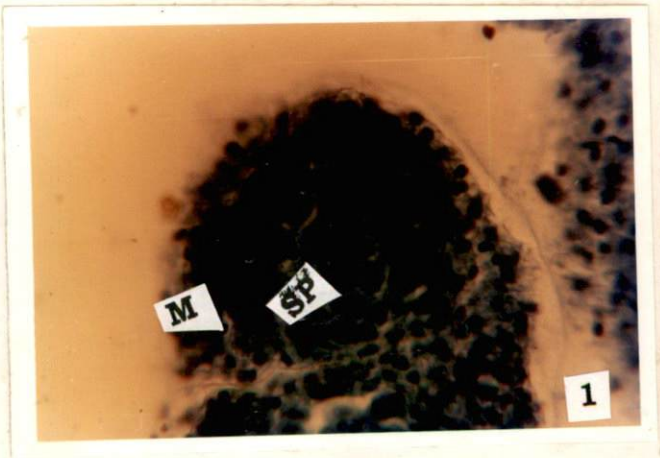
4.2 Pollen mother cells x 400

4.3 Tetrads x 400

4.4 Microspores and tapetal contents x 400

4.5 Developing pollen grains x 400

SP – Sporogenous tissue
P – Pollen mother cells
T – Tapetum
Tr – Tetrads
M – Middle wall layers
Ms – Microspores
C - connective
En – Endothecium
Ep – Epidermis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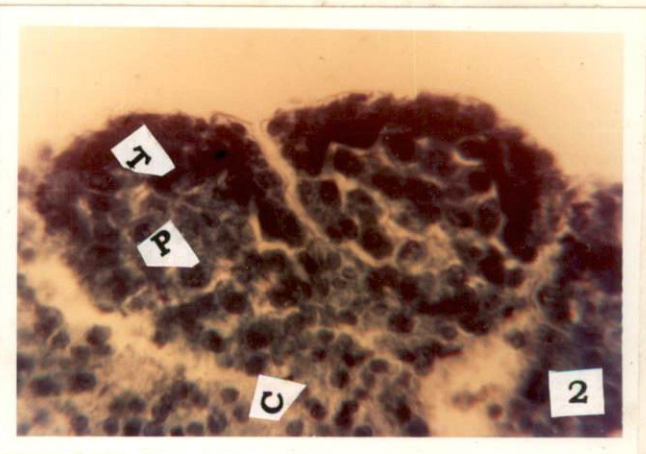
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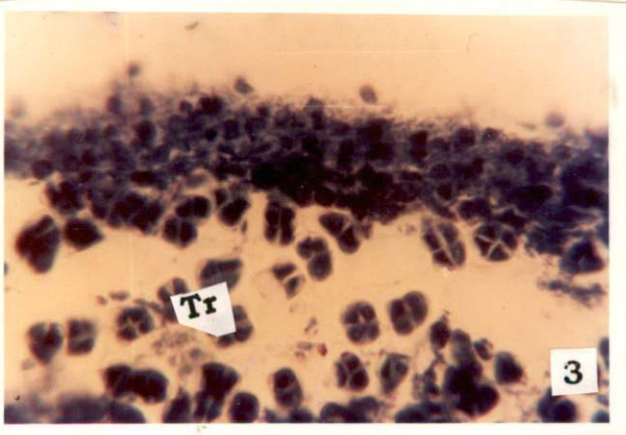
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Ms
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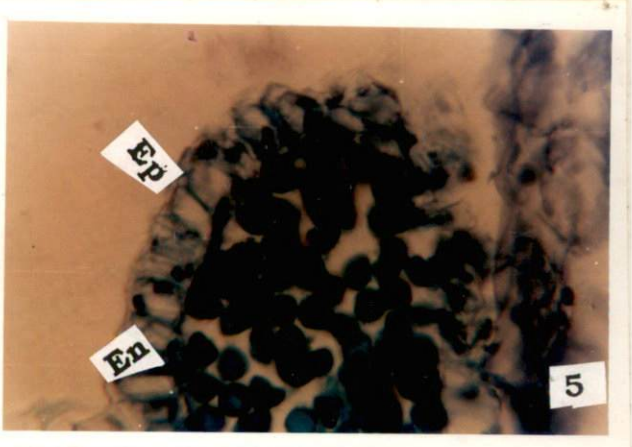
Ep
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5

4.4.3 Proteins

Mercuric Bromophenol Blue method was used to localise the proteins and the histochemical changes observed are presented in Table 8 and described as follows.

The sporogenous cells in the early stages of development were rich in proteins, while all other layers were poor in proteins (Plate 5.1).

The tapetum and PMC's in pollen mother cell stage showed intense protein content and all other layers were poor in protein (Plate 5.2). At tetrad stage, tapetum and tetrads were rich in protein content. The other components of anthers were poor in protein content (Plate 5.3).

The microspores separated from tetrads were rich in protein content and tapetum started to diffuse into anther locule. The endothecium and connective tissues were poor in protein (Plate 5.4).

The developing pollen grains were found to be poor in protein content, so also the connective and vascular strands. But the endothecium was rich in protein content. (Plate 5.5).

4.5 Histochemical changes during megasporogenesis and female gametophyte development.

In the organised embryo sac, synergids, antipodal cells, nucellus, integuments and hypostase were found to be rich in insoluble polysaccharides, while the egg was poor in insoluble polysaccharides (Plate 6.1, 6.3, 6.4).

Table 8 : Changes in protein content during microsporogenesis and male gametophyte development in 'Crinkle Red'.

Stages	Sporogenous tissue	Tapetum	Middle wall layers	Endothecium	Epidermis	Connective tissue	Vascular strand
Sporogeneous tissue	++	+	+	+	+	+	+
Pollen Mother cells	+++	+++	+	+	+	+	+
Tetrads	++	++	+	+	+	+	+
Microspores	++	D	+	+	DG	+	+
Pollen grains	+	D	DG	++	DG	+	+

\+ Poor

++ Rich

+++ Intense

Dg Degenerated

D Diffused

Table 9 : Changes in Insoluble Polysaccharides, RNA and Protein content during Megasporogenesis and female gametophyte development in 'Crinkle Red'

Stage	Macromolecular substance	Nucellus	Individual cells		Integuments	Hypostase
2- nucleate embryosac	RNA	+	++		++	
Organised embryo sac	Insoluble polysaccharides	++	Egg	+	++	++
			Synergids	++		
			Antipodals	++		
	RNA	+	Synergids	+++		
			Antipodals	++	++	++
			Polar nuclei	+		
			Synergids	++		
	Protein	+	Antipodals	++	++	++

\+ Poor

++ Rich

+++ Intense

Table 10 : The content of various macromolecular substances in embryo and endosperm of 'Crinkle Red'.

	Insoluble polysaccharides	RNA	Protein
Embryo	++	-	++
Endosperm	++	++	++

\+ Poor

++ Rich

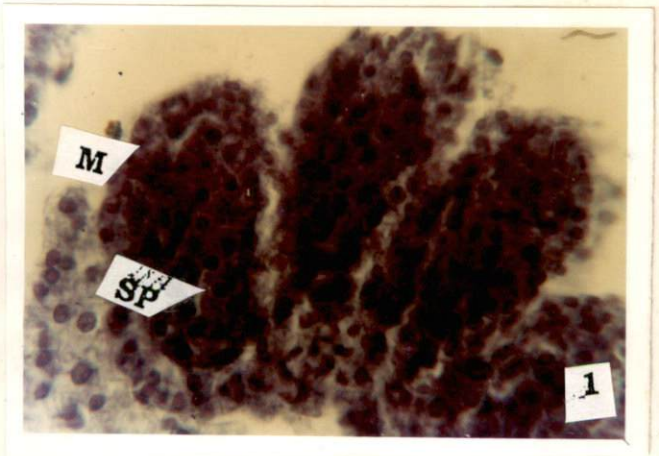
+++ Intense

PLATE – 5

Sections of the anthers tested with mercuric bromophenol blue method to localize the protein.

5.1	**Sporogeneous tissue**	**x 400**
5.2	**Pollen Mother Cells**	**x 400**
5.3	**Tetrads**	**x 400**
5.4	**Microspores**	**x 400**
5.5	**Developing pollen grains**	**x 400**

SP – **Sporogenous tissue**
P – **Pollen mother cells**
T – **Tapetum**
Tr – **Tetrads**
M – **Middle wall layers**
Ms – **Microspores**
C - **connective**
En – **Endothecium**
Ep – **Epidermis**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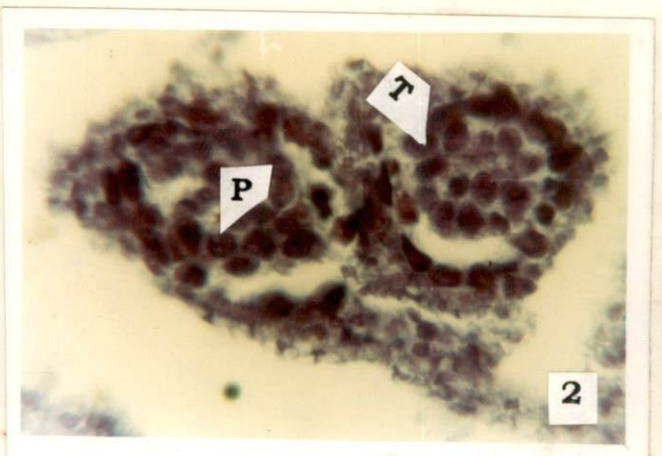
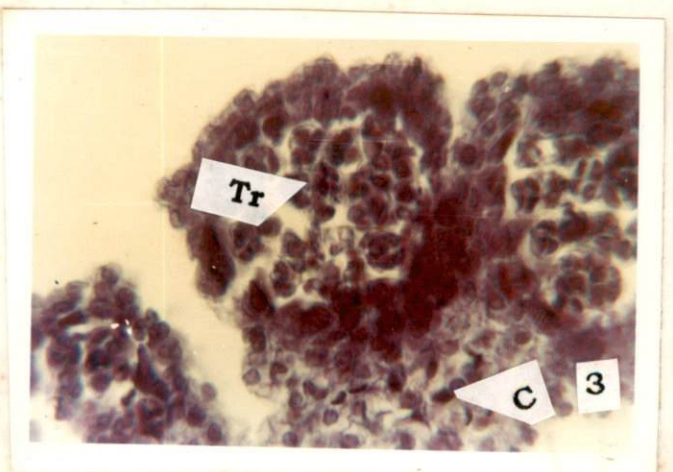
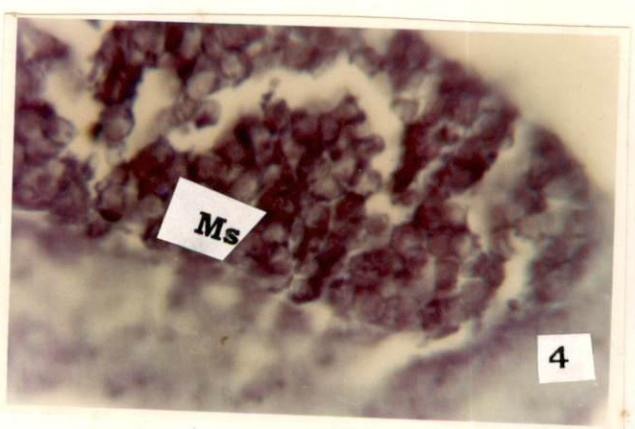

T
P
2


Tr
C
3


Ms
4

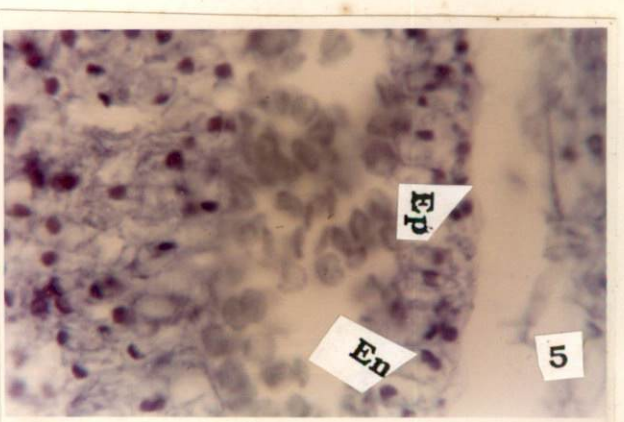

Ep
En
5

PLATE 6

Longitudinal sections of the ovules stained with per-iodic acid schiff's reagent to localize insoluble polysaccharides

6.1 Starch accumulation in embryosac x 400

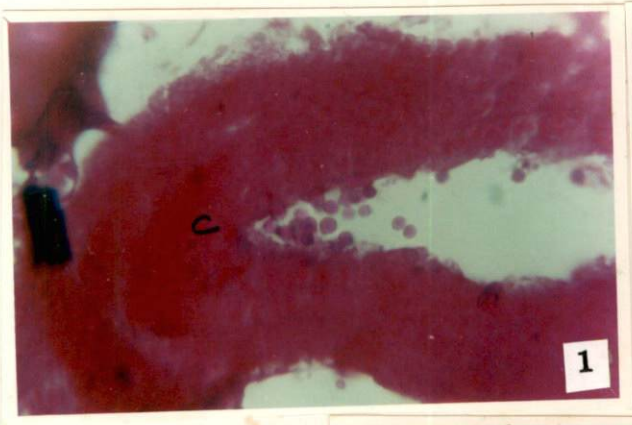
6.2 Egg x 400

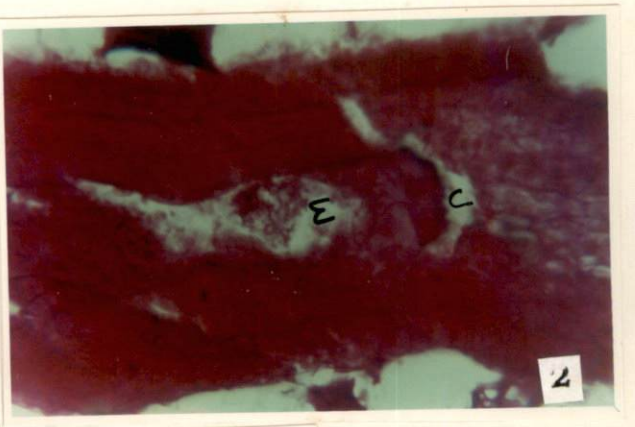
6.3 Antipodals x 400

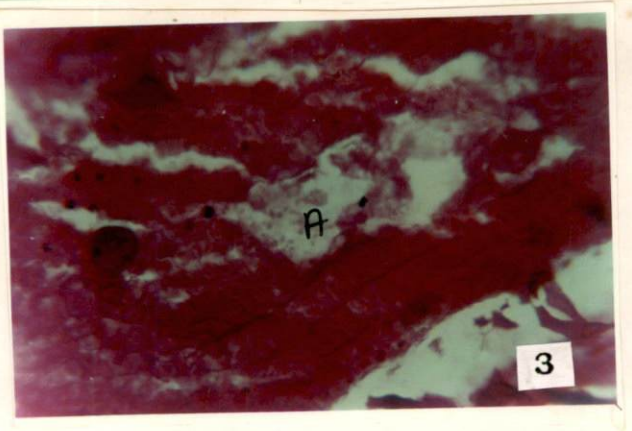
6.4 Zygote and synergids x 400

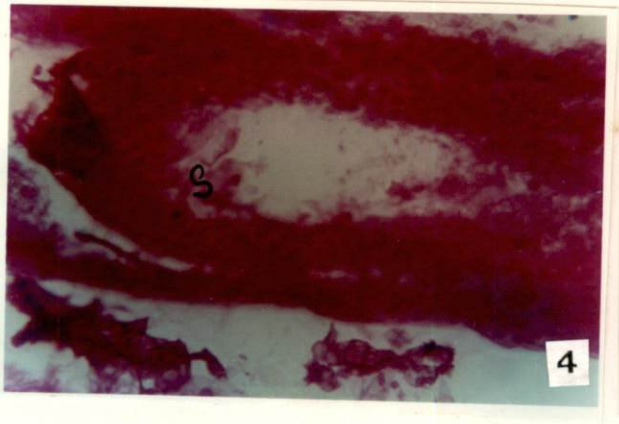
6.5 Cellular endosperm x 400

C – **Chalazal end**
Mp – **Micropylar end**
S – **Synergids**
A – **Antipodals**
En – **Endosperm**
I – **Integuments**


C
1


E
C
2


A
3


S
4

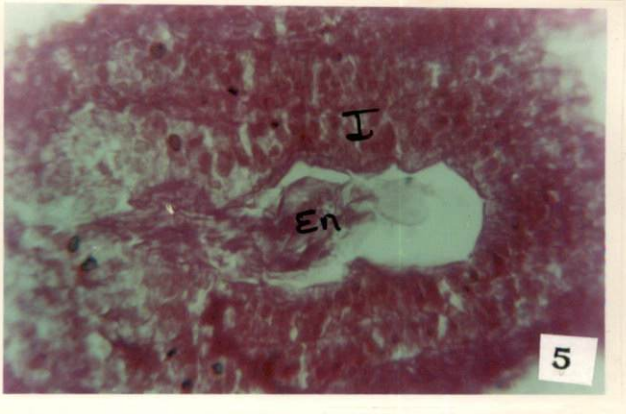
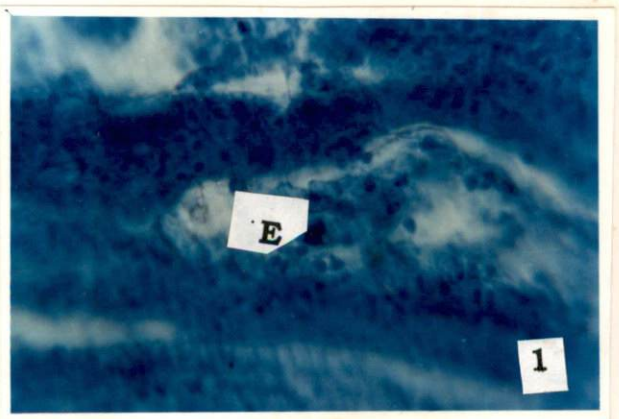

I
En
5

PLATE 7

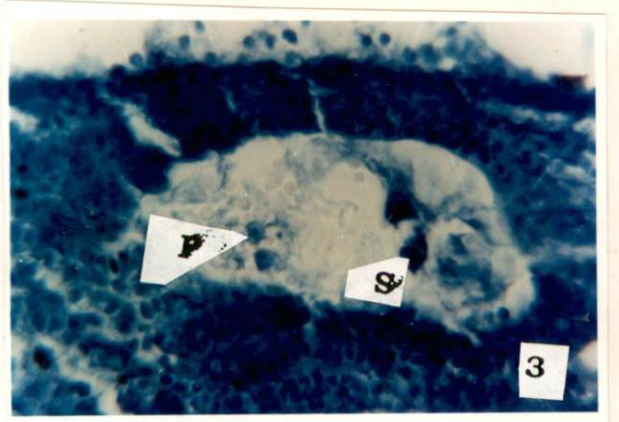
Longitudinal sections of the ovules tested with Toluidine blue to localize RNA

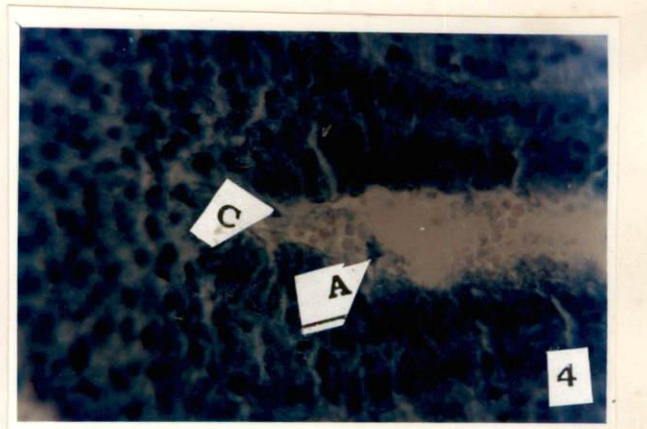
7.1	**Two nucleate female gametophyte**	**x 400**
7.2	**Eight nucleate female of gametophyte**	**x 400**
7.3	**Synergids and polar nuclei**	**x 400**
7.4	**Antipodals**	**x 400**
7.5	**Zygote**	**x 400**
7.6	**Cellular endosperm**	**x 400**

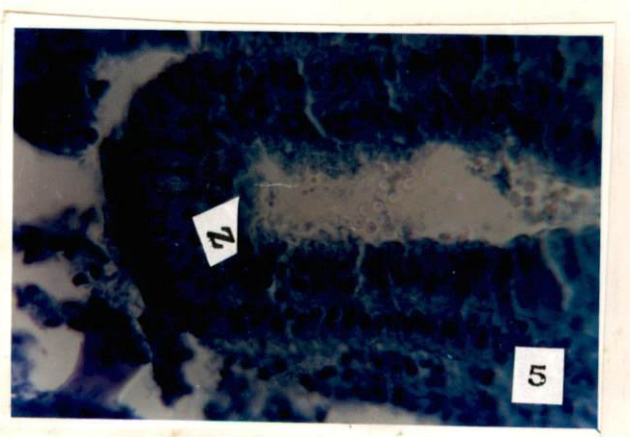
C – **Chalazal end**
Mp – **Micropylar end**
S – **Synergids**
E – **Egg**
A – **Antipodals**
Em – **Embryo**
I – **Integuments**
S – **Starch globules**


E
1


2


P
S
3


C
A
4


N
5

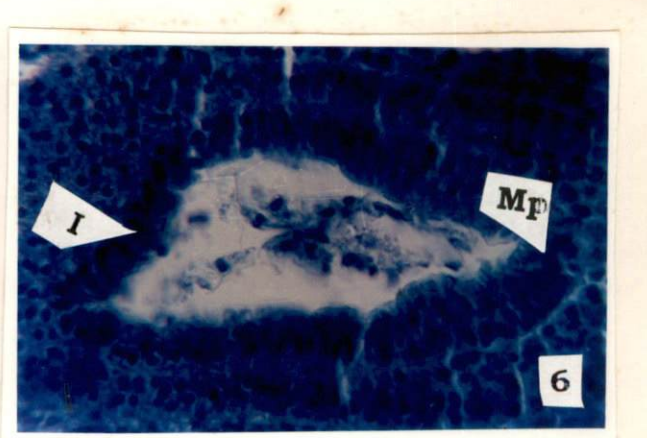

I
MF
6

PLATE 8

Longitudinal sections of ovules tested with mercuric bromophenol blue to localize proteins

8.1 Ovule with young embryo sac nucellus and integuments with intense protein x 400

8.2 Synergids x 400

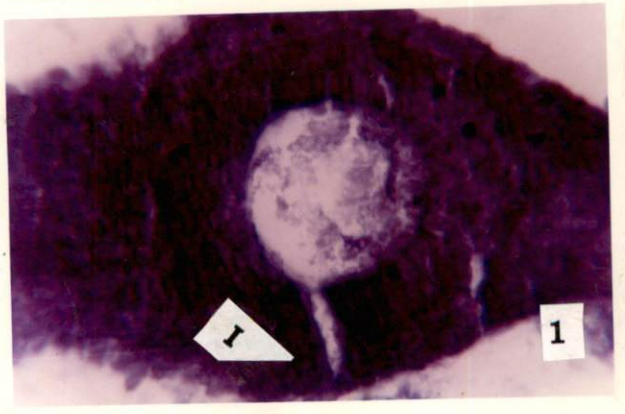
8.3 Cellular endosperm and antipodals x 400

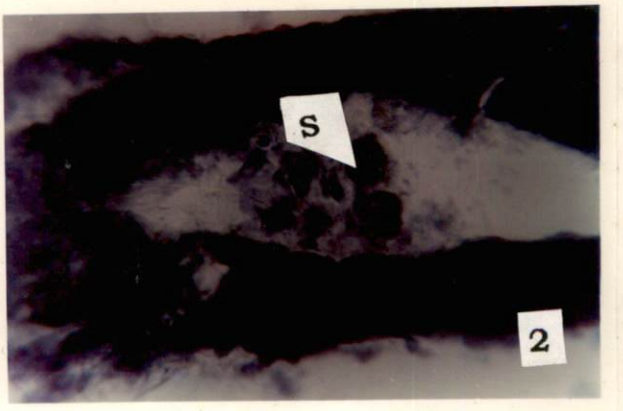
8.4 Zygote x 400

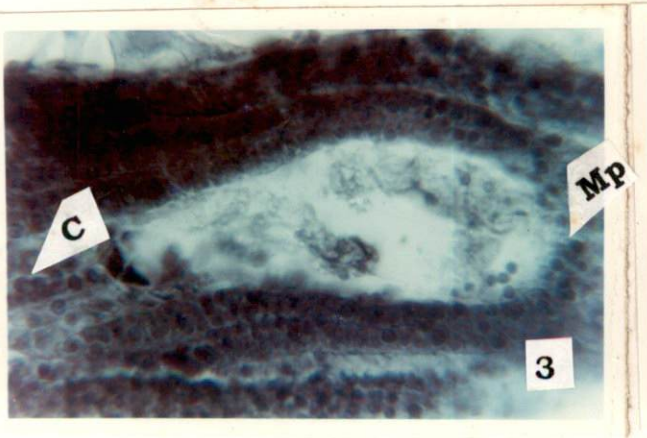
8.5 Two embryos at the Micropylar end x 400

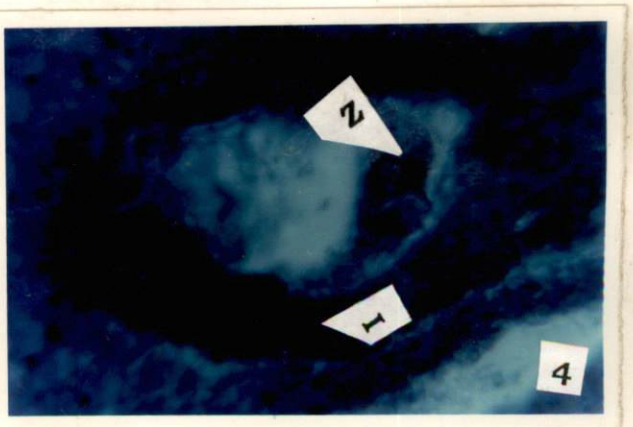
8.6 Embryo surrounded by endosperm cells x 400

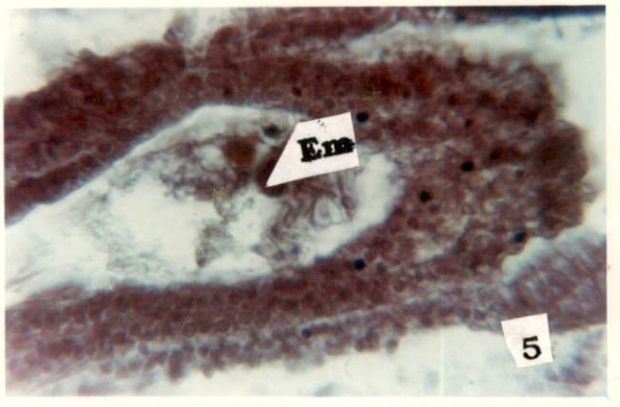
C – Chalazal end
Mp – Micropylar end
E – Egg
S – Synergids
P - Polar nucleus
Z - Zygote
I – Integument
Em – Embryo
En – Endosperm

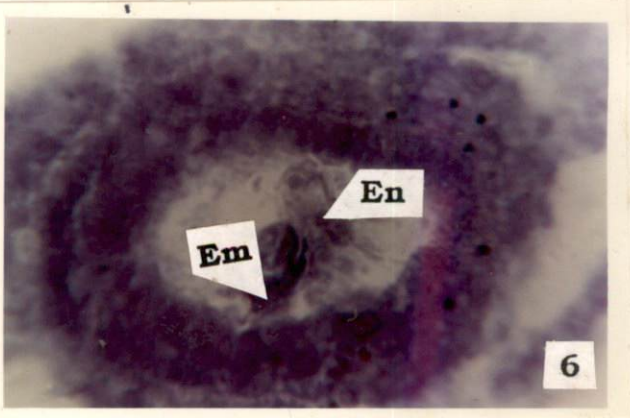

I
1


S
2


C
Mp
3


Z
I
4


Em
5


En
Em
6

The two nucleate stage embryosacs were found to be rich in RNA for egg cell and integuments, while the nucellus was poor in RNA (Plate 7.1). In the organized embryo sac, antipodals, integuments and hypostase were rich in RNA. Polar nuclei was poor, while synergids were intense in staining for RNA (Plate 7.3, 7.4, 7.5).

Nucellus stained poorly for proteins but synergids, antipodals, integuments and hypostase were rich in proteins (Plates 8.1, 8.2, 8.3). Starch accumulation was also noticed in organized embryo sac in the form of starch globules (Plate 6.1).

Endosperm was found to be rich in all the three macromolecular substances, and embryo was rich in insoluble polysaccharides and proteins (Plates 6.5, 7.6, 8.3).

DISCUSSION

V. DISCUSSION

The present investigations were carried out to study the floral biology, storage life of pollen, histology and histochemistry of anther and ovule development in *A. andreanum* cv. 'Crinkle Red'.

5.1 Floral biology

5.1.1 Flower development

The tiny bisexual flowers were arranged closely on the spadix and number of flower per cm^2 was about 26.8. There are no reports available in anthuriums however Savitha(1995) reported 13.80 flowers in *Spathiphyllum wallisii,* another member of 'Araceae'. The growth rate pertaining to either vegetative or reproductive growth is very slow in anthurium. From the stage of visible initiation inflorescence took 76.26 days to reach the female phase (stigmatic receptivity) and it continued for another 5.73 days. Anther dehiscence was observed starting from 89.33 days after visible initiation and continued upto 95.66 days. Fertilized inflorescence turned green after 181.66 days of visible initiation and berries ripened after 308.26 days of visible initiation. The number of berries in artificially pollinated inflorescence were 19 in number.

Berries took 232 days for ripening from the date of anthesis, which is in agreement with the findings of Swaminathan (1986), who reported it as, 180-200 days from the date of pollination in *Anthurium andreanum.* However, the little variability might be due to the climatic and varietal influences.

Savitha (1995) reported that, spathiphyllum inflorescence took 30•50 days for stigmas to became receptive and anther dehiscence was

around 35.57 days. Inflorescence turned green after 88.0 days and ripening of berries occurred after 155 days of visible initiation.

5.1.2 Stigmatic receptivity

The average number of days required for stigmas to become receptive was 76.26 from date of visible initiation and it continued for 5.73 days for the whole spadix. A similar observation was made by Croat (1980) in different species of *Anthurium*. He reported that, the pistillate phase was expressed by either glistening of the stigmatic surface or presence of stigmatic droplets. It lasted for 3days in *A. fragratissimum*, 2-3 days in *A. schlechtendalii* and 3-5 days in *A. binervia*. He also reported that this protracted phase of stigmatic receptivity can facilitate the hybridization programme which involves this as one of the parents.

Individual flowers starting from the base became receptive by 6.00 a.m. and receptivity lasts upto 3.36 p.m. Savitha (1995) observed in *S. wallisii* that receptivity coincides with time of anthesis. Protogyny was also observed. There was a gap of 5 days between pistillate and male phase. This effectively prevents self pollination and favours cross pollination. This was also observed by Croat(1980), in many *Anthurium* species, with a range of 0 to 5 days.

5.1.3 Anther dehiscence

The peak anther dehiscence was observed during 7.00 a.m. (52.94% of flowers), where as Mercy and Dale (1994) reported 8-10 a.m. as the peak period of anthesis and anther dehiscence. This variation might be due to influence of climatic and varietal characters. Male phase occurred 89.33 days after visible initiation and remained as such upto 95.66 days. Anthers started to dehisce from basal part of the

inflorescences and progressed upwards. A similar pattern of anther dehiscence was observed by Croat (1980) in different species of *Anthurium.*

5.1.4 Pollination and fruit set

Artificially pollinated inflorescence showed fruit set of 19 berries, but in *Anthurium,* a well fertilized inflorescence is reported to give 100-200 fruits (Mercy and Dale, 1994). This may be due to the fact that pollen grains were collected from distant places and some times used after storing in refrigerated conditions. So this might have reduced the number of berries.

5.2 Storage of pollen

The different temperature treatments in storage of pollen showed a significant reduction in pollen viability at weekly intervals, except between 1st and 2nd weeks at 0°C. The treatments consisting of low temperatures 0°C and 4°C showed a significant effect on viability as compared to the ambient storage conditions. Khosh-Khui *et al.* (1976) observed a similar results in different species of rose wherein pollen grains stored at 0°C showed longest survivability compared to 25°C.

The viability (91.51%) of fresh pollen was reduced to 8.73 per cent by 8 weeks of storage in 0°C. The pollen grains stored at 4°C turned nonviable by 7th week, while those stored at room temperature became non viable just in a week. These results indicate clearly that the higher temperatures were detrimental to the pollen viability, in 'Crinkle Red'. However, in case of anthurium there are no previous reports available on pollen storage. The deleterious effects of high temperature on pollen viability has been reported in another aroid *Spathiphyllum floribundum,* in which the pollen grains became

nonviable by 4th week of storage at 25°C and 90 per cent relative humidity. However, the pollen grains remained viable upto 24 weeks in 7°C and 90 per cent relative humidity (Henny, 1978b).

5.3 Histological and Histochemical studies

The cell is the basic unit of the living organism. So growth and differentiation of any tissue or organ can be traced to the physiological and biochemical changes at the cellular level. The study of histological and histochemical changes in a structure helps in understanding these phenomena.

5.3.1 Histological and Histochemical changes during microsporogenesis and male gametophyte development

Anther primordium consisted of differentiated archesporial cells at four corners in the hypodermal layers. These cells divided periclinally and gave rise to primary parietal cells and primary sporogenous cells. The former by repeated anticlinal and periclinal divisions gave rise to three outer most layer of cells. Anthers had four microsporangia, connected by connective tissue and transversed by vascular stands. A similar developmental process was reported in *Theriophonum minutum* (Parameswaran, 1959), *Arisaema wallichianum* (Maheswari and Khanna, 1956) and *Spathiphyllum wallisii* (Savitha, 1995).

The pollen mother cells differentiated from sporogenous tissue and innermost wall layers gave rise to thick cytoplasmic tapetal cells which formed the 'Tapetum' and all the cells of Tapetum were uniform in size, but rich in cytoplasmic contents and bigger in size and hence they were conspicuous. By meiotic divisions, PMC's became tetrads which are either isobilateral or tetrahedral tetrads. The tetrads in *Arisaema wallichianum*, an aroid was decussate, isobilateral and tetrahedral in

arrangement(Maheswari and Kahnna, 1956). The tapetum reached its maximum thickness during tetrad stage, this was in agreement with Savitha (1995) in *Spathiphyllum wallissi.*

The tapetum was of amoeboid or plasmodial type. Further during microspores stage, the tangential and radial walls of tapetal cell began disintegrating and protoplasm diffused into anther locule forming "Periplasmodium", reported in other aroids like, *Dieffenbachia senguine* (Campbell, 1900), *Symplocarpus foetidus*, *Peltandra undulata* (Duggar, 1900), *Aglaonema versicular* (Gow, 1980), *Peltandra virginica* (Gold berg, 1941), *Typhonium trilobatum* (Banerji, 1947), *Arisaema wallichianum* (Maheswari and Khanna, 1956, *Theriophonum minutum* (Parameswaran, 1959) and *Spathiphyllum wallisii* (Savitha 1995).

At the pollen grain stage, there was a marked increase in thickness of endothecium, accompanied by disappearance of tapetal contents, indicating the possibility of their utilization in the developmental process. (Savitha, 1995).

The sporogenous cells at early stages of differentiation were poor in polysaccharides, similar to the findings of Panchaksharappa and Joshi (1974) in *I. pallida,* Aswath *et al.*(1989) in *P. venusta* and Savitha (1995) in *S. wallisi,* while these cells were PAS positive in mango (Anitha Karun 1989). The sporogenous cells were rich in RNA content, confirms with the findings of Taylor (1959) in *L. longifolium,* Panchaksharappa and Joshi (1974) in *I. Pallida,* Wang *et al.* (1992) in *L. longifolium,* while the contrary reports were made by Aswath *et al.* (1989) in *P. venusta* and Savitha (1995) in *S. wallisii.* The sporogenous tissue was found to be rich in protein content, which is in agreement with Taylor (1959) in *L. longifolium,* intense in *Ipomoea horsefalliae* (Roopashri *et al.*, 1992). But Aswath *et al.* (1989) and Savitha (1995) reported poor protein content in *P. venusta* and *S. wallisii,* respectively.

At PMC stage, callose deposited around the cells separating the sister meiocytes. The tapetum became prominent due to its dense cytoplasm as well as increased size. The PMC's were rich in insoluble polysaccharides, RNA content and intense for proteins. A similar finding was reported by Aswath *et al.* (1989) in *Tecoma jasminoides* and *Arabidaea magnifica,* where as Savitha (1995) reported, that in *S. wallisii,* the PMC's were poor in insoluble polysaccharides, RNA and proteins. The tapetal cells showed intense macromolecular accumulation indicating the high metabolic activity. Similar reports of protein accumulation were given by Taylor (1959) in *L. longifolium,* Moss and Heslop-Harrison (1967) in maize. The tapetal cells were rich in all the macromolecular substances in *S. wallisii* reported by Savitha (1995), which is not in confirmation with the present study.

PMC's upon meiotic divisions I and II formed isobilateral or tetrahedral tetrads. The tapetal thickness increased further and reached its maximum. At this stage, the tapetum was rich in polysaccharides and protein while intense for RNA. Aswath *et al.*(1989), reported rich polysaccharides, intense proteins and RNA content in tapetal cells of *Arabidaea magnifica.* The tapetal cells of *Spathiphyllum wallsii* were intense for RNA, rich for protein and polysaccharides, this is in confirmation with the present study. The tetrads at this stage had intense polysaccharide, rich RNA and protein contents.

Upon disappearance of callose, the microspores released from the tetrads and tapetal contents diffused into anther locule forming periplasmodium. The microspores at this stage were rich in polysaccharides and proteins but were intense for RNA. Moss and Heslop - Harrison (1967) observed the protein accumulation in the developing spores, similar to the present case. On the contrary, Wang

et al. (1992) reported a reduction in protein content of microspores in *Lilium longifolium.* A similar finding was observed by Savitha (1995) who reported, that all the three macromolecular substances were rich in microspores in an aroid *S. wallisii.*

The microspores differentiated into pollen grains by the development of pollen wall. Before anther dehiscence, the pollen grains were intense for polysaccharides and RNA, but were poor for protein, while the endothecium showing good development was rich in polysaccharides, RNA and protein but in *Pennisetum typhoideum,* pollen was rich in starch, protein, lipids and polysaccharides (Panchaksharappa and Rudramuniappa(1972) in contrary to the present work.

Panchaksharappa and Joshi (1974) gave a similar report of PAS positive pollen grains in *I. pallida,* but these pollen grains were rich in protein, which is not in agreement with the present work. They also reported fibrous thickenings of endothecium. Grayum (1985) reported that *Anthurium* pollens were thoroughly starchless but in the present case it is reverse. A similar report of poor proteins was reported by Savitha (1995) in the developing pollen grains of *S. wallisii,* but both RNA and protein were intense in staining, while the endothecium was rich in polysaccharides, but poor in RNA and protein

5.3.2 Histochemical changes during megasporogenesis and female gametophyte development

In 2-nucleate embryo sac the egg cell was rich in RNA, while the nucellus was poor. Rich RNA content was also reported in *Dipcadi montanum* (Panchaksharappa and Joshi, 1975).

In the organized embryo sac, egg cell was poor in insoluble polysaccharides. This is in conformity with the findings of Panchaksharappa and Joshi (1975) in *D. montanum*, in which the egg cell had poor polysaccharide and RNA and intense protein. But a contrary report of rich polysaccharide was reported in Vanda (Alvarez and Sagawa. 1965).

The synergids were rich in insoluble polysaccharide and proteins but were intense in RNA. A contradictory report of poor RNA was reported in *D. montanum* (Panchaksharappa and Joshi, 1975), but rich in polysaccharides and protein. Savitha (1995) reported that synergids of *S. wallisii* were low in insoluble polysaccharide, intense in RNA and rich in proteins. The antipodals were rich in all the three macromolecules. Similar result of rich polysaccharides and proteins was reported by Panchaksharappa and Joshi (1975) in *D. montanum*, but synergids were poor in RNA. In contrary to this Savitha (1995) observed intense protein in *S. wallisii*.

The endosperm was found to be rich in insoluble polysaccharides, RNA and protein. Embryos were also found to be rich in insoluble polysaccharides and intense protein. Endosperm was of cellular type. A similar report of embryo being rich in all the macromolecules was observed in *S. wallisii* on the contrary, endosperm was found to be poor in polysaccharides, proteins and RNA (Savitha, 1995).

SUMMARY

VI SUMMARY

The floral biology, pollen storage, histological and histochemical changes in the developing anther and ovule of *Anthurium andreanum* cv 'Crinkle Red' were studied in the present investigation. The salient findings of these studies are as follows.

Floral Biology

The *Anthurium* cv,'Crinkle Red' took on an average 69.33 days for complete opening. Further 6.93 days were required for stigmatic receptivity from the date of unfurling of spathe. The pistillate phase lasted for 6.34 days on an average. After a gap of 5.73 days after stigmatic receptivity, anthers started to dehisce which continued for 4.33 days on an average in an inflorescence. The stigmas were receptive from 6.00 a.m. to 3.36 p.m. as evidenced by the presence of stigmatic droplets. Berries took 232 days to mature from the date of pollination. The peak period of anther dehiscence was 7.00 a.m.

Pollen storage

The fresh pollen had 90.33 per cent viability and showed a maximum survivability of 8.73 per cent even after 8 weeks of storage at 0^{0}C, while they turned nonviable by 7^{th} and 1^{st} week of storage period at 4^{0}C and room temperature conditions, respectively.

Histology and Histochemistry

Anther

The sporogenous cells were poor in insoluble polysaccharides, but rich in RNA and proteins. PMC's were rich in insoluble polysaccharides and RNA but intense in protein. Tapetum was stained intense in

protein, RNA and insoluble polysaccharides. The tapetum was amoeboid in type. The tetrads were isobilateral or tetrahedral. Tetrads were intense in polyaccharides, but rich in RNA and protein which is an indication of high rate of metabolism.

The microspores were released from tetrads upon dissolution of callose. Microspores were rich in insoluble polysaccharides, proteins but intense for RNA. At this stage tapetal contents diffused into anther locule forming "Periplasmodium". Pollen grains were poor in protein but had intense RNA and insoluble polysaccharides.

Ovule

The ovules were anatropous and bitegmic. In the organized embryosac, egg cell were poor in insoluble polysaccharides. Synergids were rich in insoluble polysaccharides and protein, but intense for RNA indicating high rate of metabolic activity in them. Antipodals were rich in all the three macromolecular substances.

The embryo had rich insoluble polysaccharides and proteins while endosperm was rich in all the three macromolecular substances.

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* Original not seen