CHROMOSOME BEHAVIOUR AND POLLEN ANALYSIS IN *Anthurium sp.*

BY

BINDU.M. R.

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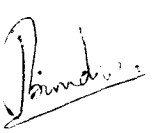
DECLARATION

I hereby declare that this thesis entitled **Chromosome behaviour and pollen analysis in *Anthurium sp.***, is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for award to me of any degree, diploma, associateship, fellowship or other similar title, of any other similar title, of any other University or Society.



BINDU. M.R.

Vellayani,
14-12-1992.

CERTIFICATE

Certified that this thesis, entitled **"Chromosome behaviour and pollen analysis in *Anthurium sp.*** is a record of research work done independantly by Kum. BINDU. M.R., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

(Dr.S.T. MERCY)

Chairman
Advisory Committee,
Professor of Botany,
College of Agriculture,
Vellayani

Vellayani,
12 '92.

APPROVED BY

CHAIRMAN

Dr. S.T. Mercy

MEMBERS

1. Dr. R. Gopimony

2. Dr. D. Chandramony

3. Dr. S. Ramachandran Nair

EXTERNAL EXAMINER :

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BINDU. M.R.

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INTRODUCTION

INTRODUCTION

The Genus Anthurium with over 700 species (Sheffer and Croat 1983) is the largest genus of the family Araceae. It is the most morphologically diverse and taxonomically complex genus of the family and comprises about one third of its total number of species (Croat, 1980). Anthurium was placed under a seperate tribe(Tribe 4) Anthuriae by Hutchinson (1934)

The most popular and economically important species of the genus are A. andreanum Lind. and A. scherzerianum Schott. Both of them possess attractive long lasting inflorescences. A andreanum is known as 'palette flower' and A scherzerianum as 'flamingo flower'. Both are having spectacular blossoms and attractive leaves. Foliage type anthuriums come under 'tail flower' group and have handsome glossy foliage and inconspicuous spadices.

A. andreanum, an important cut flower crop is a native of Columbia (Geier, 1989). The plant required high humidity and is not easy to grow but is well worth any special attention because of its magnificent 'flowers'. The name 'Anthurium' means 'tail flower' in Greek. The varieties with the unusual and attractive foliage were the first ones known and their blossoms are little more than a bit of a tail like

structures on a tall stem and hence the name.

A. andreanum and its hybrids are the best known of the anthuriums. Their fantastically beautiful 'flowers' look as if they are made from fine and glossy leather. They persist in perfection for weeks and a plant is rarely without a 'flower', for new 'flowers' develop as the old ones fade and the plant flowers all the year round.

The diversity and complexity of the genus Anthurium is evident both cytologically and morphologically. Cytological study is a valuable tool for systematic analysis.(Sheffer, Threobald and Kamemoto, 1980). Chromosome numbers for 95 different Anthurium species have been reported, which constitutes about 15% of the known species(Sheffer and Croat,1983). B chromosomes were first reported by Pfitzer(1957 b) and later confirmed by other workers.

The present work is taken up with a view to analyse the chromosome number, their behaviour during mitosis and meiosis anthesis pollen production and pollen viability in 5 different commercially important varieties of Anthurium andreanum in order to generate necessary basic information to initiate further genetic and hybridisation studies in this important commercial crop.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The importance of karyotype analysis and pollen studies in distinguishing species is well known. The role of alteration of chromosome morphology in speciation and in determining interrelationships between species varieties and even strains has been reported earlier by Sharma and Sharma (1959). It has also been reported that the different members of a species often show similarity in gross chromosome morphology, but differ from each other in small details like the centromeric position, secondary constriction, number and size of satellites and variation in total chromatin length. (Sharma and Mallick ,1965). However we cannot estimate either the amount or the directional trend of evolution by studying only the external visible characteristics of the chromosomes. Nevertheless, comparison of chromosomes of related species has in many instances revealed certain regular differences, which are often correlated with trends of specialisation in the external morphology of the plant.

The study of pollen morphology as an effective aid to plant taxonomy and phylogeny has been amply demonstrated by several workers (Erguson and Muller, 1976 ; Nair, 1979).

Exhaustive literature on karyotype analysis and pollen studies is available in various plant species. Anthurium is

one of the most cytologically analysed genus of the family Araceae. (Darlington and Wylie, 1955; Federov, 1969). However work on pollen an alysis of the genus is considerably limited.

A. CYTOLOGICAL STUDIES

1. Mitosis

a. Chromosome number

The chromosome numbers of individuals of a particular species will be same because species are reasonably constant biological entities and this stability is determined by a constancy in the numbers and kinds of genes and chromosomes (Swanson, 1968). The chromosome numbers of different species of the same genus as well as different varieties of the same species may be different. The chromosome numbers reported for some members of the genus Anthurium illustrates this point (See Table 1).

Table 1. Chromosome numbers in some species of Anthurium

Species	Chromosome Number (2n)	Authority
A. angustispadix	30	Sheffer and Croat, 1983.
A. andreanum	Ca 30	Gaiser, 1927
	30	Sheffer and Kamemoto, 1976
	30	Sheffer and Croat, 1983.
	32	Haase-Bessell, 1928

	32	Tsuchiya and Takada, 1962
	30 30+2B	Kaneko and Kamemoto, 1978
A. andreanum		
var. 'roseum'	30+0 -2B	Lalithambika, 1978
	30+1B	Satyadas, 1985
A. andreanum	30+0-2B	Sharma and Bhattacharya, 1966
var. 'rhodochlorum'	30	Satyadas, 1985
A. bakeri	Ca .30	Gaiser, 1927
	30	Sheffer and Croat, 1983
	28+1f	Sharma and Bhattacharya, 1966
	30+2B	Satyadas, 1985
A. bellum	56	Mookerjea, 1955
	28	Bhattacharya, 1977
A. circinatum	30	Sheffer and Croat, 1983
A. crassinervium	60	Sheffer and Croat, 1983
	30+2B	Lalithambika, 1978
A. crystallinum	Ca. 30	Gaiser, 1927
	30 30+2B	Sheffer and Croat, 1983
	40+0 -2B	Lalithambika, 1978
	45+0 -4B	Satyadas, 1985
A. digitatum	Ca.60	Gaiser, 1927
	30	Sheffer and Kamemoto, 1976
A. forgetti	30+0-2B	Pfitzer, 1957a
A.gracile	20 40	Sheffer and Croat, 1983
A. glaziovii	34	Mookerjea, 1955
A. grande	30	Sharma and Bhattacharya, 1966

A. harrisii	28+2B	Bhattacharya, 1977
A. hookeri	Ca.30	Gaiser, 1927
A. imperiale	30+1f	Marchant, 1973
A. lucidum	Ca. 124	Marchant, 1973
A. maximum	Ca. 30	Gaiser, 1927
A. magnificum	Ca. 30	Gaiser, 1927
	32	Haase - Bessell, 1928
	30+0-2B	Pfitzer, 1957 a
A. ornatum	30+0-2B	Gaiser, 1927
	30+0-2B	Satyadas, 1985.
A. solitarium	34	Mookerjea, 1955
	30+0-2f	Sharma and Bhattacharyya 1961
A. standleyi	60	Sheffer and Croat, 1983
A. splendidum	30+2B	Sheffer and Kamemoto, 1976
	30+4B	Bhattacharya, 1977.
A. scherzerianum	30	Sheffer and Croat, 1983
	32	Tsuchiya and Takada, 1962
A. subsignatum	Ca.30	Gaiser, 1927
	30	Sheffer and Kamemoto, 1976
	30	Lalithambika, 1978
	30	Satyadas, 1985.
A. scandens	24 48 84	Sheffer and Kamemoto, 1976
A. trinerve	24 30	Sheffer and Kamemoto, 1976
A. upalaense	30	Sheffer and Croat, 1983
A. umbrosum	30	Sheffer and Croat, 1983

A. vallense	30	Sheffer and Croat, 1983
A. veitchii	30	Sheffer and Croat, 1983
A. warocqueanum	30	Sheffer and Croat, 1983
	30+3B	Sheffer and Kamemoto, 1976
	30+2B	Stayadas, 1985
A. pentaphyllum	Ca.30	Gaiser, 1927
var. bombacifolium	60	Sheffer and Croat, 1983
Var. pentaphyllum	60	Sheffer and Croat, 1983

The somatic chromosome number of Anthurium vary from 20 (A. gracile) to 124 (A. lucidum). However, the most common number is 30 (Sheffer and Kamemoto, 1976). The majority of the counts were reported by Gaiser (1927) Pfitzer (1957), Marchant (1973) Sheffer and Kamemoto (1976) and Sheffer and Croat (1983). The presence of B chromosomes was first reported by Pfitzer (1957 c).

Chromosome numbers for the species A. andreanum were reported as 2n = 30 by Gaiser (1927), Kurakubo(1940), Itô (1942), Sharma and Bhattacharaya (1966), Sheffer and Kamemoto (1976),Sheffer and Croat (1983), Satyadas(1985), Sengupta and Chettri (1989). A somatic chromosome number of 2n = 32 was reported by Haase-Bessell (1928) and Tsuchiya and Takada (1962). Presence of 0 to 2B chromosomes was first reported by Sharma and Bhattacharya (1964) in the variety 'rhodochlorum' and Lalithambika (1978) in the variety 'roseum'. Kaneko and Kamemoto (1976) reported two B chromosomes in 'Uniwai' variety of A. andreanum and Satyadas(1985) reported one B chromosome in the variety 'roseum'.

b. B chromosomes

B chromosomes, have been reported in numerous plant taxa by Jones and Rees (1982). The first report of occurence of B chromosomes, in plants was in Zea mays by Longley (1927). The occurence of B chromosomes has been reported in some species of Anthurium such as A.andreanum (Sharma and Bhattacharyya 1961; Kaneko and Kamemoto 1978), A.crystallinum (Pfitzer, 1957 a,b ; Lalithambika 1978; Satyadas, 1985), A.forgetti (Pfitzer, 1957 a,b), A.glaziovii (Sharma and Bhattacharyya , 1961), A magnificum (Pfitzer,1957 a,b) and A.scherzerianum (Tsuchiya and Takada, 1962).

The size of the B chromosomes was highly variable, being very small in A. warocqueanum and much larger in the other species (Sheffer and Croat, 1983). Pfitzer (1957 a) reported two similar rod like B chromosomes with distinct constrictions in A. crystallinum. Sharma and Bhattacharya (1961) reported that the B chromosomes of A. andreanum var. 'roseum' are spherical accessory fragments and have no distinct constrictions marking the position of the centromere. Battaglia (1964) has suggested that B chromosomes in some Anthurium species are telocentric. Keneko and Kamemoto (1979) had reported that the B chromosomes of A. warocqueanum have submedian primary constrictions and are about half the size of the smallest A chromosomes Sheffer and Croat (1983) reported that the number of B chromosomes in the same species of Anthurium was variable, ranging from zero to four in A. crystallinum.

c.Karyotype analysis

Chromosome shape is studied at the somatic metaphase or anaphase at which the chromosomes reach their maximum contraction (Swanson, 1968). Most species of plants and animals possess a definite individuality in their somatic chromosomes which is evident in their size, shape, position of primary constriction or centromere and additional features like secondary constrictions and satellites. Furthermore closely related species are usually similar in these respects.

and distantly related ones are often recognizably different. These facts led to the early formulation of the concept of karyotype (Delaunay, 1926). Karyotype is the morphological aspect of the chromosome complement as seen at mitotic metaphase(Stebbins, 1971). The principal ways in which the karyotypes differ from each other are.

1. Basic chromosome number.
2. Form and relative size of different chromosomes of the same set.
3. Number and size of satellites and secondary constriction.
4. Absolute size of chromosomes.
5. Distribution of material with different staining properties.

(Levitzki, 1931; Heitz, 1932; Darlington 1937).

Karyological studies of the different species of the family Araceae considerably help in tracing the phylogenic relationship(Sharma and Das 1954; Mookerjea, 1955). Though the family Araceae consists of a large number of genera only a few of them have so far been karyotypically analysed and Anthurium is one of them. (Darlington and Wylie 1955; Federov, 1969).

Keneko and Kamemoto (1978) analysed the karyotype of A. andreanum var.' Uniwai' and found a chromosome number of 2n =

30 + 2B. Four chromosomes were large metacentric, two were fairly large with satellites and the rest smaller sized chromosomes.

Lalithambika (1978) had done the karyotype analysis of A. crystallinum, A. crassinervium and A. subsignatum and found that the somatic chromosome number of A. crystallinum was 45 + 0 to 2B indicating autotriploidy. The chromosome length ranged from 3 to 6.11μ. Karyotype formula was 6M + 24m + 15sm (See Table 4) for the species. B chromosomes had a length of 1.16μ.

Analysis of the karyotype of A.crassinervium revealed that the somatic chromosome number was 2n = 30+2B. Length of chromosomes ranged from 2.6 to 5.8μ. and the length of B chromosomes was 1.0μ. The koryotype formula was 4M + 14m + 10sm + 2st.

Karyotype analysis of A.subsignatum showed that the chromosome number was 2n = 30 without any B chromosomes. The chromosome length ranged from 1.73 to 3.40μ. The karyotype formula was 6M + 20m + 4sm.

Karyotype analysis of Anthurium warocqueanum (Kaneko and Kamemoto, 1979) revealed that the somatic chromosome number was 2n = 30 + 3B with 2 pairs of large chromosomes, 1 pair of

satellite chromosomes and 12 pairs of medium to small chromosomes. Chromosome length ranged from 2.8 to 6.0μ. The B chromosomes were about half the size of the smallest A chromosome ie 1.5μ. But Satyadas (1985) had reported the somatic chromosome number as 2n = 30 + 2B for the same species. According to her, the length of the chromosomes ranged from 3.5 to 7.66μ, while the two B chromosomes had an identical length of 1u. The karyotype formula was 1M + 4m + 8sm + 2 st and the species was put in the karyotype category '3B' (See Table 5).

Satyadas (1985) confirmed the chromosome number reported by Lalithambika (1978) for A.crystallinum and reported that the length ranged from 3.0 to 8.0μ. The karyotype formula for the species was 2M+7m+11sm+1st and three unpaired chromosomes with submedian centromeres. The TF% was 38.87.

According to Satyadas (1985), the chromosome number of A.digitatum was 2n = 30+0 to 4B. The absolute length of chromosomes ranged from 7.0 to15.6μ and the total length of the chromosomes of the genome complement was 129.69μ. The TF% was reported as 28.98 and it was placed in the karyotype category of '3B'. The karyotype formula for the genome worked out was 2m + 9sm +3st+1t. The length of the first pair of B chromosomes was 1.3μ and that of the second pair was 1.1μ.

The somatic chromosome number for A. subsignatum was

reported by Satyadas (1985) as 2n = 30. The absolute chromosome length ranged from 2.17 to 6.34μ. The total chromosome length of the genomic complement was 62.10μ. The TF% was 40.6 and she placed the species in the karyotype category of '2B . The idiogram formula was 3M + 8m + 3sm + 1st.

The somatic chromosome number of A.ornatum was 2n = 30+0 to 2B (Satyadas, 1985). Chromosome length ranged from 3.34 to 10.5μ and the total length of the chromosomes of the genomic complement was 85.28μ. The idiogram formula for the genome was 2M + 4m + 5sm + 4st. The TF% was 38.11 and the karyotype category was '2B'. Average length of B chromosomes was 1.00μ.

Satyadas (1985) reported the somatic chromosome number of A.bakeri as 2n = 30+2B. Absolute chromosome length ranged from 2.00 to 7.00μ and the average length of B chromosomes was 0.83μ. Total length of the chromosomes of the genomic complement was 65.19μ and the TF% was 39:12. The species was placed in the karyotype category '2B'. Idiogram formula was workedout as 1M + 8m + 6sm for the genome.

The karyotype analysis of two varieties of A.andreanum viz 'roseum' and 'rhodochlorum' revealed that both of them belong to the karyotype category '3B' (Satyadas, 1985). But the chromosome number for 'roseum' was 30+1B while that of

'rhodochlorum' was 30 without any B chromosomes. Total length of the chromosomes of the genomic complement was 51.48μ and 70.2μ respectively. The absolute length of chromosomes for 'roseum' ranged from 2.90 to 8.33μ and that for 'rhodochlorum' ranged from 2.33 to 6.00μ. The TF% was 31.13 and 36.23 respectively. The idiogram formula was 2M + 1m + 11sm + 1st and 5m + 10sm respectively.

Sengupta and Chettri (1989) reported the somatic chromosome number for A. andreanum as 2n = 30 with 4 fairly large, 22 medium sized and 4 small chromosomes. No B chromosomes were reported.

Mitotic abnormalities like presence of bridges in some species of Anthurium (Lalithambika, 1978) numerical variation in chromosome number in the somatic cells of A.crystallinum presence of two nuclei in A.andreanum and achromatic lesions in the somatic cells of A.warocqueanum (Satyadas, 1985) were also reported.

2. Meiosis

Meiotic behaviour of chromosomes in various species of Anthurium had already been studied (Lalithambika, 1978; Kaneko and Kamemoto, 1978; Marutani and Kamemoto, 1983).

Keneko and Kamemoto (1978) reported that in A. andreanum var. Kaumana bivalent pairing ranged from 15 to 8. The most common configuration was $11_{11} + 8_1$ and the mean was $11.7_{11} + 6.6_1$. The bivalents at metaphase I were either ring or rod shaped. Laggards were also reported. The maximum number of micronuclei reported was 4. But in the variety Uniwai, the chromosome pairs observed ranged from 15 to 10. The mode was 12_{11} and 11_{11} and the mean was 12.1_{11}. Lagging chromosomes and micronuclei were also encountered. The B chromosomes were paired or unpaired.

Marutani and Kamemoto (1983) reported that the three B chrosomes present in A. warocqueanum showed three different configurations -- one trivalent; one bivalent and one univalent or three univalents. Univalent B chromosomes showed a tendency to lag behind in anaphase I. Micronuclei were also observed. About 6% of pollen mother cells showed 14 bivalents + 2 univalents and 1% showed 13 bivalents + 4 univalents.

Lalithambika (1978) reported different types of meiotic abonormalities like presence of univalents, laggards micronuclei, varying numbers of bivalents, unequal seperation of chromosomes during anaphasic movement, stickiness of chromosomes etc. in some species of Anthurium.

3. Mitotic index

A high mitotic index in a system does not mean that the division is rapid, but merely indicates that mitosis occupies a large proportion of the total cell cycle (Dyer, 1979). Dyer (1979) recorded the mitotic index of Hyacinthus orientalis as shown in the following table.

Table 2. Mitotic index of Hyacinthus orientalis.

Stage	All	Inter-phase	Prop-hase	Meta-phase	Ana-phase	Telo-phase
Frequency	10461	9077	563	300	131	390
Mitotic index	100	86.8	5.4	2.9	1.2	3.7
Duration assuming mean value for whole cycle=20 hrs.	20	17.36	1.08	0.58	0.24	0.74

Selvanathan and Khanna (1990) studied the mitotic index of four cultivars of rice and reported a very low mitotic index of 3.66-4.00%.

Geier (1988) reported a very low mitotic index for callus cultures of Anthurium scherzerianum.

B. POLLEN STUDIES

Pollen grains in many instances provide many additional characteristics for determining relationship (Wodehouse, 1935). In some instances they may provide additional evidence for phylogenic trends as in some primitive genera of the Family Compositae Tribe Cichoreae (S tebbins, 1940 b).

1. Time of flower opening and anthesis

Modes of flowering behaviour probably have a direct influence on pollination biology and evolution (Croat, 1980). The process of emergence of anthers, their dehiscence and distribution of pollen is termed anthesis. The details of anthesis vary from one crop species to another. They are also greatly affected by environmental factors such as humidity and temperature (Singh, 1990).

Croat (1980) reported that Anthurium species generally had maturation of flowers initiated from the basal portion and development proceeds regularly in the direction of the apex. The pistillate phase of flowering could be distinguished by stigmatic droplets or glistening stigmas. The duration of the pistillate phase (female phase) was quite variable ranging

from only a few hrs in A. ravenii to 21-28 days in A. luteynii Seperation period for male and female phase was several days in most species whereas in a few of them, the time lag between the production of stigmatic fluid and the emergence of stamens, was so short that it was not certain whether the species involved were homogamous or protogynous. The stamens did not develop simultaneously. Usually one or both of the lateral stamens emerge first followed by the alternate pair with the anterior stamen usually preceding the posterior one. He reported that in A. paludosum usually one or sometimes two stamens emerge per flower and each stamen remained for not more than one or two days before being fully retracted.

2. Pollen morphology - size and shape

Size as well as shape of pollen grains show a broad spectrum of variability. Pollen grain size when measured was affected both by chemical treatment (Christensen, 1946) and mounting media (Anderson, 1946). Reproducible size measurements require that determinations be made under identical conditions (Stanley and Linskens, 1974).

Mc Rae (1987) reported that the pollen grain length in lilies ranged from 0.067-0.1mm in diploids (2n = 24) and from 0.060-0.113mm in triploids (2n = 36) and from 0.090-0.5mm in tetraploids (2n = 48).

Shim et al. (1988) reported that the pollen grains of Hibiscus syriacus, H. rosa-sinensis and H. mutabilis were spherical, echinate and periporate.

Kaseko (1990) studied pollen grains of different species of Tulipa and reported that they differ in aperture type, exine sculpturing, shape and to a lesser extent in exine thickness and size.

Tarasevich (1989) reported that different sections of Anthurium were heterogeneous for pollen grain morphology and that Anthurium was unique in the family Araceae in possessing pore apertures.

3. Pollen production per flower

The yield of pollen varies with species as reported by Stanley and Linskens (1974). Pohl (1937) reported that Zea mays produce 10^7 pollen grains per plant while Vallisneria produces 72-144 pollen grains per plant. Beri and Anand (1971) compared the pollen production of 22 varieties of wheat and reported that pollen grains per anther varied from 581 to 2,153. Oberle and Goertzen (1952) reported that the variety 'Winesap' of Pyrus communis produces 400 pollen grains per anther while the variety 'Delicious' usually produces 7000

Pollen grains per anther. Gilbert and Breen (1987) reported that if 75% or more of the flowers of strawberry had at least some healthy anthers, pollen production averaged > 240,000 pollen grains/flower. About 400-500 pollen grains per anther had been reported in rice by Mercy and Zapata (1987).

4. Pollen fertility

The capacity of the pollen to germinate and grow is to be assessed. Appearance of the pollen alone, even at collection time is not always a good index of viability (Stanley and Linskens, 1974). So pollen fertility is tested either by using specific stains or by in vitro growth studies.

Mitu and Acatrinei (1974) reported that the germination of pollen grains was proportional to pollen grain stainability.

Pearson and Harney (1984) reported that there was a positive and significant correlation between pollen staining and germination in Rose.

Lalithambika (1978) reported that the pollen sterility of different species of Anthurium vary from 63% (A.cordatum) to 96.5% (A.veitchii), Satyadas (1985) had also got similar results and in her study the pollen sterility varied from 67% (A.warocqueanum) to 80% (A.ornatum). Lalithambika (1978) reported a pollen sterility of 70-75% for A.andreanum which was later confirmed by Satyadas (1985).

In most pollen viability tests, a small sample of the pollen grains is germinated and the percent of grains producing tubes after a given time is calculated. This percent is considered as an index of viability of the pollen sampled. Such tests assume that the optimum conditions have been established for the in vitro test, so that germination approximates that on the plant. (Stanley and Linskens 1974).

Rao and Chin (1973) reported that the pollen culture media sucrose and stigmatic extract were more effective in promoting pollen germination of orchid hybrids than were inorganic salts and growth substances.

Mercy et al.(1976) had reported that the best medium for pollen germination and tube growth in two species of Cicer ie., C. arietinum and C. soongaricum was a combination of 0.5M sucrose with 100 ppm boric acid. They obtained a maximum germination percentage of 91.9% and 80.1% and maximum tube length of 12.5 and 11.8 respectively after 3 hrs for the two species

Markose and Aravindakshan (1987) reported that a medium containing 20% sucrose 1% agar and 100 ppm boric acid was best for pollen germination of shoe flower.

Rana et al. (1989) compared the pollen germination in 10,15 and 20% sucrose solution for Impatiens balsamina and reported that the best concentration was 20% in which the germination percentage was 70.2%.

Parfitt and Ganeshan (1989) while comparing different procedures for estimating viability of Prunus pollen reported that hanging drop slide and agar plate germination procedures were more reliable than different staining methods.

Choudhary (1991) reported that 15% sucrose with 75 ppm boric acid was the best medium for germinating pollen grains of Gladiolus cv. Melody.

Stomata

Stomata are the small pores bordered by guard cells found in the epidermis of leaves stems and other green plant parts through which gaseous exchange occurs. (Ben Hill et al., 1960).

The average stoma size is only about 18u long and 6u wide. By actual measurement the average area of the opening in 37 kinds of cultivated plants has been found to be 92 sq.u (Ben Hill et al., 1960).

Chen et al.(1982) reported that the average stoma size of an aneuploid developed by somatic hybridization between

52.0 μx 37.6 μ while that of the parents was 35 μ x 27 μ and 35.8 μ x 29.0 μ respectively.

Moyazaki et al.(1985) reported that the stomata on the third leaf from the top were shorter in diploid (26-32 μ) than in triploid varieties of taro ie. 36-39 μ. It was concluded that stomatal length could be used to distinguish diploids from polyploids.

Chen et al.(1986) reported that the average guard cell length in a haploid japonica hybrid of rice was 20.18 μ and that of a diploid hybrid was 26.17 μ.

Sun et al.(1986) reported the stomata number and stomatal length of four cultivars of rice. The stomatal number / mm^2 varied from 405-470 and the length varied from 23.6-24.6μ.

Pant et al.(1989) had studied the stomata in four varieties of sugarcane and reported that the mean number of stomata per unit area ranged from 175.40-212.82. Stomatal frequency was considered as one of the indices of drought tolerance.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was undertaken in the Department of Agricultural Botany, College of Agriculture, Vellayani during the period 1990-'92 in order to analyse the chromosome behaviour and pollen characters of five commercial cultivars of Anthurium andreanum Linden.

MATERIALS

The material selected for the study was five varieties of Anthurium andreanum (Table 3). The plants were purchased from commercial nurseries where they were being maintained through clonal propagation by suckers. The plants were raised in pots. Pots were filled with a bottom layer of broken bricks and a middle layer of coconut husk pieces on which the plants were placed and then a top layer consisting of river sand small charcoal pieces, and powdered cowdung was poured around the base of the plants to anchor the roots and help the plants to stand erect. Care was taken to keep the potting mixture porous to allow good drainage. Plants were kept under 70-75% shade, natural humidity of 70% and a temperature ranging from 25°C to 32°C. Watering was given twice daily and limited to once in a day during rainy season.

Some characters of the varieties of Anthurium andreanum.

Table 3. The varieties and some important characters of A. andreanum

Variety	Plant size	Suckering habit	Leaf shape	Spathe colour	Spathe character
A. andreanum Var. Honeymoon Red	Large	Shyly Suckering	Large heart shaped leaf with overlapping base	Red	Large size smooth and glossy surface
A. andreanum Var. White (album)	Medium	Profusely Suckering	Large triangular	White	Medium sized, prominently veined with smooth surface.
A. andreanum Var. Lady Jane (Pink)	Small	Profusely suckering	Triangular and narrow	Dark Pink	Small sized with smooth glossy surface
A. andreanum Var. Chillired	Medium	Shyly suckering	Heart shaped narrow leaves	Dark Red	Medium sized with blistered, veined surface
A. andreanum Var. Pink	Large	Profusely suckering	Large heart shaped	Dark pink	Large sized with smooth and prominently veined surface

METHODS

A. CYTOLOGICAL STUDIES

1. Mitosis

Young actively growing root tips were collected between 11.00 AM and 12.30 PM which was found to be the peak time for division. The collected root tips were washed in water and then subjected to a pretreatment with 0.002 M 8-hydroxy quinoline for 5 hrs at 10°C. The root tips were then washed in water and fixed for 24 hrs in Carnoy's fluid(3 alcohol : 1 acetic acid : 1 chloroform to which a few drops of ferric chloride was added as mordant) at room temperature. Fixative was changed once or twice to clear the cytoplasm and the roots were then washed and stored till needed in 70% ethyl alcohol.

For squash preparation, the root tips were hydrolysed in IN HCl and kept for 5 minutes at a temperature of 60°C. Hydrolysed roots were transferred to 2% acetocarmine at 60°c for five minutes. The terminal 1mm of deeply stained portion of the root tip alone was taken for squashing.

The slides were observed under a magnification of 1000X on a Nikon microscope. Various division stages were observed and the chromosome behaviour was recorded.

For karyomorphological studies, only those cells showing well dispersed chromosomes with straight or almost straight arms were used for making measurements and drawing (Matern and Simak, 1968). Such stages were selected and photomicrographs were taken with a biological microscope (Nikon Optiphot) and Microflex UFX-II. Nikon Plan 100 x (1.25 oil immersion) objective in combination with Nikon CF PL field lens (5 X) was used. Metaphase chromosomes were measured from photographic enlargements. The chromosomes were then matched into pairs. The mean values for the long and short arms of each pair were calculated from ten photographs for each variety. The pairs were then arranged in the order of decreasing length and idiograms were drawn.

Karyotype analysis

The following parameters were worked out for all the five varieties of Anthurium andreanum under the present study.

a. Relative chromosome length (RCL)

For comparing size relative chromosome length was used which was calculated by the following formula.

$$\text{Relative chromosome length (RCL)} = \frac{\text{Individual chromosome length} \times 100}{\text{Total length of the haploid chromosome complement.}}$$

(Levan & Hsu, 1959)

b. Arm ratio

The location of the centromere can be expressed as a ratio called the arm ratio. It was calculated as,

$$\text{Arm ratio (r)} = \frac{\text{Length of long arm (l)}}{\text{Length of short arm (s)}}$$ (Levan *et al*, 1964)

Depending on the values of arm ratio, the chromosomes are classified. The chromosomes are referred as strictly median (M) when arm ratio is 1.0, median centromere (m) when arm ratio is 1-1.7, submedian centromere (sm) when arm ratio is 1.7-3, subterminal centromere (st) when arm ratio is 3-7 and terminal centromere (t) when arm ratio is 7. (Levan, Fredga and Sandberg, 1964. Table 4).

Table 4. Nomenclature of Chromosomes according to Levan, Fredga and Sandberg, 1964.

Centromeric position	Arm ratio	Symbol
Median *sensu stricto*	1.0	M
Median region	1.0-1.7	m
Submedian	1.7-3.0	sm
Subterminal	3.0-7.0	st
Terminal region	7.0	t
Terminal *sensu stricto*	∞	T

c. Centromeric index (F%)

Centromeric index was calculated by the formula.

Centromeric index (F%) = $\frac{100S}{C}$ 'S' is the length of short arm and 'C' is the total length of the chromosome(Shindo and Kamemoto, 1963).

d. TF %

TF% which is the ratio in percentage of the total sum of the short arm length to the total length of the chromosome of cell, was calculated. TF% indicates the degree of asymmetry of the karyotype. A TF% of 50 indicates that all the chromosomes have median centromere and therefore there is an absolute symmetry of the karyotype. A TF% of zero indicates that all the chromosomes have terminal centromeres so that the karyotype is completely asymmetrical.

$$TF\% = \frac{\text{Total short arm length} \times 100}{\text{Total chromosome length}}$$ (Huziwara 1962).

(e) Categorisation of karyotype asymmetry.

Karyotype asymmetry has been categorised on the basis of the scheme proposed by Stebbins (1958) in Table 5

Table 5. Categorisation of karyotype asymmetry(Stebbins, 1958)

Ratio Largest / Smallest Chromosome	Proportion of Chromosomes with arm ratio <2:1			
	0-0	0.01-0.5	0.51-0.99	1.0
< 2:1	1A	2A	3A	4A
2:1 - 4:1	1B	2B	3B	4B
> 4:1	1C	2C	3C	4C

2. Meiosis

Young spadices were fixed between 10 AM and 11 AM in 1:1 acetic - alcohol fixative to which a trace of ferric chloride was added as mordant. The fixative was changed once or twice which helped to clear the cytoplasm and afer 24 hrs of fixing, the spadices were washed and stored in 70% alcohol. The anthers were dissected out under a dissection microscope from the flower buds.

Dissected out anthers were placed on a clean slide in a drop of 2% acetocarmine. Smear preparations were made by gently tapping the anther with the end of a steel rod. The anther walls and other debris were removed. A cover glass was gently put over the smear and the slide was made

semipermanent. The prepared slides were examined under a magnification of 1000 x in a light microscope. Different types of meiotic abnormalities were recorded and photomicrographs were taken.

3. Mitotic Index

The slides prepared for mitosis were used for calculating the mitotic index. The cells at different dividing stages and the total number of cells were counted from ten fields in each slide. For all the five varieties, ten slides each were used for the observation. Mitotic index was calculated using the following formula.

$$\text{Mitotic index} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells counted}}$$

Aceto-orcein (2%) also tried in cytological studies and was found as good as acetocarmine (2%)

B. POLLEN STUDIES

1. Time of flower opening and anthesis

The date of appearance of flower buds on the leaf axil, date of spathe opening, date at which the female phase starts and ends, date of initiation as well as the end of male phase

etc. were recorded. Data from five spadices were recorded for each of the five varieties and the average for each phase was calculated.

2. Pollen morphology - size and shape

The pollen grains from freshly dehisced anthers were collected after anthesis, mounted on water and observed under a microscope. Size of fifty fertile pollen grains was recorded for each variety using an ocular micrometer and the average was computed.

3. Pollen production per flower.

Haemocytometer was used to study the pollen production per flower. For this spadices in which the female phase was over, were collected. Mature but undehisced anthers were dissected out. A suspension of pollen grains was made in 1 ml. of water from the 4 anthers of a flower for each variety. The coverglass was placed over the counting chamber of the haemocytometer and a drop of suspension was placed at the edge of the coverglass which would be drawn rapidly into the space between the coverglass and grid. Overflow was not allowed into the moat. The Pollen grains in the four corner groups and in the centre group of the haemocytometer were counted. The grains lying on the line were counted if more than 50% was inside the square. The pollen grains in all the five groups

were added up. The following formula was used to determine the number of pollen grains per flower.

Number counted x 50 = Number/mm^2 x 1000 = Number of grains/ml
= Number of pollen grains/flower.
(Nuckles and Kuc, 1990)

4. Pollen fertility

a. By acetocarmine staining method.

Pollen grains were collected during the male phase from all the five varieties and stained with 1:1 glycerine - acetocarmine(2%). Five slides were made for each variety and from each slide, ten microscopic fields were scored and the data recorded. Unstained, undersized, partially stained and shrivelled pollen grains were scored as sterile and the uniformly stained, properly filled pollen as fertile. Fertility of each variety was estimated as percentage of the number of fertile pollen grains to the total number pollen grains scored. Photomicrographs were taken in a Nikon microscope.

b. By *in vitro* pollen germination method.

Fresh pollen grains were put into a culture medium containing 0.5 M sucrose and 100 ppm boric acid in cavity slides and kept them covered in a petridish, then incubated at

room temperature for four hours. Five slides were made for each variety. The slides were examined under a light microscope to observe the pollen tube growth. The total grains in ten fields in each slide and the grains germinated were counted. Then pollen fertility was calculated using the following formula.

$$\text{Pollen fertility} = \frac{\text{Number of grains germinated} \times 100}{\text{Total number of grains}}$$

Stomata

For counting the number of stomata per unit area and measuring their size, peelings from abaxial sides of the leaves were taken from five leaves in each variety. Peelings were examined under a magnification of 100 x and 400 x. Size was measured using an ocular micrometer and photomicrographs were taken.

RESULTS

R E S U L T S

An experiment was conducted at the College of Agriculture, Vellayani to study the chromosome behaviour and pollen characters of five varieties of Anthurium andreanum L. The data collected were analysed and the results are presented below.

A.MORPHOLOGICAL STUDIES

The plants belonging to the genus Anthurium have short and erect stems with very short internodes which give the plants a compact, stocky appearance. Petioles are slender and longer than leaf blades. Leaf blade is broad, oblong to ovate or cordate in shape, prominently veined with pointed or acute tip and broad base which is usually lobed. In some varieties, the two basal lobes of the leaves overlap. Blades are glossy and light green to dark green in colour.

The anthurium 'flower' is actually a spadix with a fleshy, rod like floral axis known as the 'candle', bearing about 50-150 sessile flowers and a bright subtending spathe. The candle may be straight or curved. The spathe is broad and cordate - ovate or narrow and triangular, deeply or lightly veined, smooth or blistered and varying in size from

Figure 1 Anthurium andreanum var. Honeymoon Red

Figure 2 Anthurium andreanum var. White (album)

small to medium to large. It is brilliantly coloured in shades of white, pink, salmon, orange, red or deep maroon.

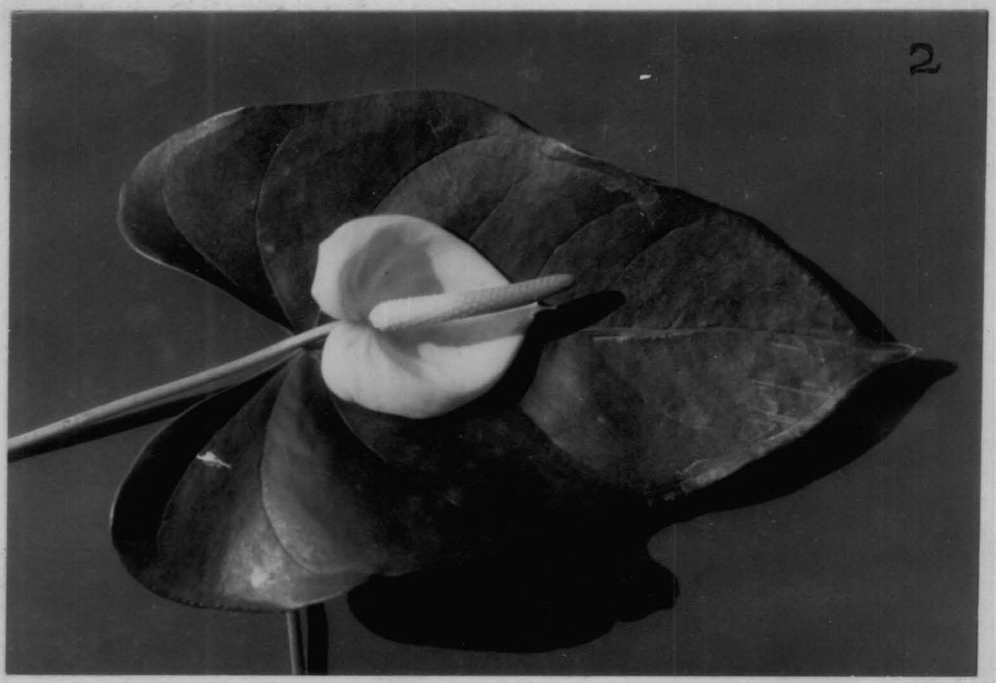
Flowers are bisexual and protogynous. They are closely embedded in spiral rows on the fleshy candle. Flowers are regular and superior with four tepals. There are four free stamens alternating with the tepals. Ovary is ovoid and two celled with one or two ovules in each locule. Stigma is two lobed. The fruit is a berry.

Some important morphological characters were studied in five varieties of the species Anthurium andreanum and the results are presented in the table 6.

1. A. andreanum var. Honeymoon Red

This is a fairly large variety with an average height of 70.0 cm. Leaves are large, more or less cordate with acuminate tips and overlapping leaf bases. Average leaf size is 18.0 x 15.3 cm and the length of the petiole is 28.1 cm. Tender leaves are purplish green in colour. Spathe is bright red and fleshy with a smooth glossy surface and has a size of about 8.0 cm length and 6.5 cm width. Pedicel has an average length of 38.0 cm. The candle is red in colour and of about 8.0 cm long. (Figure 1).


1


2

2. A. andreanum var. White (album)

The plants are medium in size with an average height of 60.0cm. Leaves are cordate, entire with acute to slightly acuminate tips and with more or less leathery leaf blades. Average size of leaf is 16.5x13.6cm and the tender leaves are light green in colour. Petiole is slender and long (28.0-30.0cm). The spathe is white in colour, cordate with undulating margin and has a prominently veined smooth surface. Average size of spathe is about 7.0cm length and 5.0cm breadth with long (41.0-45.0cm) pedicels. The candle is light yellow in colour and has an average length of 7.0cm (Figure 2).

3. A. andreanum var. Lady Jane (Pink).

The plants are smaller with an average height of 45.0cm. Leaves ovate, entire with acute to acuminate leaf tips. Average size of the leaf is 15.4x8.7cm and length of petiole is about 16.3cm. Tender leaves are light green in colour. Spathe is narrower, triangular or lanceolate with a size of 6.5x3.5cm. The average length of pedicel is 17.0cm. It is pink in colour with a glossy and smooth surface. The candle is light pink, and is about 4.0cm long (Figure 3).

4. A. andreanum var. Chillired.

Plants are medium in size with an average height of

Figure 3 *Anthurium andreanum* var. Lady Jane (Pink)

Figure 4 *Anthurium andreanum* var. Chillired


3


4

Table 6. Some important morphological characters of five varieties of A.andreanum
(Data as average of five measurements each)

Variety	Plant height cm	Leaf size cm	Length of petiole cm	Spathe size cm	Length of pedicel cm	Length of candle cm
A.andreanum var.Honeymoon Red	70.0	18.0x15.3	28.1	8.0x6.5	38.0	8.0
A.andreanum var. White(album)	60.0	16.5x13.6	28.2	7.0x5.0	42.0	7.0
A.andreanum var.Lady Jane(Pink)	45.0	15.4x 8.7	16.3	6.5x3.5	17.0	4.0
A.andreanum var. Chillired	55.0	13.5x 9.0	31.5	7.0x4.6	39.0	5.2
A.andreanum var. Pink	85.0	26.0x23.0	53.7	10.4x9.7	55.0	9.5

Figure 5 *Anthurium andreanum* var. Pink

5

55.0cm. Leaves are comparatively narrow and are with a size of 13.5x9.0cm. Petiole is about 31.5cm long. Tender leaves are purplish green in colour. Spathe is red, thinner than that of Honeymoon Red and with a blistered and veined surface. The average size of spathe is 7.0x4.6cm. The pedicel is having an average length of 39.0cm. The candle is slender, white, with an yellow band at the tip and is about 5.2cm long.

(Figure 4).

5. A. andreanum var. Pink

The plants are large in size with an average height of 85.0cm. Leaves are cordate, entire with acute to slightly acuminate tips. The average size of the leaf is 26.0x23.0 cm and the tender leaves are brownish green in colour. The petiole is slender with an average length of 53.7cm. The spathe is smooth with prominent veins and is light pink in colour. The average size of spathe is 10.4x9.7cm with a long pedicel (55.0cm). The candle is light pink to reddish brown and 9.5cm long.

(Figure 5).

B. CYTOLOGICAL STUDIES

Mitotic and meiotic behaviour of the chromosomes were studied and the results are analysed and presented below.

1. Mitosis

a. Chromosome number

Mitosis of the root tip cells was studied in all the five varieties of Anthurium andreanum. For all the varieties, somatic chromosome number of 30 + 2B was recorded. (Plate 1. Fig 1-5). Mitosis was regular. Very little abnormalities were recorded. The variety Pink showed late seperation of chromosomes (Plate 4 Fig 1). Excepting for this, all dividing cells observed, appeared normal.

Two B chromosomes were seen in all the five varieties irrespective of the time of sampling and tissues studied. The B chromosomes of the five varieties in the present study were seen to be deeply stained, smaller in size than the smallest A chromosome of the genome and were either round (Plate 1 Fig 2,5) or rod shaped (Plate 1 Fig 1,3,4).

b. Karyotype analysis

The karyotype of all the five varieties was analysed. Two difficulties encountered in karyotype analysis were (1) differences has to be sought in the gross morphology of the chromosomes, as all the varieties had the same chromosome number (2n=30+2B) (2) difficulty in identifying true pairs using conventional techniques because of the presence of several chromosomes of equal length within the genome.

PLATE 1

PHOTOMICROGRAPHS OF MITOTIC CHROMOSOMES

- Metaphase polar view

2n = 30 + 2B

Fig 1 *Anthurium andreanum* var. Pink

Fig 2 *Anthurium andreanum* var. Honeymoon Red

Fig 3 *Anthurium andreanum* var. Lady Jane

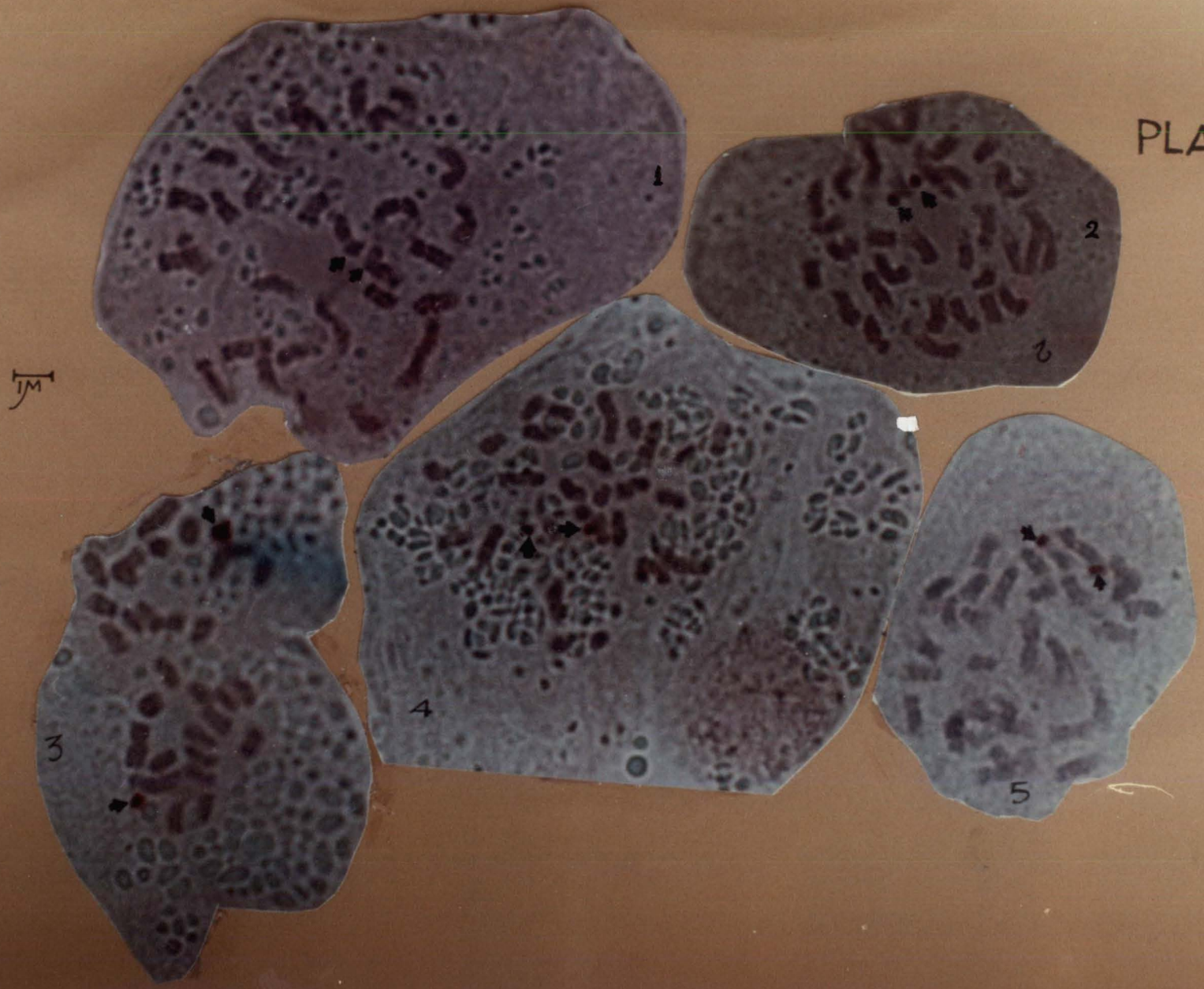
Fig 4 *Anthurium andreanum* var. Chillired

Fig 5 *Anthurium andreanum* var. White

B chromosomes are pointed out

Magnification scale is given in the plate.

PLATE.1



The data on karyotype analysis are tabulated and presented on table 7-11 and Plate 2 Fig 1-5. Since all the five varieties had the same chromosome number (2n =30+2B), the karyophotograph of the variety, Pink has been shown as a representative figure (Plate 3 Fig 8,10).

1. Anthurium andreanum var. Honeymoon Red

Length of the chromosomes ranges from 2.50 to 6.78μ (Table 7). Total length of the chromosomes (TCL) of the genomic complement is 56.10 μ . The relative chromosome length (RCL) ranges from 4.50 to 12.09. The arm (r) ranges from 1.00 to 3.10. The F% varies from 24.46 to 50.00. The B chromosomes show a uniform length of 1.10μ and are round in shape. The idiogram formula for the variety is 1M + 6m + 7sm + 1 st (See table 4) and the variety has a

TF% ($\frac{\text{Total short arm length x 100}}{\text{Total chromosome length}}$) of 39.10. The variety belongs to the karyotype category of '3B' as per the classification of Stebbins (1958) (Plate 1 Fig 2 Plate 2 Fig 1)

2. A andreanum var White (album)

Length of the chromosomes ranges from 2.17 to 5.85μ (Table 8). The TCL of the genomic complement is 58.71 μ The RCL ranges from 3.80 to 10.21 and the r value ranges from 1.00 to 3.19. The limits of F% range are 23.77 and 50.00. The B chromosomes record a uniform lengh of 1.00μ and are rod shaped. The TF% is 36.55. The idiogram formula is 1M + 7m +

PLATE 2

KARYOMORPHOLOGY OF FIVE VARIETIES

OF *Anthurium andreanum*

Fig 1 *Anthurium andreanum* var. Honeymoon Red

Fig 2 *Anthurium andreanum* var. White (album)

Fig 3 *Anthurium andreanum* var. Lady Jane (Pink)

Fig 4 *Anthurium andreanum* var. Chillired

Fig 5 *Anthurium andreanum* var. Pink

2μ

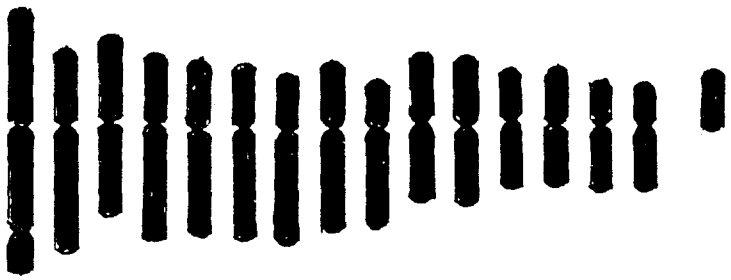


Fig 1

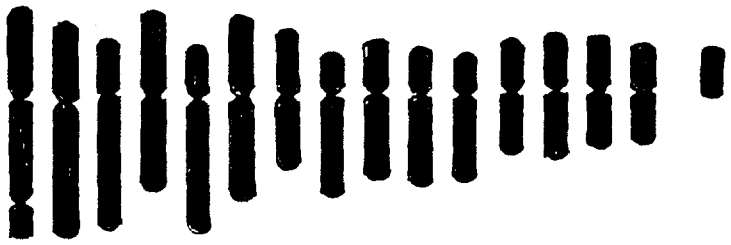


Fig 2

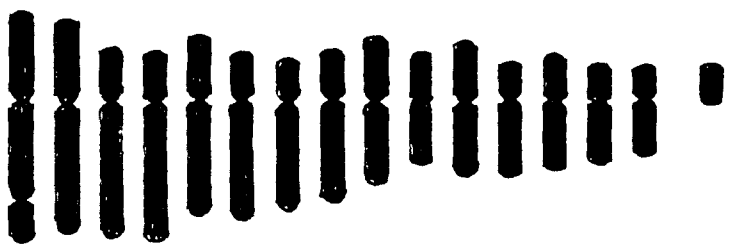


Fig 3

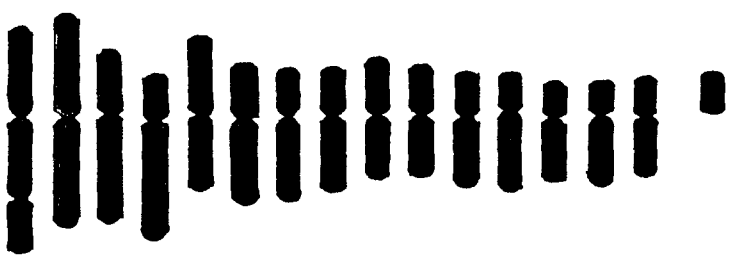


Fig 4

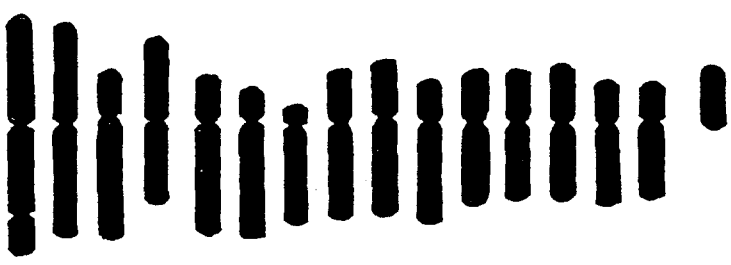


Fig 5

PLATE 2

Table 7. Description of karyotype

(I) Anthurium andreanum var. Honeymoon Red

Table 7. Description of karyotype

Chromosome Number	Total length of chromosomes in μ			RCL	r	F%	Centromeric location
	Long arm	Short arm	Total				
1	3.78 (2.8+0.98)**	3.00	6.78	12.09	1.26	44.24	m
2	3.20	2.05	5.05	9.01	1.56	40.59	m
3	2.33	2.33	4.66	8.30	1.00	50.00	M
4	2.92	1.68	4.60	8.20	1.73	36.52	sm
5	2.90	1.60	4.50	8.00	1.81	35.56	sm
6.	2.88	1.46	4.34	7.70	1.96	33.64	sm
7	3.15	1.02	4.17	1.40	3.10	24.46	st
8	2.74	1.43	4.17	7.40	1.91	34.29	sm
9	2.68	1.16	3.84	6.80	2.30	30.24	sm
10	1.92	1.75	3.67	6.50	1.10	47.68	m
11	2.04	1.63	3.67	6.50	1.25	44.41	m
12	1.62	1.21	2.83	5.00	1.33	42.76	m
13	1.58	1.25	2.83	5.00	1.26	44.17	m
14	1.73	0.93	2.66	4.70	1.85	34.96	sm
15	1.66	0.84	2.50	4.50	1.98	33.60	sm
B			1.10				

Total chromosome length (TCL) = 56.1 μ

Idiogram formula = 1M + 6m + 7sm + 1st

$TF\% \left(\frac{\text{Total short arm length} \times 100}{\text{Total chromosome length}} \right) = 39.10$

** Satellite chromosomes

Karyotype category - 3B

RCL - Relative chromosome length. r - Arm ratio. F% - Centromeric index.

Table 8.Description of karyotype

(2) A. andreanum var. White (album) Table 8. Description of karyotype

Chromosome Number of the genome	Length of Chromosomes in μ			RCL	r	F%	Centromeric location
	Long arm	Short arm	Total				
1	3.65 (2.9+0.75)**	2.18	5.83	10.21	1.67	37.39	m
2	3.75	1.75	5.50	9.63	2.14	31.82	sm
3	3.48	1.34	4.82	8.44	2.61	27.80	sm
4	2.41	2.41	4.82	8.44	1.00	50.00	M
5	3.56	1.11	4.67	8.18	3.19	23.77	st
6	2.63	2.04	4.67	8.18	1.29	43.68	m
7	1.97	1.69	3.66	6.41	1.17	46.17	m
8	2.58	0.92	3.50	6.13	2.79	26.29	sm
9	2.33	1.17	3.50	6.13	2.00	33.43	sm
10	2.41	0.93	3.34	5.85	2.61	27.84	sm
11	2.38	0.80	3.17	5.55	2.99	25.24	sm
12	1.46	1.38	2.83	4.95	1.07	48.76	m
13	1.52	1.30	2.83	4.95	1.16	45.94	m
14	1.32	1.18	2.50	4.38	1.11	47.20	m
15	1.24	0.93	2.17	3.80	1.34	42.86	m
B			1.00				

Total chromosome length (TCL) = 57.12 μ

Idiogram formula - 1M + 7m + 6 sm + 1st

$TF\% = \left(\frac{\text{Total short arm length} \times 100}{\text{Total chromosome length}}\right) = 36.55$

Karyotype category - 3 B

** Satellite chromosomes.

RCL - Relative chromosome length. r - Arm ratio. F% - Centromeric index.

6sm + 1st. It belongs to the karyotype category of '3B' (Stebbins, 1958) (Plate 1 Fig 5. Plate 2 Fig 2).

3. A. andreanum var. Lady Jane (Pink)

In this variety, the length of the chromosomes ranges from 2.00 to 5.95 μ. (Table 9) and TCL of the genomic complement is 57.81 μ. The RCL ranges from 3.46 to 10.29 and the r value ranges from 1.25 to 3.20. The F% varies from 23.77 to 44.48. The B chromosomes record a uniform length of 0.83 μ and are rod shaped. TF% is 33.73 and idiogram formula is 4m + 9sm + 2st. It belongs to the karyotype category '3B' (Stebbins, 1958) (Plate 1 Fig 3 , Plate 2 Fig 3)

4. A. andreanum var. Chillired

This variety shows a chromosome length that ranges from 2.20 to 5.56 μ (Table 10). TCL of the genomic complement is 51.02 μ. RCL varies from 4.31 to 10.90 and the r value ranges from 1.00 to 3.15. The TF% ranges from 24.47-50.00. The length of the B chromosomes is 1.00 μ and they are round in shape. The TF% is 37.89. The idiogram formula is 1M + 6m + 7sm + 1 st. This variety also belongs to the karyotype category '3B' (Stebbins, 1958) (Plate 1 Fig 4 , Plate 2 Fig 4)

5. A. andreanum var. Pink

Length of the chromosomes ranges from 2.80 to 6.31 μ

Table 9. Description of karyotype

(3) A. andreanum var. Lady Jane (Pink)

Chromosome number of the genome	Length of Chromosome in μ			RCL	r	F%	Centromeric Location
	Long arm	Short arm	Total				
1	3.74	2.21	5.95	10.29	1.69	37.14	m
2	3.37	1.96	5.33	9.22	1.72	36.77	sm
3	3.52	1.15	4.67	8.08	3.05	24.63	st
4	3.56	1.11	4.67	8.08	3.20	23.77	st
5	2.78	1.55	4.33	7.49	1.80	35.80	sm
6	3.09	1.24	4.33	7.49	2.50	28.64	sm
7	2.78	1.06	3.84	6.64	2.62	27.60	sm
8	2.50	1.17	3.67	6.35	2.14	31.88	sm
9	2.21	1.46	3.67	6.35	1.51	39.78	m
10	1.18	1.45	3.33	5.76	1.29	43.54	m
11	1.76	1.41	3.17	5.48	1.25	44.48	m
12	1.89	0.94	2.83	4.89	2.00	33.21	sm
13	1.83	1.00	2.83	4.89	1.81	35.34	sm
14	1.64	0.86	2.50	4.32	1.91	34.40	sm
15	1.30	0.70	2.00	3.46	1.85	35.00	sm
B							

Total chromosome length (TCL) = 57.81 μ

Idiogram formula - 4m + 9sm + 2st

$TF\% = \left(\frac{\text{Total short arm length} \times 100}{\text{Total chromosome length}}\right) = 33.73$

** Satellite chromosomes

Karyotype category - 3 B

RCL - Relative chromosome length. r - Arm ratio. F% - Centromeric index.

Table 10. Description of karyotype

(4) A. andreanum var. Chillired

Chromosome number of the genome	Length of the chromosome in μ			RCL	r	F%	Centromeric location
	Long arm	Short arm	Total				
1	3.46 (2.16 + 1.3)**	2.10	5.56	10.90	1.64	37.77	m
2	2.77	2.61	5.38	10.54	1.06	48.51	m
3	2.78	1.60	4.38	8.58	1.73	36.53	sm
4	3.12	1.00	4.12	8.08	3.15	24.27	st
5	1.83	1.83	3.66	7.17	1.00	50.00	M
6	2.15	1.18	3.33	6.53	1.81	35.44	sm
7	2.09	1.08	3.17	6.21	1.93	34.10	sm
8	1.92	1.08	3.00	5.88	1.78	36.00	sm
9	1.61	1.29	2.90	5.68	1.25	44.48	m
10	1.60	1.23	2.83	5.55	1.31	43.46	m
11	1.74	1.09	2.83	5.55	1.60	38.52	m
12	1.76	0.90	2.66	5.21	1.95	33.83	sm
13	1.74	0.76	2.50	4.90	2.30	30.40	sm
14	1.80	0.70	2.50	4.90	2.60	28.00	sm
15	1.32	0.88	2.20	4.31	1.50	40.00	m
B			1.00				

Total chromosome length (TCL) = 51.02 μ

Idiogram formula - 1M + 6m - 7sm + 1st

$$TF\% = \left(\frac{\text{Total short arm length} \times 100}{\text{Total chromosome length}}\right) = 37.89$$

Karyotype category - 3 B

** Satellite chromosome. RCL - Relative chromosome Length. r - Arm ratio. F% - Centromeric index.

Table 11 Description of karyotype

(5) A. andreanum var. Pink

Chromosome number of the genome	Length of the chromosome in μ			RCL	r	F%	Centromeric location
	Long arm	Short arm	Total				
1	3.50	2.80	6.31	10.75	1.25	44.37	m
2	3.2	2.66	5.86	9.98	1.20	45.39	m
3	3.04	1.16	4.20	7.10	2.62	27.62	sm
4	2.10	2.10	4.20	7.10	1.00	50.00	M
5	2.96	1.10	4.06	6.91	2.69	27.09	sm
6	3.04	0.80	3.84	6.54	3.80	20.83	st
7	2.56	0.16	3.72	6.33	2.21	31.18	sm
8	2.44	1.28	3.72	6.33	1.91	34.41	sm
9	2.40	1.32	3.72	6.33	1.81	35.48	sm
10	2.56	0.94	3.50	5.96	2.72	26.86	sm
11	2.10	1.16	3.26	5.55	1.81	35.58	sm
12	2.02	1.24	3.26	5.55	1.63	38.04	m
13	1.96	1.30	3.26	5.55	1.51	39.88	m
14	2.10	0.94	3.04	5.18	2.23	30.92	sm
15	1.86	0.94	2.80	4.77	1.979	33.57	sm
B			1.16				

Total chromosome length (TCL) = 58.71 μ

$TF\% = \left(\frac{\text{Total length of short arm} \times 100}{\text{Total chromosome length}}\right) = 35.59$

Idiogram formula - 1M + 4m + 9sm + 1st

Karyotype category - 3 B

RCL - Relative chromosoem length. r - Arm ratio. F% - Centromeric index.

(Table 11). TCL of the genomic complement is 58.71 μ. The RCL and r value range from 4.77 to 10.75 and 1.00 to 3.80 respectively. The F% shows a range of 20.83 to 50.00. The B chromosomes have a uniform length of 1.16μ and are rod shaped. The TF% of this variety is 35.59 and the idiogram formula is 1M + 4m + 9sm + 1st. It belongs to the karyotype category '3B' (Stebbins, 1958) (Plate 1 Fig 1, Plate 2 Fig5)

The summary of the karyotype analysis of the five varieties of A. andreanum is tabulated and presented in table 12.

The highest average chromosome length and total chromosome length are recorded in the variety White (album) (3.91 and 58.71 μ respectively) and the lowest in thevariety Pink (3.40 and 51.02 respectively). The total chromosome length of Pink is significantly lower than that of all other varieties. The variety Honeymoon Red records the largest relative chromosome length (7.14) and Chilliered has the smallest (6.66).The variety Pink has the largest arm ratio (2.025) and Lady Jane has the smallest (1.03). When the F percent is compared, White (album) (37.21) has the largest value and Lady Jane (34.13.) has the smallest value. Most of the chromosomes of all the five varieties studied are having a high F percentage indicating they are either with median or with submedian centromeres. Based on F percentage,

Table 12. Summary of karyotype data of five varieties of A.andreanum

Variety	2n	ACL μ	TCL μ	RCL	r	F%	Karyotype/ idiogram formula	Asymmetry category	TF%
Honeymoon Red	30+2B	3.74	56.10	7.14	1.69	36.46	1M+6m+7sm+1st	3B	39.10
White (album)	30+2B	3.91	58.71	6.74	1.88	37.21	1M+7m+6sm+1st	3B	36.55
Lady Jane(Pink)	30+2B	3.81	57.12	6.72	1.03	34.13	4m+9sm+2st	3B	33.73
Chillired	30+2B	3.85	57.81	6.66	1.77	34.90	1M+6m+7sm+1st	3B	37.89
Pink	30+2B	3.40	51.02	6.66	2.03	34.75	1M+4m+9sm+1st	3B	35.59

ACL - Average chromosome length
TCL - Total chromosome length
RCL - Relative chromosome length
r - Arm ratio

$$F\% = \frac{\text{Short arm length} \times 100}{\text{Total chromosome length}}$$

$$TF\% = \frac{\text{Total Short arm length} \times 100}{\text{Total Chromosome Length}}$$

PLATE 3

PHOTOMICROGRAPHS OF MEIOSIS AND MITOSIS

Fig 1 Diakinesis (var. Pink)

Fig 2 Diakinesis (var. Lady Jane)

Fig 3 Diakinesis (var. Honeymoon Red)

Fig 4 Diakinesis (var. Chillired)

Fig 5 Diakinesis (var. White)

Fig 6 A normal cell at telophase I (var. Pink)

Fig 7 A normal cell at prophase II (var. Pink)

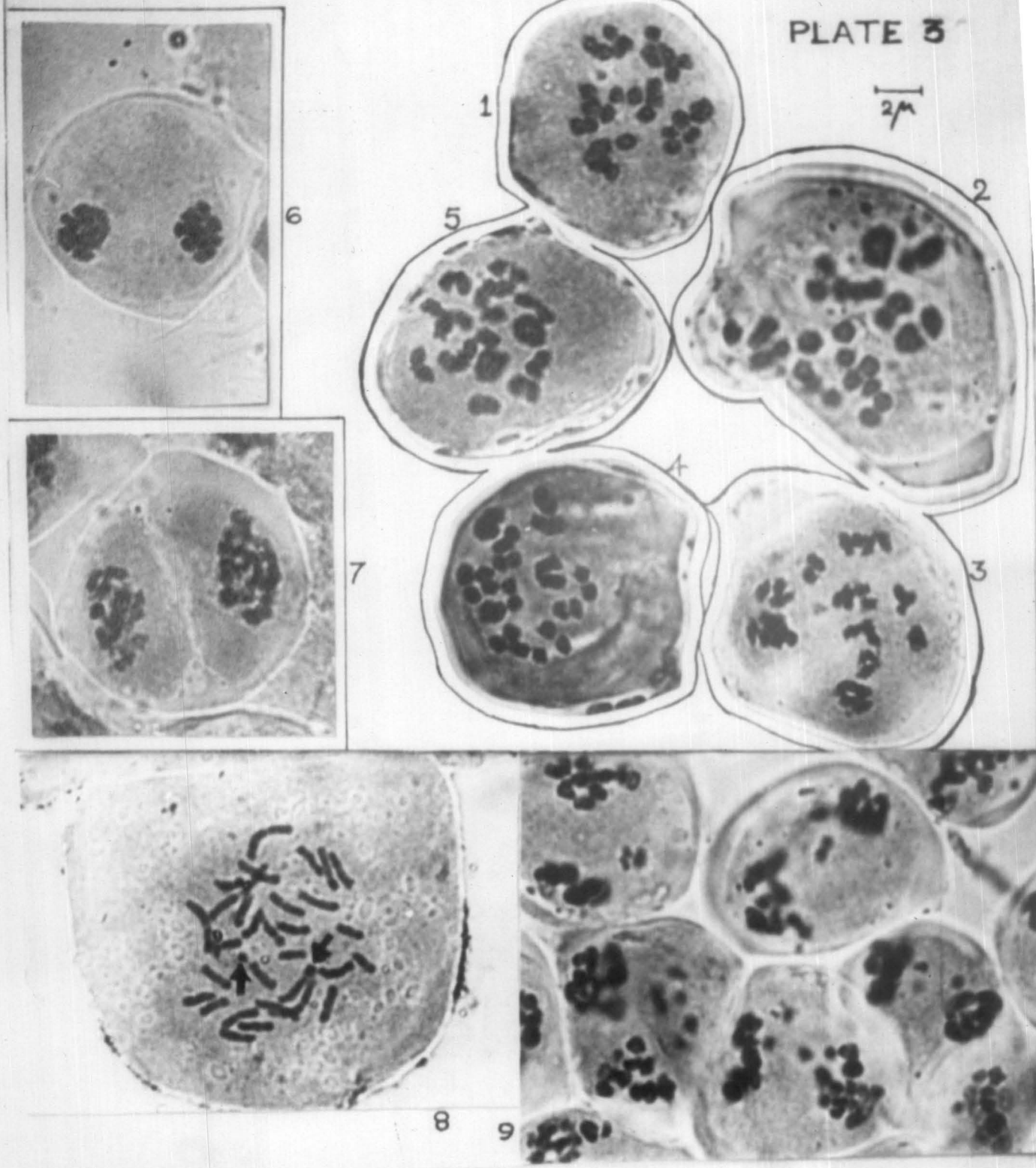
Fig 8 Mitosis 2n = 30 + 2B (var. Pink)
(B chromosomes are pointed out)

Fig 9 Laggards (var. Lady Jane)

Fig 10 Karyophotograph (var. Pink)

Magnification scale is given in the plate

PLATE 3



the chromosomes were classified (Shindo and Kamemoto, 1963) as median (45-50%), submedian (30-45%) and values below 30 indicate subtelocentric and telocentric. Based on this classification, of the 15 pairs of somatic chromosomes of Honeymoon Red, 12 pairs are submedian, 2 pairs are median and one pair is subtelocentric. In the variety White (album), 5 pairs of chromosomes are submedian, 5 pairs median and 5 pairs subtelocentric. The variety Lady Jane has 11 pairs of submedian chromosomes and the rest being subtelocentric. Two pairs of chromosomes of the variety Chillired are with median, 10 pairs with submedian and the rest with subtelocentric centromeres. Ten pairs of chromosomes of the variety Pink are submedian, two pairs median and the rest are subtelocentric. It is clear that as the F percentage decreases, the chromosoems become more and more asymmetrical. The TF% percent is highest in the variety Honeymoon Red (39.10) and is lowest in the variety Lady Jane (33.73). The idiogram formulae of all the five varieties are different. However, in all the varieties, the m and sm types chromosomes predominate. Though all the five varieties show variation in the above mentioned characters, all of them belong to the same asymmetry category 3B.

Meiosis

Meiosis in pollen mother cells of all the five varieties

PLATE 4

PHOTOMICROGRAPHS OF MEIOTIC ABNORMALITIES

Fig 1 Unequal seperation (var. Lady Jane)

Fig 2 Unequal seperation (var. Pink)

Fig 3 Precocious seperation (var. White)

Fig 4 Unequal seperation and laggards (var. White)

Fig 5 Chromosome elimination (var. Honeymoon Red)

Fig 6 Laggards (var. White)

Fig 7 Laggards (var. Chillired)

Fig 8 Unequal seperation with laggards (var. Pink)

Fig 9 Unequal seperation (var. White)

Fig 10 Chromosome elimination (var. Chillired)

Magnification scale is given in the plate

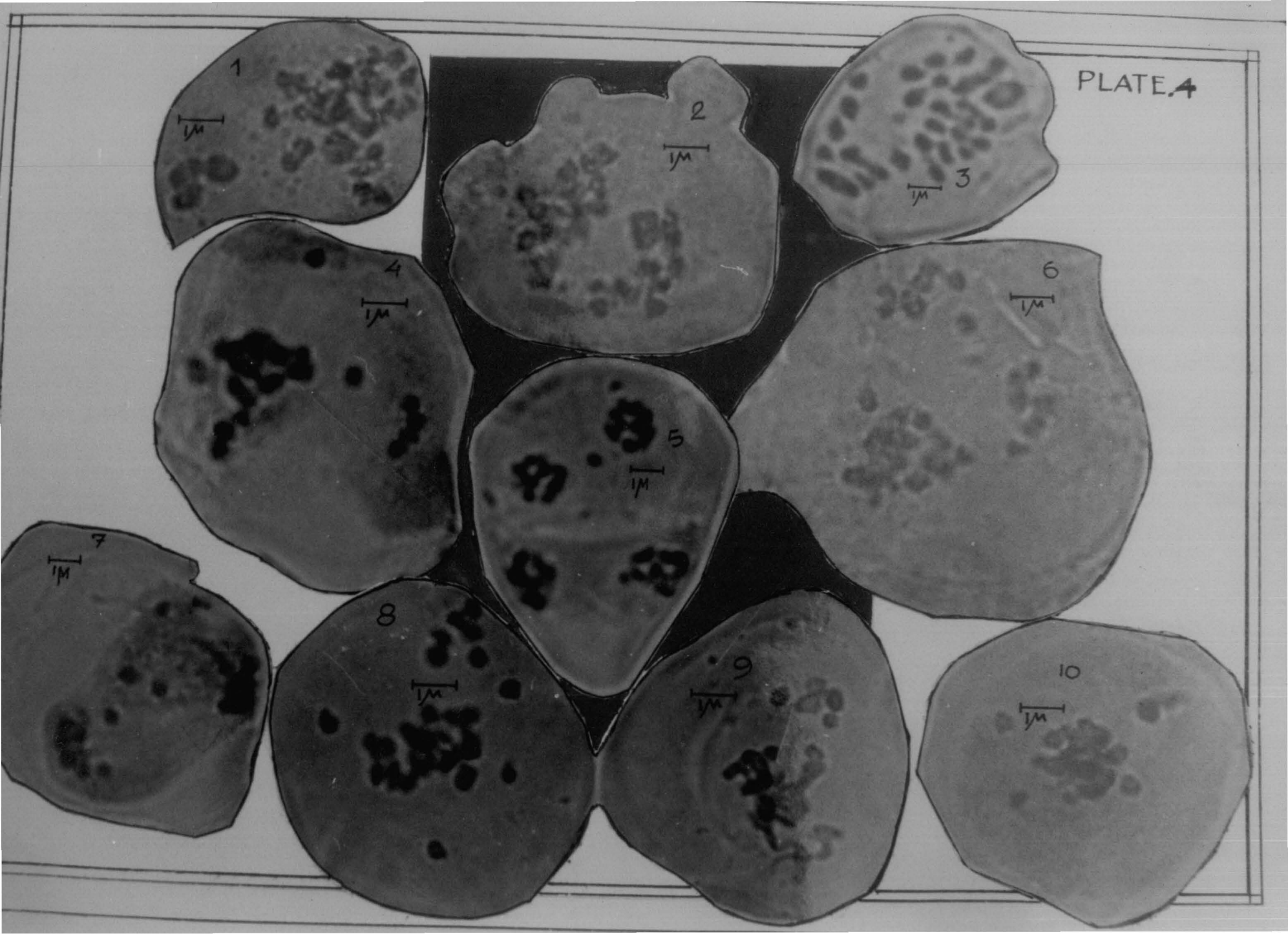


PLATE. 4
1
1μ
2
1μ
3
1μ
4
1μ
5
1μ
6
1μ
7
1μ
8
1μ
9
1μ
10
1μ

was studied. The normal and abnormal behaviour of chromosome were observed are presented below.

1. Univalent formation

At diakinesis and metaphase I, a low frequency of pollen mother cells showing univalents is found in all the five varieties (Table 13). Varying numbers of univalents (2-8) have been observed. The B chromosomes of the varieties White, Chillired and Honeymoon Red are found as univalents on either side of the metaphase plate (Plate 6 Fig 1,2,4). But the B chromosomes of the varieties Pink and Lady Jane are found to be often paired (Plate 6 Fig 3,5).

2. Unequal segregation

All the varieties show unequal segregation at anaphase (Table 13 Plate 4 Fig 1,2,6 Plate 5 Fig 8).

3. Clumping

During metaphase I, all the somatic chromosomes in the five varieties are clumped together. (Table 13 Plate 6)

4. Laggards

Varying numbers of laggards (1-10) are seen in the pollen mother cells of the five varieties during anaphase I (Table 13). Both the B chromosomes and the A chromosomes appear to form laggards. However, the B chromosomes are always found as laggards, never orienting themselves in the metaphase plate (Plate 6 Fig 1 to 5). The frequency of cells with fewer number of laggards (one or two)(Plate 3 Fig 9),

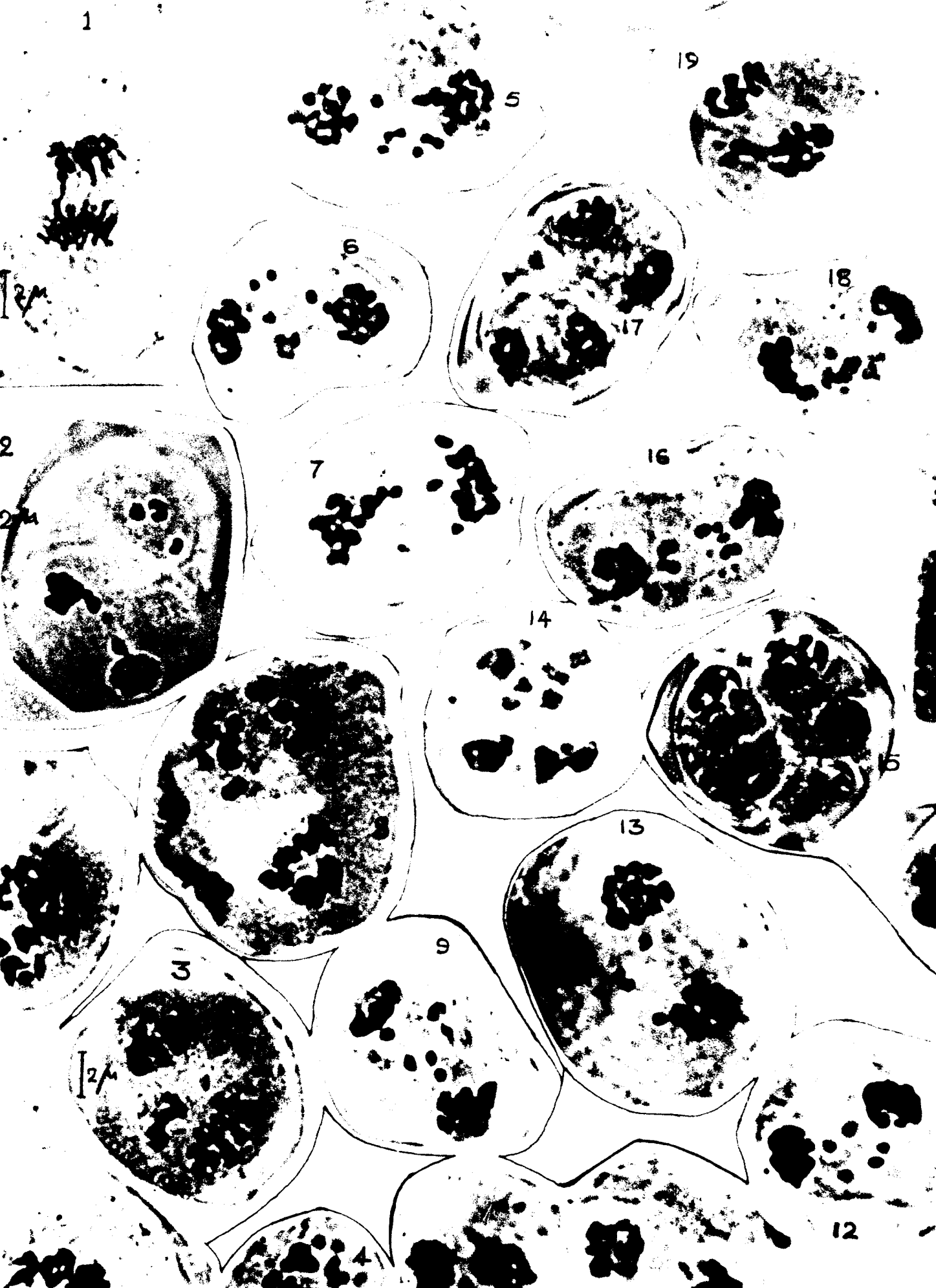

1
19
5
6
18
17
2
7
16
14
15
13
9
3
12
4

Table 13. Chromosomal aberrations in the pollen mother cells.

Variety	Number of cells observed	Normal cells	Clumping	Unequal segregation	Presence of laggards					Univalents
					1	2	3	4	>4	
Honeymoon Red	225	53	31	72	18	17	12	10	14	25
White (album)	190	48	27	53	22	19	17	8	12	14
Lady Jane(Pink)	260	67	33	68	12	17	13	6	4	31
Chillired	315	81	38	118	33	28	14	21	35	42
Pink	420	108	53	163	61	38	24	18	22	47

PLATE 6

PHOTOMICROGRAPHS OF MEIOSIS SHOWING B CHROMOSOMES

Fig 1 var. Honeymoon Red

Fig 2 var. Chillired

Fig 3 var. Pink

Fig 4 var. White

Fig 5 var. Lady Jane

Magnification scale is given in the plate

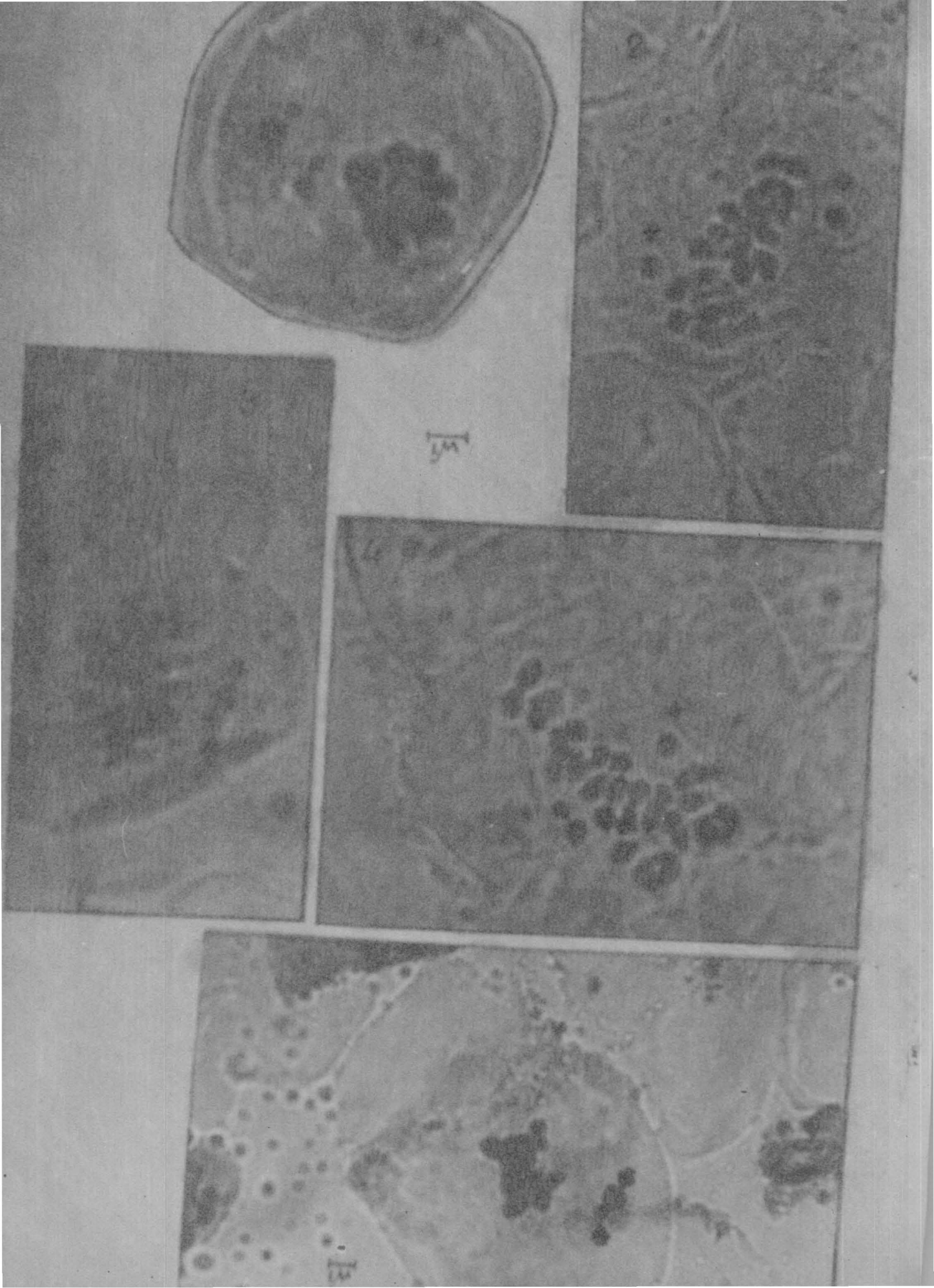


Plate 5 fig 11,19) is higher than those with a higher number (Plate 4 Fig 7, Plate 5 Fig 5,6,7,9,10,12,13,16).

5. Micronuclei

Micronucleus is observed in all the five varieties at telophase II. (Plate 5 Fig 2,15).

Apart from the above mentioned abnormalities, precocious seperation of the bivalents (Plate 4 Fig 3) and chromosome elimination (Plate 4 Fig 5) are also observed.

3. Mitotic index

The mitotic index reported for the five varieties are given in the table 14.

Of the five varieties studied, the mitotic index is highest for variety Pink and lowest for Chillired. This may be due to the fact that the mitotic cycle extends for a longer period for Pink when compared to Chillired. The varieties Lady Jane, album and Honeymoon Red have intermediate mitotic indices. However there is no statistically significant difference among the five varieties.

C. POLLEN STUDIES

The time of flower opening, anthesis, pollen production

Table 14. Mitotic indices of the five varieties of Anthurium andreanum

Variety	Mitotic index		
	Range	Average	Average in %
Honeymoon Red	0.06-0.14	0.12	12.00
White (album)	0.08-0.16	0.12	12.00
Lady Jane (Pink)	0.08-0.12	0.11	11.00
Chillired	0.10-0.12	0.11	11.00
Pink	0.10-0.15	0.14	14.00

per flower, size and shape of pollen and pollen viability were studied and the results are analysed and presented below.

1. Time of flower opening and anthesis.

The time of flower opening and anthesis of all the five varieties of A. andreanum was observed and recorded. The spathe covering the candle begins to open about 3-4 weeks after the appearance of the tip of the spadix in the leaf axil. The spathe takes about one week for opening out fully. About 50-150 flowers are found on each candle. They are closely packed in slanting rows. The flowers are bisexual, regular and protogynous. The flowers mature first from the base and proceeds upwards in an acropetal succession in all the five varieties studied. The styles are short and stigmas generally capitate. When receptive, they project out slightly on the surface of the tepals which remain closed.

Female phase

Female receptivity is identified by the presence of honey dew or stigma droplets and insect activity (bees, ants etc). Some inflorescences never exhibit a female phase. From the observations on five varieties in the present study, it is clear that the female phase for the species, A.andreanum varies from 3 to 12 days.

Interphase

The stigmatic droplets dry up before any stamens emerge.

The seperation period or the interphase between the female phase and male phase for the species ranges from 4 to 7 days normally. During rainy season, the interphase is prolonged and or the male phase is completely suppressed. Sometimes a few stamens appear on the candle irregularly.

Male phase

Male phase is identified by the appearance of stamens on the candle. Anther exertion starts from the base and proceeds regularly towards the apex and thus one candle may be in male phase for 3 to 7 days. The four stamens of each flower are clustered around the stigma.

Anthesis as well as anther dehiscence occured during the early morning hours.

The duration of the different phase of flower maturity such as female phase, interphase and male phase of the varieties, is studied and the results are presented below in Table 15.

From the above data it is evident that all the varieties are protogynous. The varieties having comparatively large candles like Pink and Honeymoon Red take more period for flower maturity than those with smaller candles.

In the variety Honeymoon Red, the female phase is for

8-10 days. The interphase is for 4-7 days and the male phase, is for 4-6 days. The average period of flower maturity is 16-23 days.

The female phase in the variety White is for 5-8 days, the interphase is for 5-7 days and the male phase is for 4-6 days. The duration of flower maturity is 14-21 days.

The variety, Lady Jane takes 3-5 days for completing female phase. The duration is 4-6 days and 3-4 days for interphase and male phase respectively. The period of flower maturity ranges from 10-17 days.

The female phase in the variety, Chillired is for 4-6 days, the interphase is for 4-7 days and the male phase is for 4-6 days. On an average 12-19 days are taken for flower maturity.

The variety Pink is in female phase for 8-12 days. The interphase is for 5-7 days and the male phase is for 4-7 days. On an average, the period of flower maturity for Pink is 17-26 days.

The total number of days taken for complete flower maturity in the five varieties of A.andreanum in the present study is statistically compared. The mean number of days taken by the varieties is tabulated (Table 15). The

Table15. Duration of floral maturity phases in five varieties of A. andreanum

Variety	I Female phase in days	II Inter phase in days	III Male phase in days	Number of days taken for flower maturity
Honeymoon Red	8 - 10	4 - 7	4 - 6	19.5
White (album)	5 - 8	5 - 7	4 - 6	18.0
Lady Jane (Pink)	3 - 5	4 - 6	3 - 4	12.8
Chilli red	4 - 6	4 - 7	4 - 6	15.5
Pink	8 - 12	5 - 7	4 - 7	22.3
CD				5.116

statistically analysed results are given in Apendix - I

The varieties show statistically significant variation, when the duration for flower maturity is compared. The variety Lady Jane has the lowest value (12.75 days). This variety significantly differs from the varieties Pink, Honeymoon Red and White, but is on par with the variety Chillired in flower maturity. The highest number of days for flower maturity is required by the variety Pink (22.25 days) but it is not significantly different from Honeymoon Red and White.

2. Pollen morphology - size and shape.

The pollen grain size of all the five varieties was measured and the results are presented in Table 16. The average pollen grain size of the five varieties when compared is not significantly different. The variety, Lady Jane with the smallest sized spathe and candle has the largest pollen grains (87.2 x 86.4μ) while the variety Pink with the largest spathe and candle has the smallest (81.8 x 68.0μ) pollen grains. The pollen grain size of the other three varieties come in between.

The five varieties of Anthurium andreanum under the present investigation have more or less round pollen with a single germpore.

Table 16. Pollen grain size in five varieties of A.andreanum

Variety	Size in μ		
	Range		Average
	Length	Breadth	Length x Breadth
Honeymoon Red	64-88	64-80	81.0x74.1
White (album)	64-92	64-80	82.6x74.0
Lady Jane (Pink)	80-112	64-96	87.2x86.4
Chillired	74-96	64-76	83.6x72.0
Pink	72-88	64-72	81.8 x68.0

3. Pollen production per flower

The pollen production by an anther and four anthers of one flower was studied in all the five varieties. The results, are presented in Table 17.

From the data, it is clear that the varieties show statistically significant variation in their ability to produce pollen grains. When all the five varieties are considered together, they produce on an average about 6000 pollen grains per a single flower.

The variety, Lady Jane produces the lowest amount of pollen per anther (1213). The variety Pink produces maximum number of pollen grains per anther (1725). The pollen production capacity of Pink is on par with Honeymoon Red while Chillired is on par with White and White is on par with Lady Jane.

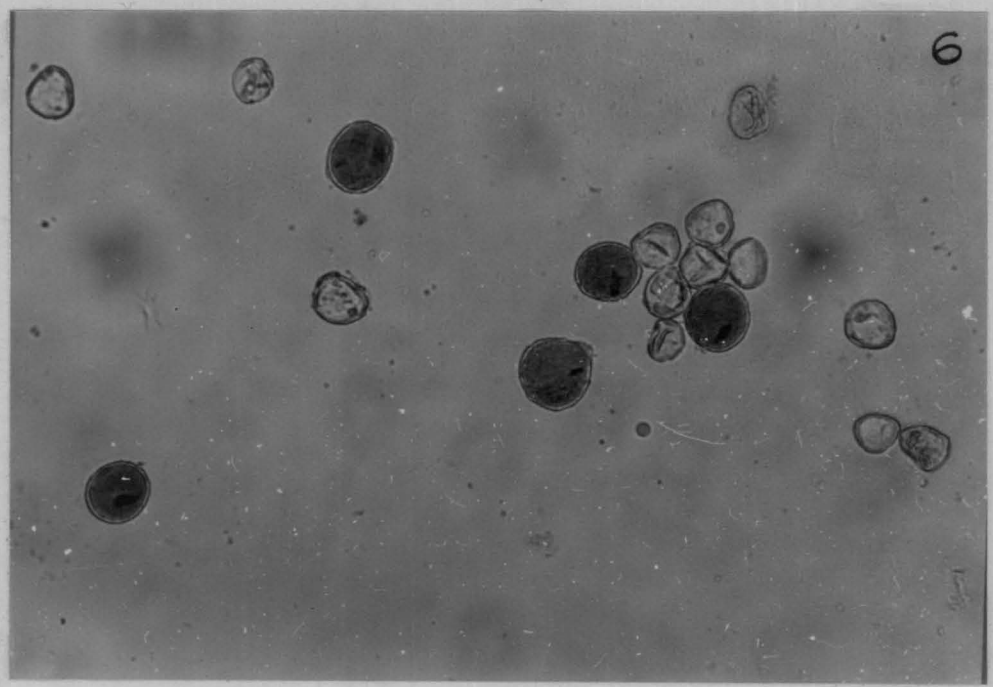
4. Pollen fertility

Pollen fertility was studied by both acetocarmine staining method and *in vitro* pollen germination method. The results are presented in table 18 (Fig 6,7,8).

The variety Pink is having the highest fertility by acetocarmine staining method (28.8%) (Fig 6) and *in vitro*

Figure 6 Photomicrograph of pollen stainability (var. Pink) (2675 x)

Figure 7 Photomicrograph of pollen stainability (var. Honeymoon Red) (2675 x)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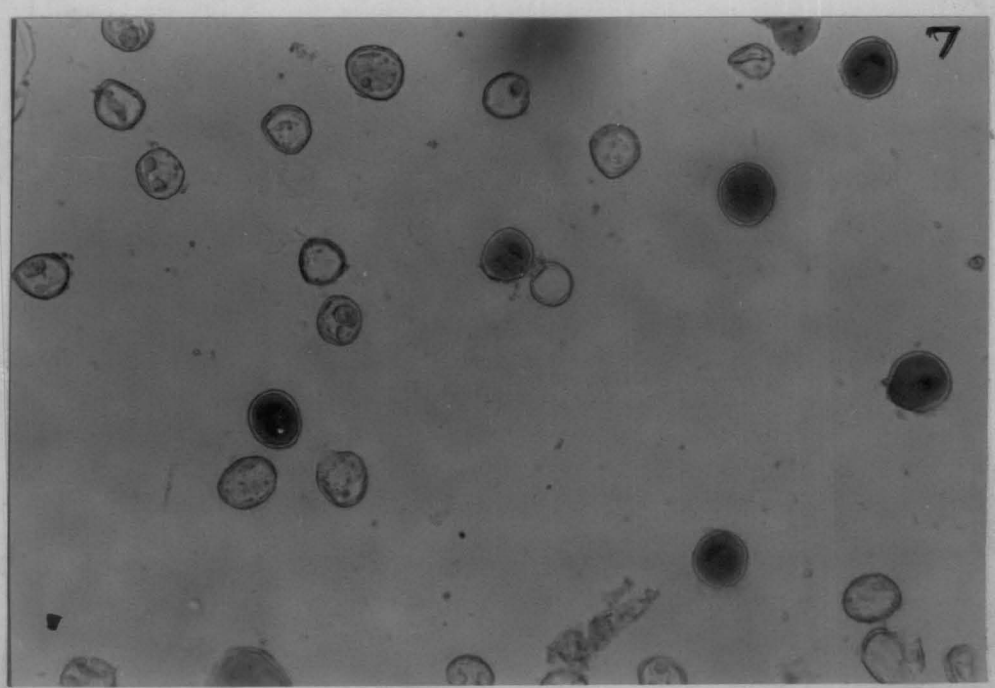
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Table 17. The pollen production of five varieties of A.andreanum

Variety	Pollen production per single flower	Pollen production per anther	Average pollen production per anther
Honeymoon Red	6000-7200	1500-1800	1650
White (album)	4000-6000	1200-1500	1313
Lady Jane (Pink)	4000-5600	1000-1400	1213
Chillired	4800-6400	1200-1600	1438
Pink	6400-7200	1600-1800	1725
CD			168.6392

Figure 8 Photomicrograph of a germinated pollen grain (var. Pink) (3685 x)

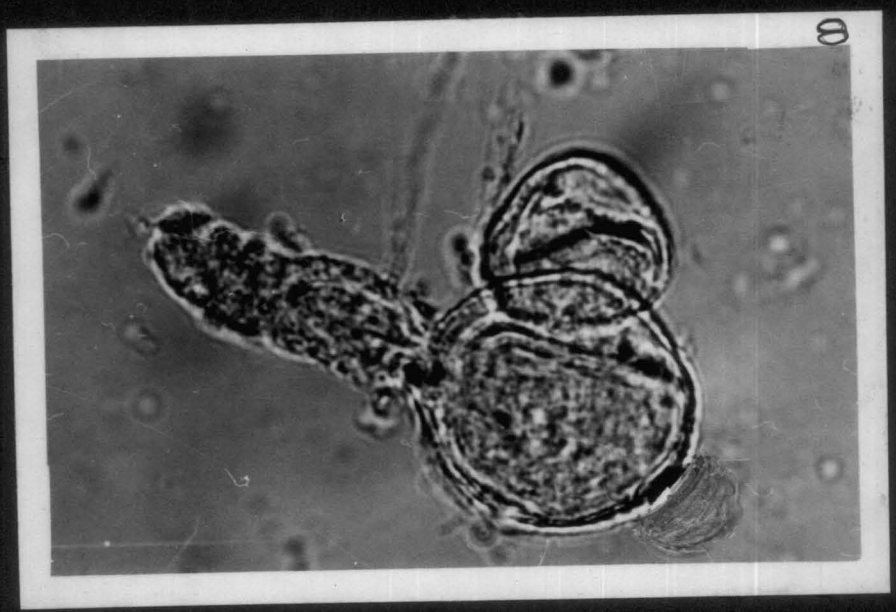
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Table18.Pollen fertility % of five varieties of A.andreanum

Variety	Pollen Fertility %	
	by aceto carmine staining method	by in vitro pollen germination method
Honeymoon Red	20.4	11.6
White (album)	24.2	13.9
Lady jane Pink	21.4	9.7
Chillired	21.7	10.4
Pink	28.8	17.9

tube growth studies (17.9%) (Fig 8). Lowest pollen fertility by acetocarmine staining method is recorded in the variety Honeymoon Red (20.4%) (Fig 7) and by in vitro pollen tube growth studies, it is recorded in the variety Lady Jane (9.7%).

When pollen fertility by acetocarmine method is compared for the five varieties, the ferlity % of Lady Jane and Chillired is lower ie. 21.4% and 21.7% respectively. In in vitro pollen tube growth studies also, similar low values are (9.7% and 10.4%) obtained for these two varieties.

Stomata

The stomata in all the five varieties under the present study are of paracytic type (Fig 9) in which the stoma is accompanied on either side by one or more subsidiary cells parallel to the long axis of the pores and guard cells.

The stomatal size and distribution of all the five varieties were scored and the results are presented in table 19.

It is clear that the varieties differ widely in stomatal size and distribution. The variety Honeymoon Red is having the smallest stoma (Fig 10) while the variety Lady Jane (Fig 11) has the largest. Pink has the minimum number of stomata per unit area while Lady Jane has the maximum number, when all the five varieties are considered together.

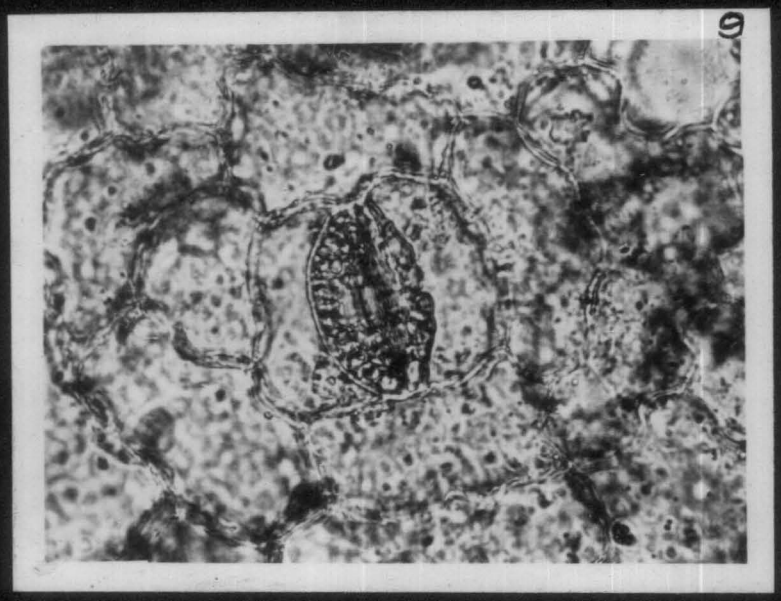
Figure 9 Paracytic type of stomata of A. andreanum (1560 x)

Figure 10 A single stoma (var. Honeymoon Red)

(3900 x)

Figure 11 A single stoma (var. Lady Jane)

(3900 x)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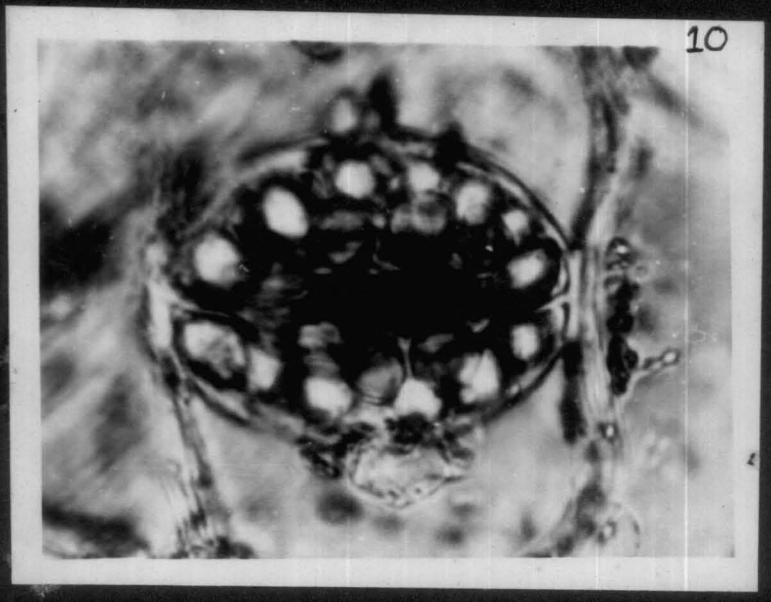
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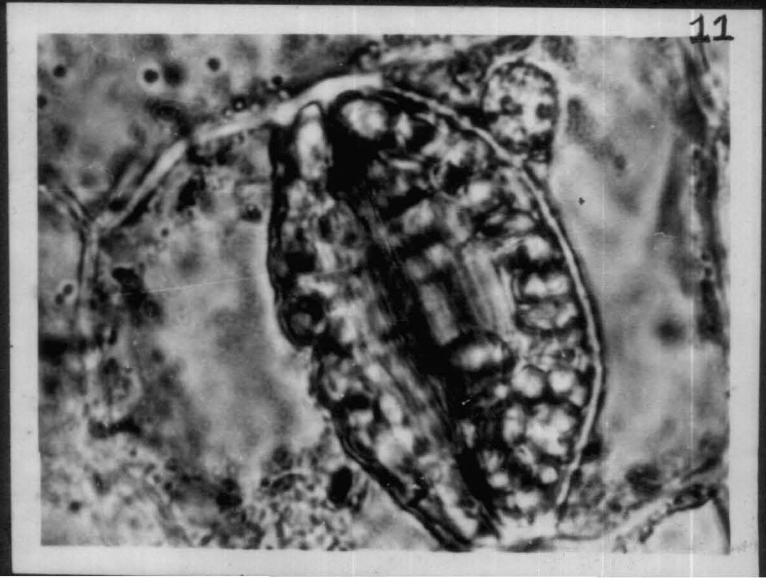
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Table19.Distribution and size of stomata

Variety	size in μ	Distribution
Honeymoon Red	123.2x64.0	24
White (album)	190.4x94.4	28
Lady Jane(Pink)	166.4x84.8	40
Chillired	150.4x89.6	32
Pink	196.8x107.2	20

Distribution per unit area (10x X 10x) was taken

DISCUSSION

DISCUSSION

The importance of studying morphological characters in identifying species as well as varieties is well known. Several workers have distinguished different varieties of crop plants based on their external appearance. In the genus Anthurium also, different varieties can be identified and classified on the basis of external morphology. Some of the characters studied in the present investigation have been studied earlier by Lalithambika (1978) and Satyadas (1985) in two other varieties of A. andreanum ie 'roseum' and 'rhodochlorum'. All the five varieties used in the present study have clear morphological character variations so that the varieties can be identified even in the absence of their typical spadices. This behaviour is probably because of the hybrid nature of the species which is further confirmed by cytological and pollen studies.

Mitosis of the root tip cells was studied in all the five varieties of Anthurium andreanum. For all varieties a somatic chromosome number of 2n = 30+2B was recorded. This is in accordance with the earlier observation of Kaneko and Kamemoto (1978) in the variety 'Uniwai' of Anthurium andreanum, though the chromosome number of the five varieties under the present study has not been previously reported. Sharma and

Bhattacharya(1961) had reported a chromosome number of 2n=30+0 to 2B in varieties 'roseum' and 'rhodochlorum'. The somatic chromosome number of 'roseum' was later confirmed as 30+0 to 2B by Lalithambika (1978). However Satyadas in 1985 reported the somatic chromosome number of 'roseum' as 2n = 30+1B and that of 'rhodochlorum' as 2n = 30 without any B chromosomes. A chromosome number of 2n=30 has already been reported by several workers such as Gaiser (1927), Kurakubo (1940), Itô (1942), Simmonds (1954), Pfitzer (1957a), Sharma and Bhattacharya (1961) and Sheffer and Kamemoto (1976) for various varieties of Anthurium andreanum. Haase-Bessell (1928) and Tsuchiya and Takada (1962) reported a chromosome number of 2n=32 without any B chromosomes in Anthurium andreanum. Sheffer and Kamemoto (1976) suggested that the satellites in Anthurium species are often loosely attached, and therefore possibly in earlier counts of 2n=32, the two satellites were counted as separate chromosomes. But studies of Kaneko nd Kamemoto (1978), Lalithambika (1978) and Satyadas (1985) clearly demonstrated the presence of B chromosomes in different varieties of A.andreanum. Kaneko and Kamemoto (1978) suggested that the discrepancy in chromosome counts by previous workers might be attributed to the occurence of B chromosomes and the number 2n=32 reported earlier for A.andreanum probably represents 2n=30+2B.

Sheffer and Croat (1983) reported that the most common somatic number of the genus Anthurium is 30. Four polyploid series are discernible from the composite chromosome distribution in the genus. In terms of somatic chromosome numbers the four series are (1) 20-40-60 (2) 24-30-48-84 (3) 28-56 and (4) 30-60-90 - ca.124. They supported the contention presented by Sheffer and Kamemoto (1976) that the basic chromosome numbers for the genus were 5,6 and 7 with 15 being a secondary basic number (x_2). Based on chromosome numbers, crossability studies and gross morphological studies, Sheffer and Kamemoto (1976) proposed a basic chromosome number of x=6 for the genus Anthurium unlike the other members of the family Araceae where a basic number of x=7 has been reported (Jones 1957; Larsen 1969; Marchant 1973; Raven 1975).

Sheffer and Croat (1983) suggested that the existing species in Anthurium would be palaeopolyploids (palaeoaneuploids), which observation is consistent with the statement of Goldblatt (1980) that most of the genera in Araceae were palaeopolyploids.

Sheffer and Croat (1983) also proposed that 16% of the Anthurium species are intrageneric polyploids when chromosome number above 2n = 30 were considered as polyploids. Accordingly the existing species of Anthurium were apparently built on the basic numbers n=10, 12, 14 and 15 which seemed to

to suggest a small role for polyploidy in speciation other than the initial palaeopolyploidy. If species with n = 15 were considered as polyploids as suggested by Goldblatt (1980), then 98% of the species in Anthurium would be intrageneric polyploids. This situation seems to be unlikely in the light of the observations on chromosome behaviour and secondary associations of chromosomes made on the five varieties of A.andreanum in the present study, which is discussed at the end of this chapter.

Other than the palaeoaneuploidy that involved changes in x=5, 6, and 7, aneuploidy is apparently an uncommon phenomenon in the genus Anthurium. Some of the reported aneuploids perhaps reflect the counting of B chromosomes, satellites or chromosome fragments as A chromosomes. Sheffer and Croat (1983) reached the conclusion that paleoaneuploidy, polyploidy and B chromosomes are basic features of the genus, whereas subsequent recent aneuploidy is not.

Satyadas (1985) visualised an amphidiploid origin of x=15 through natural hybridisation between forms with n = 7 and n = 8 followed by chromosome doubling, in the early evolution of the genus, Anthurium. The basic numbers of 7 and 8 exist in the family.

All the five varieties in the present investigation had two B chromosomes each. Sheffer and Croat (1983) reported that

B chromosomes are basic features of the genus Anthurium. B chromosomes or accessory chromosomes are nonhomologous chromosomes which are found over and above the normal zygotic chromosome complement. They differ from the basic chromosomal complement mainly with respect to inconstancy in number, smaller size and greater degree of heterochromatisation (Stebbins, 1971). According to him, the number of accessory chromosomes may differ from one tissue to another of the same organism, and from one individual to another of the same population. This discrepancy in the B chromosome number had been observed in A.crystallinum (Lalithambika, 1978). Kaneko and Kamemoto (1979) reported that the B chromosomes of Anthurium warocqueanum were found deeply stained and different from the somatic set in size and morphology. The B chromosomes of the five Anthurium varieties in the present study were also seen to be deeply stained, smaller in size than the smallest A chromosome of the genome and were either round or rod shaped.

B chromosomes differ from the chromosomes of the standard complement in their genetic constitution which does not strongly influence the individual (Stebbins, 1971). However Bolkhovskikh (1989) had reported that the B chromosomes of Ornithogulam abionicum influenced the size and DNA content of the autosomes. Marutani and Kamemoto (1983) found that B chromosomes showed abnormal behaviour during meiosis in Anthurium warocqueanum.

The principal physiological effects of Bchromosomes are on the overall vigour of the plant as well as on pollen fertility (Stebbins, 1971). Kaneko and Kamemoto (1979) reported from preliminary observations that B chromosomes had no effect on plant morphology in Anthurium warocqueanum.

According to Stebbins (1971), the facts that B chromosomes show no signs of homology with chromosomes of the basic set (few exceptions not withstanding) and that they are completely different in size and morphology, suggest that in most species which have them, they are not recently derived from the basic genome. This seems to be applicable to the accessory chromosomes of A.andreanum also.

According to Moss (1966), the presence of B chromosomes in parents increased the variability of their progenies over and above the variability directly determined by the A chromosomes. The wide range of morphological and physiological variations shown by the different cultivars of A.andreanum may probably be due to the presence of B chromosomes also to some extent.

Lalithambika (1978) and Satyadas (1985) reported that the B chromosomes of Anthurium do not conform to any set or clear cut pattern with regard to their occurence, number, size, shape, behaviour and effects. Contrary to this report, in the present investigation, a constant number of B chromosomes ie.

two, was recorded for all the five varieties. Their size is smaller than the smallest A chromosome of the genome for each variety. In populations of Crepis capillaris, Parker et al. (1989) reported that the B chromosomes were extremely stable between roots and between sampling times, which proved the stability of B chromosomes. In the present study also, a constant and stable number of B chromosomes was found in root cells as well as pollen mother cells and also at all the various sampling times.

Regarding the origin of B chromosomes, Kapoor (1978) reported that the B chromosomes of Solidago sempervirens and S.uliginosa arose either by misdivision or by disarticulation at the weak secondary constriction in the chromosomes with satellites. This observation was generally supported by the size of supernumerary chromosomes which was approximately that of satellites. A similar origin of B chromosomes was also reported by Markarian and Schaeffer (1958) in Anthoxanthum odoratum and Secale cereale and Kapoor (1973) in Clintonia borealis. The origin of B chromosomes where the short arms and satellites in a complement are intact as in Solidago macrophylla and S.juncea had been explained on the basis of chromosome rearrangements especially reciprocal translocation (Kapoor, 1978). The possible method of origin of B chromosomes in Anthurium andreanum is yet to be investigated. The presence of intact satellite chromosomes in the complement rules out their origin from the satellites.

Eventhough, all the five varieties in the present study are morphologically and physiologically diverse, all of them had a constant and stable chromosome number of 30+2B

The karyotype analysis of the five varieties had been done and the varieties were compared in respect of length of the chromosomes and presence of satellites, total chromosome length of the genomic complement (TCL), relative chromosome length (RCL), F percentage, arm ratio (r) and centromeric position, idiogram formula and karyotype asymmetry category, TF percentage and accessory or B chromosomes.

All the five varieties recorded a pair of satellite chromosomes. This finding is in conformity with the report of Kaneko and Kamemoto (1978) in Anthurium andreanum var. 'Uniwai' and 'Kaumana', where they reported a pair of fairly large chromosomes with satellites. Satyadas (1985) also found two satellite chromosome in A. andreanum var. 'roseum'. But in the variety, 'rhodochlorum', she reported two pairs of satellite chromosomes and the secondary constriction was on the longer arm of the chromosomes. The secondary constriction in all the five varieties in the present study is on the longer arm of the chromosomes confirming the earlier reports. However a contradictory observation was made by Satyadas (1985) in A.andreanum var. 'roseum'. Here the position of secondary

constriction is on the short arm of the satellite chromosomes. According to Stebbins (1971), the difference in the number and position of satellites reflects difference in the location and size of nucleolar organiser regions.

The length of the chromosomes in the five varieties studied ranged from 2.00 to 6.78μ. In general, the variety, Honeymoon Red has the longest chromosome of the complement ie 6.78μ and the variety Lady Jane has the shortest chromosome ie 2.00μ. A much higher value of 8.33μ for the largest chromosome was recorded for another variety A.andreanum var. 'roseum', (Satyadas,1985). The average length of the chromosomes among the five varieties ranged from 3.40 to 3.91μ. The highest value is for the variety White(album) and the lowest value is for Pink.

Such variations in chromosome length among varieties have also been reported in other crops. Sreekumari and Mathew (1991) while studying two morphotypes of taro (Colocasia esculenta (L) Schott) reported that the length ranged from 1.66 to 3.60μ for type I and 1.33 to 3.00 μ for type II. Similar small variations in chromosome lengths were reported by Zhan et al.(1988) in four cultivars of Arachis hypogaea. The variation in chromosome lengths may be due to the various types of chromosome rearrangements in the course of evolution.

Total chromosome length of lthe genomic complement among the varieties ranged from 51.02 to 58.71μ. The largest total chromosome length was recorded in the variety album and smallest is in Pink. The five varieties under the present study exhibit significant variation in TCL. The TCL of Pink is significantly lower than the other varieties. The present observation of TCL of the five varieties is in confirmity with the report of Satyadas (1985) in Anthurium andreanum var. 'rhodochlorum' in which the TCL was 51.48u. But she reported a much higher value of 70.20μ in A.andreanum var. 'roseum'. The total mass of the chromosomes in a nucleus is closely correlated with its DNA content (Stebbins, 1971). It suggests that at any particular stage of development, the ratio of DNA to protein is relatively constant in eucaryotic organisms. Because of this correlation, by examining the chromosomes, we can make reasonable inferences about the DNA content of an organism and hence the amount of genic material which it possess. Such comparisons must be made at comparable developmental stages and under similar environmental conditions. Stebbins (1971) proposed that differences in absolute chromosome size between related species or genera probably reflects different amount of gene duplication either in tandem fashion or through polytene multiplication of chromonema. This can be the reasonfor the differences in total chromosome lengths observed in the five varieties under the present study.

Absolute chromosome length can vary appreciably from cell to cell due to differential effects of pretreatment (Tjio and Hagberg 1951; Schlarbaum and Tscuchiya 1984). Relative length, based on a value of 100 for the haploid complement, was instead used to designate size class (Battaglia, 1955). The RCL value of the five varieties of A.andreanum varied from 3.46 for the smallest chromosome (var.Lady Jane) to 12.09 for the largest chromosome (var. Honeymoon Red). The average RCL value for the whole genomic complement ranged from 6.66 (var.Chillired) to 7.139 (var.Honeymoon Red). As postulated by Delaunay (1926), there will be reduction in chromosome size of the chromosomes with advancing evolution which was also reportd in Crepis by Babcock and Cameron (1934). By these criteria the variety Honeymoon Red with an RCL of 7.139 appears to be the least evolved among the five varieties while Chillired (RCL-6.66) is the most evolved.

Most of the chromosomes of all the five varieties studied are having a high F percentage indicating they are either with median or with submedian centromeres. Based on F percentage, the chromosomes were classified (Shindo and Kamemoto, 1963) as median (45-50%) submedian (30-45%) and values below 30 indicate subtelocentric and telocentric. Based on this classification, of the 15 pairs of somatic chromosomes of Honeymoon Red, 12 pairs are submedian, 2 pairs are median and one pair ssubtelocentric. In the variety White (album), 5 pairs of chromosomes are submedian, 5 pairs median and 5 pairs

subtelocentric. The variety Lady Jane has 11 pairs of submedian chromosomes, the rest being subtelocentric. Two pairs of chromosomes of the variety Chillired are with median, 10 pairs with submedian and the rest with subtelocentric centromeres. Ten pairs of chromosomes of the variety Pink are submedian, two pairs median and the rest are subtelocentric. It is clear from these observations that as the F percentage decreases, the chromosomes become more and more asymmetrical. The average F% among the varieties varied from 34.132(Lady Jane) to 37.213(White).

In the morphologic identification of chromosomes, the location of the centromere is the most useful landmark, and one which is characterised by great constancy (Levan et al., 1964). Based on the values of arm ratios and following the nomenclature proposed by Levan et al.(1964), each and every chromosome of all the five varieties have been identified in the study and properly named. Though most of the chromosomes of the five varieties are with either median or submedian centromeres and thus the individual chromosomes are more or less symmetrical, still there are subtelocentric chromosomes also in varying numbers in each of the varieties. In addition, there is a significant difference in sizes of chromosomes within the genomic complement. The presence of karyotype differences even between the varieties of the same species suggests that karyotype analysis may not be useful in the delimitation of the

taxa but would be helpful in assessing the evolutionary trends in the group of Anthurium andreanum as a whole. Such a behaviour was reported by Rao et al.(1980) in Solanum spp.

According to Levan (1935) primitive types have median or submedian centromeres, while with advancing evolution, the chromosomes become subterminally constricted indicating an evolution from symmetry to asymmetry. As the location of centromeres in the chromosomes of the five varieties of Anthurium andreanum under this study appears to change from median to submedian and submedian to subtelocentric positions leading to asymmetry of the karyotype, such an evolutionary trend as suggested by Levan (1935) seems applicable in Anthurium also. Based on centromeric position, the most evolved variety appears to be Lady Jane withmost chromosomes having submedian centromeres and some [illegible] having subtelocentric centromeres. According to Stebbins (1971), increasing asymmetry of the chromosomes can be due to a shift of centromeric position from median to subterminal or terminal position or by a pericentric inversion. The reason for increasing asymmetry in Anthurium has yet to be identified.

The centromeric positions of the individual chromosomes of the genomic complement are correlated together to constitute a karyotype or idiogram formula for each variety. The idiogram formula is different for the five varieties under the present

study. So it will be difficult to work out a general formula for the species A.andreanum. However, the number of chromosomes with median or submedian centromeres is more for all the five varieties when compared to the number of subtelocentric chromosomes. Thus in spite of the individual differences in idiogram formulae of the five varieties, all appear to belong to 3B karyotype asymmetry category.

As the value of TF% (the ratio of total short arm length to the total chromosome length) decreases, the karyotype becomes more and more asymmetrical. Increasing asymmetry, results from pericentric inversions and unequal translocations involving chromosome arm (Stebbins, 1971). According to him, the increasing asymmetry of karyotype, therefore, takes place without changing the number of centromeres or the number of independant chromosomes. Honeymoon Red having the highest TF% is probably the most primitive among the five varieties while Lady Jane with the least TF% appears to be the most advanced.

All the varieties studied, possess a stable number of accessory of B chromosome ie two irrespective of the varietal type and showed a uniform length in each variety within a range of 0.83-1.16μ. They are smaller than the smallest A chromosome in all the five varieties. Battaglia (1964) suggested that the B chromosomes of Anthurium are telocentric. But Sharma and Bhattacharya (1961) and Satyadas (1985) observed that the B chromosomes in some varieties of A.andreanum had no centromeres.

In the present study, the B chromosomes were always found as laggards never orienting themselves in the metaphase plate. This indicates that they probably are acentric but unlike strictly acentric fragments which are usually lost during division, these B chromosomes were invariably included in the daughter nuclei at the end of division. This fact seems to point out that the B chromosomes in these varieties are probably telocentric though evidences for the presence of telocentric centromeres could not be obtained.

B chromosomes in all the five varieties were found to divide normally in mitosis as in A. warocquenum as reported by Marutani and Kamemoto (1983). They proposed that the occurence of bivalent B chromosomes indicates that they are homologous or partially homologous and hence have a common or similar origin. In the present study also paired B chromosome bivalents were often observed indicating partial or complete homology and a common or similar origin.

Cytological observation on five varieties of Anthurium, studied indicate that , the karyotypes of all the varieties are apparently similar in gross karyomorphology as they are all in the same karyotype asymmetry category (3B) and have comparable TF%. The chromosomes are small sized with the average chromosome length ranging from 3.40 to 3.91μ. Karyotypes of all the varieties are predominated by m and sm

type chromosomes. However changes in gross chromosome morphology among the varieties appear to be extensive enough that a general karyotypic formula cannot be constructed.

When the idiogram formula and TF% were considered together, the variety Lady Jane is the most evolved and the variety Honeymoon Red is the least evolved. While the chromosome lengths and arm ratio were considered, the variety Pink is most evolved and the variety White is least evolved. With regard to the character, relative chromosome length, Chillired with the lowest value seems to be most evolved while Honeymoon Red with the highest RCL value is least evolved.

The presence of karyotype differences between the varieties of the same species suggests that karyotype analysis would be helpful in assessing the evolutionary trends in the species. The constancy in the somatic and B chromosome number, and lack of evidence of large differences in the karyotypes and the existence of structural differences in chromosome morphology between the species suggest that specialisation in this species has been principally effected by structural alterations of chromosomes such as pericentric inversions, unequal translocations, centric shifts etc.

While distinctive karyotypic differences signal, differences in the genetic make up of the chromosomes, identical or nearly

identical chromosome morphology is not an indication of homology (Smith and Levin, 1967). Levin (1966) has shown that decreased fertility and decreased chiasma frequency may be apparent in hybrids between karyotypically similar taxa, even when evidences of large structural differences in chromosomes are lacking. This presents a difficulty in the proper interpretation of relationships based upon karyotypes. This is applicable in the present study also. It is critical that one must evaluate this evidence along with all other evidence before taxonomic decisions are reached. Karyotype data are probably useful when they can be coupled with data on meiosis in hybrids (Smith and Levin, 1967).

Observations on the meiotic behaviour in the five vareties revealed the pattern of secondary asociation among the chromosomes.

According to Dyer (1979), the possible reasons for univalent formations were attributable to three factors acting seperately or together, ie physical factors within the environment, the genotype within the individual and the number and distribution of homologous segments within the complement. According to him, environmental disturbances such as extremes of temperature during meiotic prophase can inhibit pairing or chiasma formation. It is known that various aspects of chromosome behaviour, including homologous pairing, chiasma

formation and distribution, co-orientation and disjunction are controlled by genes on the chromosomes themselves and mutations at these loci can result in partial or total inhibition of the events in some individuals. Univalents occur consistently, even in plants grown under natural condition and the most likely explanation for this is the lack of homology.

Formation of univalents was reported in species such as Anthurium crystallinum and A.warocqueanum (Lalithambika, 1978), Marutani and Kamemoto (1983) reported that in Anthurium species with two B chromosomes, the B chromosomes may or may not be paired. The paired B bivalents generally undergo normal disjunction at anaphase - I. However, in some cases, the paired B chromosomes lag at or near the metaphase plate or move toward the same pole. The behaviour of unpaired B chromosomes at anaphase I is more unpredictable than that of paired ones. They may move toward either pole, lag or the two sister chromatids of each B chromosomes may seperate to opposite poles. Varying numbers of univalents ranging from 2-14 were reported in Anthurium andreanum var. Kaumana and 3-10 in var. Uniwai' by Kaneko and Kamemoto (1978). In the present study also varying numbers of univalents(two to eight) have been observed. The possible reason for univalent formation in all the five varieties studied, is probably lack of homology. Though the two B chromosomes of the varieties Pink and Lady Jane are found to

be often paired which suggests a possible homology, the B chromosomes of the other varieties, ie White, Chillired and Honeymoon Red are seen unpaired on either side of the metaphase plate.

All the varieties showed unequal segregation at anaphase I. This can be due to the varying numbers of laggards or movement of B chromosomes 'enbloc' to one pole as suggested by Lalithambika (1978) and Satyadas (1985). Marutani and Kamemoto (1983), while studying the transmission of B chromosomes in Anthurium warocqueanum reported that the association of B chromosome with a part of an A chromosome at the centre of the cell causes an uneven distribution of the A chromosomes, possibly leading to the formation of a micronucleus at the end of meiosis. Such micronuclei have also been observed in all the varieties under the present investigation.

During metaphase I, all the chromosomes of the five varieties are clumped together. According to Lalithambika (1978) in Anthurium andreanum, 15 seperate bivalents are very rare and this species showed the extreme case of clumping and stickiness. She also reported clumping and stickiness in other species of Anthurium such as, A.crystallinum. A crassinervium and A.veitchii, and suggesteed that the clumping and stickiness might be due to some metabolic or physiological disturbances. Darlington (1937) pointed out that the surface properties of the chromosomes are important in maintaining the sp acing between the chromosomes. The same may probably be the cause for clumping and stickiness observed here.

Varying numbers of laggards (1 to 10) were seen in the pollen mother cells of the five varieties during meiosis. At anaphase I, the bivalents seperate and move towards the poles in most cases. The univalents fail to become oriented on the metaphase plate and may be seen in the spindle or oriented on the metaphase plate outside the spindle (Lalithambika, 1978). At late anaphase, they may pass to the pollen without dividing or they lag behind or form laggards which are eventually lost. But Marutani and Kamemoto (1983) reported that the single B chromosome present in the offsprings of two Anthurium warocqueanum parents with three B chromosomes each, frequently lagged at or near the metaphase plate. Laggards were also reported in some species of Anthurium like A.crystallinum, A. crassinervium, A.tetragonum, A.veitchii, A.ornatum and A.andreanum (Lalithambika, 1978).

The presence of micronuclei was observed in all the five varieties at telophase II. Either the lagging univalents or the lagging B chromosomes that could not be included in normal chromosome complement formed micronuclei. Lalithambika (1978) observed that the lagging chromosomes in both meiosis I and meiosis II formed micronuclei. Marutani and Kamemoto (1983) reported that the irregular distribution and lagging of the B chromosomes of A.warocqueanum at anaphase I often lead to the formation of micronuclei.

Lalithambika (1978) suggested that the meiotic

abnormalities in Anthurium such as univalent formation, abnormal segregation, clumping, lagging of chromosomes at anaphase, early seperation etc may be due to physiological or metabolic disturbances, which may be caused by abnormal environmental conditions. She pointed out that anthuriums are introduced plants and the sensitivity of the different species to the changed environment varies, as shown by the variation in frequencies of the aberrations by the different species in her study. But in the present study, the irregularities in meiosis of the two varieties suggest a hybrid origin of A. andreanum as proposed earlier by Kaneko and Kamemoto (1978), rather than simple physiological causes alone.

Lack of secondary association, multivalent formation or regular stickiness of chromosomes to form secondary chromosome groups shows that the species A. andreanum is a diploid one with a basic chromosome number of x = 15. This conclusion is supported by the presence of 15 regular bivalents in diakinesis in all the five varieties under study. However, natural hybridisation seems to have played an important role in its origin and speciation as shown by the wide range of variability within the genus. The role of polyploidisation in its evolution (Sheffer and Croat 1983) has not been traced out.

From the cytological studies, it is clear that all the varieties of A. andreanum in the present study have a constant

somatic chromosome number of 30+2B. The number and occurence of B chromosomes are stable irrespective of the varietal types, tissues and sampling times in the present study.

The mitotic index of Anthurium andreanum has not been reported earlier. Dyer (1979) had reported that the typical value of mitotic index in an asynchronous system in general was about 10%. In the present study a much higher value of 14% was obtained for A.andreanum var. Pink and all the other four varieties showed higher mitotic indices than 10%. The present investigation indicates that, in genral, the mitotic division cycle in A.andreanum takes longer time. This is in agreement with the observation of Swanson (1982) that duration of the cell cycle increased in proportion to the number of B chromosomes.

The studies on the five varieties reveal that the anthurium flowers are bisexual, regular and protogynous. The species in the present investigation ie A.andreanum has not been included among the protogynous species of Anthurium reported and listed by Croat (1980). The flowers mature first from the base and proceeds upwards in an acropetal successin in all the five varieties studied. Croat (1980) had earlier reported a similar behaviour in some other species of Anthurium such as A.purpureospathum, A.ravenii etc.

Receptivity of stigma is identified by the presence of

honey dew or stigma droplets and insect activity (bees, ants etc). Such observations have also been made by Dauman (1931) and Croat (1980). Croat (1980) made this observations in species like Anthurium luteynii, A. purpureospathum etc. Croat (1980) reported that there are other species in which the stigmas do not secrete droplets, but instead they have a glistening appearance and are often found exerted and thus are assumed to be receptive by these indications eg. A.armeniense, A.caperatum, A.fatoense, A.panduriforme etc. Some inflorescences never exhibit a female phase. From the observations on five varieties in the present study, it is clear that the female phase for the species, A.andreanum varies from 3 days to 12 days. In most of the species of Anthurium such as A.panduriforme, A.oerstedianum, A.hacumense, A.pittieri etc. studied by Croat (1980), the duration of female phase recorded, is in conformity with the present observations. Species showing female phase as short as half a day (A.ravenii) to 1-2 days (A.kunthii, A.lentii etc.) or as long as 21 days (A.ochranthum, A.cotobrusii) to 28 days (A.caperatum, A.luteynii) were also reported by Croat (1980).

The stigmatic droplets dry up before any stamens emerge out. The seperation period or the interphase between the female phase and male phase for the species ranged from 4 days to 7 days normally. But in most of the species of

Anthurium listed by Croat, the dry period was for 1 to 2 days only. (A.hacumense, A.kunthii, A.lentii etc). The long interphase period is probably due to changed environmental conditions. In a few species, the interphase period is so short that it is not certain whether the species involved were homogamous or protogynous. eg. A.cotobrusii (Croat, 1980). Croat (1980) also reported the interphase in A.pittieri as, long as 10 to 20 days. During rainy season, the interphase is prolonged and or the male phase is completely suppressed. Sometimes a few stamens appear on the candle irregularly.

Male phase is identified by the appearance of stamens on the candle. Croat (1980) observed varying degrees of exertion of stamens on the surface of the candle ranging from 0.5-1.5mm. But in A.pittieri, anthers were not exposed. Anther exertion starts from the base and proceeds regularly towards the apex and thus one candle may be in male phase for 3-7 days. Croat (1980) listed several Anthurium species showing such abehaviour and also found that in some species, the inflorescence may be in male phase for several weeks. Instead of regular anther extrusion on the candle, it was scattered in A.caperatum, sporadic in A.luteynii (Croat, 1980). He also observed that in A.hacumense, the anther extrusion started from the middle and proceeded towards the

ends. The stamens of A.andreanum are clustered around the pistil. A similar behaviour was observed in the species A.lentil by Croat (1980). Species showing the spacing of stamens as cluster (A.hacumense), tight cluster (A.ravenii), loose cluster (A.panduriforme), tight circle over the pistil (A.kunthii), open circle (A.lancetillense) or on four sides of pistil (A.luteynii) was also reported by Croat (1980)

Anthesis as well as anther dehiscence occur during the early morning hours. The former is affected by low temperature and the latter by relative humidity. Change in atmospheric humidity is the most frequent causative agent leading to hygroscopic shrinkage and anther wall rupture (Stanley and Linskens, 1974). In Psidium (Seth 1962; Sandhu 1988), some species of Saccharum (Kainth and Tariq, 1969), Annona reticulata (Farooqi et al., 1970) and bhindi (Mishra and Singh, 1988), the anthesis and anther dehiscence are favoured by low temperature of the day. But Boyer (1970) reported that in Pinus, low temperature will both delay and extend the period of dehiscence. Stanley and Linskens (1974) reported that in most plants, anthers dehisce in the morning; in some plants, at two peaks during the day; and in a few plants anthers dehisce at night. In mango (Singh, 1954) citrus (Singh and Dhuria,1960) and Dioscorea (Abraham and Nair, 1990), anthesis is favoured by the high temperature of the day.

Anthurium andreanum is a protogynous species and is thus highly adapted for cross pollination. Natural pollination is through the agency of insects ie entomnophilous. As reported by Croat (1980), excessive hybridisation is not apparent in this genus. The easily accessible sexual parts of flowers present no physical barriers to pollination and different varieties are frequently not separated temporally. For high seed setting, we have to resort to hand pollination. Anthesis and anther dehiscence are favoured by environmental conditions like temperature and relative humidity which is evident from the suppresssion of anthesis during rainy season.

The average pollen grain of the five varieties when compared, is not significantly different. The variety Lady Jane with the smallest sized spathe and candle has the largest pollen grains (87.2x86.4μ) while the variety pink with the largest spathe and candle has the smallest (81.8x68.0μ) pollen grains. The pollen grain size of the other three varieties comes in between these two.

The average pollen grain size of the species A. andreanum is 83.2x75.7μ. Wodehouse (1935) reported that, in majority of the anemophilous plants, pollen grain size is within the limits of 17.0 to 58.0μ. Species with smaller or larger grains are generally zoophilous or entomophilous. Pollen grains

of A.andreanum are larger in size and are entomophilous.

Singh (1962) while studying 10 varieties of litchi reported that the size of pollen grains ranged from 19.9-35.2µ. ie in size the pollen grains are similar in all the 10 varieties. According to him, this finding is important in view of the fact that size differences in pollen in species are generally correlated with polyploidy or aneuploidy and it suggests a stability in chromosome number in the 10 varieties. A stable chromosome number of 30+2B in A.andreanum is reflected in the similar size of pollen grains in the five varieties under the present investigation. But Singh (1960) studied 16 varieties of Prunus persica and reported a wide range (38.4-51.2µ) of averge pollen grain size. Some authors reported highly significant positive regression coefficient for pollen size within various species (Nissen 1950, Gould 1957; Bell 1959; Kapadia and Gould 1964). Harris (1956) reported that in families like Fagaceae, intraspecific vaiation in size is greater than interspecific variation.

Some other factors are also attributed to size difference. The pollen grains of plants like Petunia vary from flower to flower (Harris, 1956). In some plants, variation is found even from anther to anther on individual

plants. (Kuprijanov, 1940). Several authors reported that the pollen size changes over the flowering periods (Krumbholz 1926; Schwanitz 1952). The possible size variation in pollen grain affected by the above mentioned factors has not been recorded in the present study.

Apart from the above mentioned factors, some external factors such as temperture, mineralnutrition and water conditions also have an influence on the size difference. Since all the five varieties studied are grown under similar environmental and management conditions, the effect of external factors on size variation can be ruled out.

The small variation in pollen grain size exhibited by these varieties can be explained as natural intraspecific differences usually associated with cultivars belonging to the same species.

The pollen grains of all the five varieties are more or less round with a single germ pore. Croat (1980) reported the pollen grain shape of Anthurium as suboblate, three or four porate with equatorial pores and a reticulate incomplete tectum. According to Wodehouse (1935), the pollen grains of the same species and of closely related species tend to be alike. If the environmental factors are uniform, the degree of their similarity is a measure of thier closeness of relationships. For example, the pollen grains of tansy,

chrysanthemum, camomile, and daisy (all belong to the same tribe of the family Compositae) are so much alike that they can scarcely be distinguished from each other. They all have a thick, coarsely granular exine, bearing sharp conical spines and their surface is co vered with a copious layer of oil. They all have three characteristic germinal furrows, each enclosing a round germ pore. The similarity of these species is clearly a manifestation of their relationship and such characters, are purely hereditary. With regard to the external morphology of pollen grains, the five different varieties of Anthurium andreanum studied here are more or less similar in their external morphology. Thus the varietal identification by studying the pollen morphology under a light microscope is not possible here as in crops such as Chysanthemum morifolium (Shim et al.,1989), sour cherry (Pradescu et al., 1985) etc where the external morphology can be established as a varietal character.

Stanley and Linskens (1974) reported that the yield of pollen grains varies with the species. According to them, the anemophilous species always outproduce entomophilous species and the hydrophilous species usually produce very few pollen grains. Since Anthurium andreanum is an entomophilous crop, the pollen production is not as high as that of some varieties of anemophilous wheat (Beri and Anand, 1971) but higher than that of hydrophilous Vallisneria (Pohl, 1937).

The variety, Lady Jane produces the lowest amount of pollen per anther (1213), probably because it has the smallest sized flowers and anthers among the five varieties studied. The variety Pink produces maximum number of pollen (1725) per anther which may be due to the larger size of the flower.

Stanley and Linskens (1974) proposed that the differences in pollen production can be quite great on the same plant in successive years. So quantities measured in one year need not be a good index of a given plants production capacity in successive years. They also reported that the capacity to produce pollen is primarily under genetic and physiological control. Beri and Anand (1971) while comparing 22 varieties of wheat reported that the taller varieties produced larger florets and longer filaments with bigger anthers. Plant height and anther size were positively correlated with the amount of pollen shed. Similar results were obtained in the present study of Anthurium as well. The larger varieties, Pink and Honeymoon Red produced comparatively higher amount of pollen grains than the other varieties. A higher amount of pollen is produced by the plant during the initial years of its life (Stanley and Linskens, 1974). This can be a reason for a comparatively high amount of pollen production by all the plants under the study as they were all three to five years old young plants.

Pollen fertility was tested by using acetocarmine staining and in vitro pollen germination methods.

In acetocarmine staining method, acetocarmine preferentially stains the chromosome or nucleus (Owizarzak, 1952). Most stains are not sufficiently accurate when compared to germination tests to give other than crude estimates of pollen viability(Vazhnitskaya 1960; Nagorajan et al 1965).

In in vitro pollen germination method, it is assumed that the optimum conditions must have been established so that germination approximates that on the plant (Stanley and Linskens, 1974). From various reports it was clear that he pollen grains of most of the plant species will germinate in a medium containing sugar which is supplemented with boron or calcium. The sweet stigmatic droplets in several species of Anthurium (Croat, 1980) shows that it is sugary in nature. Stanley and Linskens (1974) reported that some chemicals stimulating germination in vitro like boron, calcium, magnesium were similar or identical to factors found in style tisssue or stigmatic fluid in which the pollen naturally germinates. Reports of various workers on in vitro pollen germination of various crops such as litchi (Singh, 1962), Olive(Porlingis and SFAkiotakis, 1968). Cucumis melo (Aswathy, 1969), bhindi (Dubey, 1969), Java plum (Misra, 1972), sugarcane (Moore and Jung, 1974), cardamom (Parameswar

and Venugopal, 1975), Cicer spp (Mercy et al., 1976), cocoa(Ravindran, 1977), date palm (Boughediri and Bounaga, 1987), shoe flower (Markose and Aravindakshan, 1987), guava (Sandhu et al., 1988), Gladiolus (Choudhary, 1991) show that for obtaining good in vitro germination of pollen, the medium should be supplemented with sugar and boron. In the present study good pollen germintion is obtained for all the varieties in a medium containing 0.5M sucrose and 100 ppm boric acid which is incubated at room temperature for 5 hrs.

The results show that the variety Pink has high pollen fertility among the five varieties and the variation shown are statistically significant from that of Honeymoon Red and Chillired.

The percentage of fertile grains examined by acetocarmine stainability test is higher than that recorded by artificial germination in culture media. But they are positively correlated. From the results of the five varieties studied, it is clear that the pollen fertility of A.andreanum by acetocarmine staining method vary from 20.4-28.8% which is in conformity with the observations of Lalithambika (1978) and Satyadas (1985). Pollen fertility, as determined by in vitro pollen germination counts ranged from 9.7-17.9%. A higher pollen fertility by acetocarmine staining method than by in vitro tube growth was also reported in crops like Psidium (Seth, 1962), mango (Singh, 1961) and litchi (Singh,

1962).

Mitu and Acatrinei (1974), based on thier studies on pear cultivars reported that pollen germination in pear cultivars was proportional to stainability. Most of the reports doubted the reliability of acetocarmine or any other staining methods for determining the actual viability of pollen. Prolingh (1956) was however of the opinion that staining method may be considered as an index of pollen viability rather than a reliable index of pollen germinability. According to Seth (1962) it was quite desirable to record pollen fertility by staining method for a rough estimate of the range of pollen viability. Pearson and Harney (1984) reported that there was a positive and significant correlation between pollen staining and germination in Rose. Parameswar (1974) reported that although 85.2% of cardamom pollen grains appeared fertile in the acetocarmine staining technique, the maximum germination in an artificial medium containing 20% sucrose and 1% agar solultion was only 70%. He proposed that the testing of the viability of pollen may be done either by germinationof pollen *in vitro* or by estimating the fruit set after dusting receptive stigmas with pollen. The present study also indicates that acetocarmine staining method only gives an approximate estimate of pollen viability while the *in vitro*

pollen germination method gives actual pollen germination and as such the latter is a functional test of pollen fertility.

The high pollen sterility observed in this species can be due to the high degree of meiotic abnormalities such as clumping, lagging of chromosomes at anaphase, unequal segregation, precocious disjunction of chromosomes, chromosome elimination through micronuclei etc. Gardner et al. (1952) recognised sterility as a condition frequently associated with hybridity. The high pollen sterility in the cultivated species of A.andreanum can be due to its hybrid nature. Frost (1943) while studying sterility of pollen grains of Washington Navel Sweet orange, reported that the high sterility can be due to complete degeneration of pollen mother cells without cell division. Degeneration of pollen mother cells was not observed in any of the varieties of A.andreanum studied here.

As the five varieties in this study have been grown under identical environmental conditions and the pollen is collected from them at the same period, the effect of adverse environmental conditions like water stress, nonavilability of nutrients etc influencing pollen sterility measurements can be ruled out. Randolph (1941) reported that when B chromosomes are too many, they adversely affect vigour and fertility. Whether the B chromosomes of A.andreanum

affect the fertility or not, has yet to be investigated.

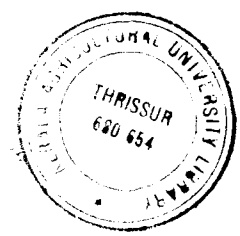
From the studies of different characteristics of pollen, it is clear that A.andreanum is a highly cross pollinated crop. Fruit set under natural condition was not observed in Lady Jane, Chillired and Honeymoon Red. A low percentage of natural fruit set was occasionally noted in A.andreanum var. White while in Pink natural fruit set was often high. This has been achieved probably through insect activity at female phase. There are reports that the plants are bee - pollinated in their native habitat. In the present study, ants were often observed moving about on candles in female phase. Fruit set can be considerably enhanced by artificial hand pollination.

The size and distribution of stomata showed a range of variation among the five varieties studied. Size varied from 123.2x64.0μ (Honeymoon Red) to 196.8x107.2μ (Pink) and the number of stomata per unit area varied from 20 (Pink) to 40 (Lady Jane). The variation in stomatal size and distribution indicates that the varieties are distinctly different in physiological characters as well.

The studies on various aspects of flowering behaviour such as anthesis, pollen production capacity, pollen viability etc. are useful for crop improvement research. As

the genetic potential of this commercially important cut flower crop Anthurium andreanum, remains unexploited, it is hoped that the detailed study of the floral biology and the standardisation of pollination techniques will stimulate new interest in Anthurium breeding.

When the diverging picture of homology indicated by karyotype analysis is correlated with the wide spectrum of meiotic abnormalities exhibited by the five varieties of Anthurium andreanum under the present study, it appears that the species, A.andreanum is a much evolved one with a basic hybrid nature. The basic chromosome number is 15. The high percentage of pollen sterility confirms the hybridity of the species.



SUMMARY

SUMMARY

Karyotype analysis and pollen studies are two important aids to assess evolutionary trends and systematic position of a crop. Detailed studies on these aspects are negligible in nthurium andreanum, the species under the present investigation. Anthurium is a valuable cut flower crop whose genetic potential has not yet been fully exploited.

In the present experiment, cytological and pollen studies were conducted in five commercially important varieties of A. andreanum viz. Honeymoon Red, White (album), Lady Jane, Chillired and Pink.

Some important morphological characters such as plant height, leaf size and shape and spathe and candle characters were studied. These studies have indicated that the varieties can be clearly differentiated from one another on the basis of morphology alone even without considering floral characters.

Under cytological studies, mitotic and meiotic behaviour of the chromosomes was analysed and the mitotic index was recorded. All the varieties recorded a somatic chromosome number of 30+2B chromosomes. Mitosis was normal and except

for a few cases of late seperation, not much chromosomal aberrations were noted.

All the five varieties studied had a stable number of two accessory B chromosomes, irrespective of the varietal type, time of sampling and tissues studied. The B chromosomes were smaller than the smallest A chromosome. They were either acentric or telocentric and rod shaped or round.

Under the karyotype analysis, total chromosome length (TCL), average chromosome length (ACL), relative chromosome length (RCL), arm ratio (r), centromeric index (F%), ratio of total short arm length to total chromosome length (TF%) and idiogram formula of all the five varieties were recorded and compared.

On the basis of TCL, ACL, and r, the variety Pink appears to be most advanced. When the RCL is taken in to consideration, the variety Chillired is found to be most advanced. The variety Lady Jane had the lowest F% and TF% and didnot have any chromosomes with median (M) constrictions. With regard to these criteria, Lady Jane appears to be most advanced among the group. When the karyotype asymmetry as a whole is considered, all the varieties are found to be on par, falling in the '3B' category which represents a high

position in the ladder of evolution among flowering plants.

Meiotic studies of the five varieties revealed a high percentage of abnormalities during the division stages. The abnormalities recorded were univalent formation, unequal segregation, presence of micronuclei and laggards, precocious disjunction of bivalents and chromosome elimination.

Both the B chromosomes and some of the A chromosomes were seen to form univalents in anaphase I and II in all the five varieties. This high frequency of univalents may be due to the lack of homology between the chromosome pairs which is often associated with hybrids. The presence of the B chromosomes and the univalents often lead to the unequal segregation of chromosomes. This explains the very high degree of pollen sterility observed in all the five varieties.

All the varieties under the present study had a high mitotic index ranging between 0.11 and 0.14 . This high value may probably due to the presence of B chromosomes, which causes an increase in the duration of the cell cycle.

All the five varieties were protogynous with a distinct interphase. This adaptation is helpful for natural cross

pollination. However only the variety Pink normally set seeds through natural crossing carried out by insect activity. A small amount of natural crossing was observed in White (album) also.

Pollen was uniformly round shaped with a single germ pore. With regard to pollen size, the variety Lady Jane had the largest pollen. Pollen production capacity of the varieties varied significantly. The varieties Pink and Honeymoon Red with larger flowers and anthers produced the highest amount of pollen. The lowest amount was produced by the variety Lady Jane, which had the smallest flowers and anthers.

When pollen fertility was tested by in vitro pollen germination method, the percentage was lower (9.7 to 17.9) than that obtained by acetocarmine staining method(21.4 to 28.8) As in vitro germination is a functional test, it is more reliablein determining pollen viability.

Though all varieties had paracytic type of stomata, when their size and distributi was studied, the five varieties showed clear differences ce again indicating the range of variability among them.

Correlating the divergent picture of homology indicated

by karyotype analysis with the wide spectrum of meiotic abnormalities exhibited by the five varieties of Anthurium andreanum under the present study, it appears the species A.andreanum is of a much evolved hybrid nature with a basic chromosome number of 15. The high percentage of pollen sterility indicating lack of homology between chromosome pairs and the wider variation in the size and distribution of stomata also confirm the hybridity of the species. This abundance of genetic variability present within the species points out the scope for achieving crop improvement through controlled hybridisation and selection.

R E F E R E N C E S

Abraham, K. and Nair, G.P. (1990). Floral biology and artificial pollination in Dioscorea alata L. Euphytica. 48 : 45-51.

Anderson, S.T. (1946). In: Stanley, R.G. and Linskens, H.F. (Eds) : Pollen Biology Biochemistry Management, p.26. Springer - Verlag Berlin Hiedel berg, New York.

Aswathy, D.N. (1969). Pollen viability, Fruit set and fruit development studies of Cucumis melo L var. Utilissimus. Plant. Sci.1 : 57-59.

Babcock,E.B. and Cameron, D.R. (1934). Chromosomes and phylogen y in Crepis II. The relationships of 108 species. Univ. Calif. Publ. Agr. Sci. 6 : 287-324.

Battaglia, E. (1955). Chromosome morphology and terminology. Caryologia. 8: 178-187.

Battaglia, E. (1964). Cytogenetics of B chromosomes. Caryologia 17 : 245-299.

Bell, C.R. (1959). In : Stanlely, R.G. and Linskens, H.F. (Eds): Pollen Biology Biochemistry Management, p.26. Springer - Verlag Berlin Heidelberg, New York.

Ben Hill, J., Overhots, L.O., Popp, H.W. and Grove, A.R.(1960). Botany. Mc Graw Hill Book Co. Inc. p.54.

Beri, S.M. and Anand, S.C. (1971). In : Stanley, R.G. and Linskens H.F. (Eds) : Pollen Biology Biochemistry Management P.31. Springer - Verlag Berlin Hiedelberg New York.

Bhattacharya, G.N. (1977). A cytological study on the tribe Anthurieae (Araceae). Bull. Bot. Soc. Bengal.30: 51-56.

**Bolkhovskikh, 2.V. (1989). Cytological study of some Ornithogulum abionicum population from the Armenian SSR. Botanicheskii Zhurnal. 74 (II): 1612-1617.

Boughediri, L. and Baunaga, N. (1987). in vitro germination of date pollen and its relation to fruit set. Datepalm Journal. 5(2) : 120-127.

Boyer, W.D. (1970). In : Stanley, R.G. and Linskens, H.F. (Eds): Pollen Biology Biochemistry Management, p.24 Springer - Verlag Berlin Heidelberg New York.

Chen, C.G., Chen, J.J. and Huang, C.S. (1986). Ploidy identification by stomatal size of rice plantlets derived from anther culture. Journal of Agrl. Res. of China. 35 (1) : 37-44.

Chen, J.Y., Zhao, S.M., Xu, J.X. and Wang, P.T. (1982). Obser vations on the pollen, fruits, seeds and stomata of somatic hybrids between N.tabaccum and N. rustica. Hereditas. 4 (3) : 31-32.

Choudhary, M.L. (1991). Response of sucrose and boric acid on pollen viability of Gladiolus spp. Haryana. J. of Hort. Sci. 20 (1-2) : 73-76.

Christensen, B.B. (1946). In : Stanley, R.G. and Linskens, H.F. (Eds) : Pollen Biology Biochemistry Management, p.26. Springer - Verlag Berlin Heidelberg New York.

Croat, T.B. (1980). Flowering behaviour of the neotropical genus Anthurium (Araceae). Amer. J. Bot. 67 (6) : 888-904.

Darlington, C.D. (1937). Recent advances in cytology (2nd Edn). Blakiston, Philadelphia. 671pp.

Darlington, C.D. and Wylie, A.P. (1955). Chromosome atlas of flowering plants. George Allen and Unwin Ltd, London.

** Dauman, E. (1931). Nectarabscheidung in der Blutenregion einiger Araceen. Planta. 12 : 39-52.

** Delaunay, L. (1926). Phylogenetische chromosomen verkurzung. Zeitschr. Zellf. u. mikr. Anat. 4 : 338-364.

Dubey, R.S. (1969). Studies on the artificial germination of okra pollen grains. Indian Journal of Hort. 26 : 181-183.

Dyer, F.A. (1979). Investigating chromosomes. Edward Arnold (publishers) Ltd. London - p.157.

Farooqi, A.A. Parvatikar, S.R. and Nalawadi, U.G. (1970). Preliminary studies on the problem of 'fruit set in Annona reticulata.L. 1. Floral biology. Mysore.J. of Agric. Sci. 4 : 44-53.

**Federov, A.A. (1969). Chromosome numbers of flowering plants. Academy of Science of USSR.

Ferguson and Muller. (1976). In: Nair, P.K.K and Lawrence, R. Advances in pollen spore research, p.1. Scholar Publishing. Home India.

Frost, H.B. (1943). Seed repoduction In : Webber H.J. and Batchelor, L.D. (Eds): Citrus industry I. Univ. Calif. Press, Berkeley.

** Gaiser, L.O. (1927). Chromosome numbers and species characters in Anthurium. Proc. Trans. Roy. Soc. Canada. 21 : 1-137.

Gardner, V.R, Bradford, F.C. and Hooker Jr. H.D. (1952). Fundamentals of Fruit Production. Mc Graw Hill, New York.

Geier, T. (1988). Ploidy variation in callus and regenerated plants of Anthurium scherzerianum Schott. Acta Horticulturae. 226 : 293-298.

Geier, T. (1989). Anthurium. In : Ammirato, P.V., Evans, D.A., Sharp, W.R. and Bajaj, Y.P.S. (Eds): Handbook

of plant cell cultures. vol.5. Ornamental species, p.228-252 Mc Graw Hill Publishing Company, New York.

Gilbert, C. and Breen, P.J. (1980), Low pollen production as a cause of fruit malformation in strawberry. J. of Amer. Soc. for Hort. Sci. 112 (1) : 56-60.

Goldblatt, P. (1980). Polyploidy in angiosperms : monocotyledons. In : Lewis, W.H. (Ed) : Polyploidy : biological relevance, p.219-239. Plenum Press, New York.

Gould, F.W. (1957). In : Stanley, R.G. and Linskens, H.F. (Eds): Pollen Biology Biochemistry Management, p.28.Springer - Verlag Berlin Hiedelberg New York.

Haase - Bessell, G. (1928). Karyologische unter suchum gen an Anthurium andreanum and A.magnificum. Planta. 6: 767-789.

Harris, W.F. (1956). In : Stanley, R.G. and Linskens, H.F. (Eds) : Pollen Biology Biochemistry Management, p.28. Springer - Verlag Berlin Hiedelberg New York.

Heitz, E. (1932). Die Herkunft dier Chromocentren. Planta. 18 : 517-636.

Hutchinson, J. (1934). The families of flowering plants : II Monocotylendons. London Mac Millan and Co.Ltd. London.

Huziwara, Y. (1962). Karyotype analysis in some genera of Compositae IX-Chromosomes of European Species of Aster. Bot. Mag. Tokyo. 75 : 143-149.

Itô, T. (1942). Chromosomen und sexualitat von der Araceae I Somatische chromosomenzahlen einigen arten. Cytologia. 20 : 313-325.

John, A.Q., Saini, S.S. and Sharma, P.P. (1988). Floral biology studies in pansy (vida x wittrockiana Gams). Haryana Journal of Hort. Sci. 17: 1-2.

**Jones, G.E. (1957). Chromosome numbers and phylogenetic relationships in Araceae. Ph.D Thesis, University of Virginia, Charlottesville.

Jones, R.N. and Rees, H. (1982). B chromosomes. Academic Press, London.

Kainth, N.A. and Tariq, A.H. (1969). Preliminary studies on anthesis and pollination in the genus Saccharum. W.Pakist. J.Agric. Res. 7 (4) : 147-151.

Kaneko, K. and Kamemoto, H. (1978). Cytological studies of 'Kaumana' and 'Uniwai' Anthurium. J.Amer. Soc. Hort Sci. 103 (5) : 699-701.

Kaneko, K. and Kamemoto, H. (1979). Karyotype and B chromosomes of Anthurium warocqueanum. J. of. Heredity. 70 (4) : 271-272.

Kapadia, Z.J. and Gould, R.W. (1964). In : Stanley, R.G. and Linskens, H.F. (Eds): Pollen Biology Biochemistry, Management, p.28. Springer - Verlag Berlin Heidelberg, New York.

Kapoor, B.M. (1973). Cytology and supernumerary chromosome fragments in Clintonia borealis. Caryologia. 26 : 457-462.

Kapoor, B.M. (1978). Supernumerary chromosomes of some species of Solidago and a related taxon. Caryologia. 31 (3) : 315-330.

** Kaseko, V.N. (1990). Possible trends of the evolution of the pollen grain wall in the genus Tulipa (Liliaceae). Botanicheskii Zhurnal. 75 (7): 929-942.

Kumbholz, G. (1926). In : Stanley, R.G. and Linskens, H.F. (Eds): Pollen Biology Biochemistry Management, p.29. Springer - Verlag Berlin Heidelberg New York.

Kunta, S. (1988). Variation and evolution in the karyotype of Lycoris, Amaryllidaceae. VII. Modes of karyotype alteration within species and probable trend of karyotype evolution in the genus. Cytologia. 53 (2) : 323-335.

Kuprijanov, S.I. (1940). In : Stanley, R.G. and Linskens, H.F. (Eds) : Pollen Biology Biochemistry Management, p.28. Springer - Verlag Berlin Hiedelberg New York.

Kurakubo, Y. (1940). Uber die chromosomenzahlen von Araceae - Arten. In : Darlington, C.D. and Wylie, A.P (Eds): Chromosome atlas of flowering plants, George Allen and Unwin Ltd. London.

Lalithambika, K. (1978). Cytological studies on twelve species of anthurium with special reference of B chromosomes. M.Sc. thesis, University of Kerala, India.

**Larsen, K. (1969). Cytology of vascular plants III. A study of Thai aroids. Dansk Bot. Ark. 27: 39-60.

Levan, A. (1935). Cytological studies in Allium VI. The chromosome morphology of some diploid species of Allium. Hereditas. 20 : 289-330.

Levan, A. Fredga, K. and Sandberg, A. (1964). Nomenclature for centromeric position on chromosome. Hereditas. 52 : 201-220.

Levan, A. and Hsu, T.C. (1959). The human idiogram. Hereditas. 45 : 665-674.

** Levin D A (1966) The Phlox pilosa complex Crossing and chromosome relationships. Brittonia 18 : 142-162

Levitzki, G.A. (1931), The Karyotype in systematics. Trudy Prikl. Bot. 27: 220-240.

Longley, A.E. (1927). Supernumerary chromosomes in Zea mays. J.Agr. Res. 35 : 769-784.

Marchant, C.J. (1973). Chromosome variation in Araceae: V.Acoreae to Lasiae. Kew Bull. 28 : 199-210.

Markarian and Schaeffer, S. (1958). A possible origin of super fragment chromosomes. J.Hered. 49: 3-7.

Markose, B.L. and Aravindakshan, M. (1987). Pollen biology in shoe flower. Agrl. Res. Journal of Kerala. 25 (1) : 5-10.

Marutani, M. and Kamemoto, H. (1983). Transmission and significance of B chromosome in Anthurium warocqueanum. Amer. J. Bot. 70 (1) : 40-46.

Matern, B. and Simak, M. (1968). Statistical problems in karyotypic analysis. Hereditas. 59: 280-288.

** Mc Rae, J.F. (1987). Stomata size and pollen characteristics as an indication of chromosome number in lilies. In : Lily year book of the American lily society.

Mercy , S.T. Kakar , S.N. and Varghese , T.M . (1976) . Studies on nutritive requirement and preservation of pollen in Cicer arietinum and Cicer soongarium . Physilogy of sexual reproduction in flowering plants .Kalyani publishers , New Delhi , India . p 21-26

Mercy , S.T. and Zapata , F J (1987) Initiation of Androgenesis in Rice (Oryza sativa L var Taipei 309). Proc. Indian Natn. Sci. Acad. 53(3). 253-258.

Mishra, R.S. and Singh, D.N. (1988). Studies in floral biology of okra under Varnasi conditions. The Punjab Hort. Journal. 28 (3-4): 231-237.

Misra, R.S. (1972). Effect of agar, boron and growth regulators in germination of Java plum (Syzygium cuminii.L). Indian Journal of Agrl. Science. 42 (1) ; 16-20.

Mitu, M. and Acatrinei, G. (1974). Pollen germinating ability in some foreign pear cultivars. Facultatea germinativa a polennului la citeva soiuri straine de par Lucrari Stiintifice, Institutual Agronomic Jon Jonesiu de la Brad. 1 : 275-279.

Mookerjea, A. (1955). Cytology of different species of Aroids with a view to trace the basis of their evolution. Caryologia. 7 : 221-291.

Moore, P.H. and Jung, W.L. (1974). Studies on sugaracane pollen. In vitro germination of pollen. Phyton Argentia. 32 (2) : 147-157.

Moss, J.P. (1966). The adaptive significane of B chromosome in rye. Hereditas. 29 : 91-112.

Moti. (1972). Studies on the morphology and viability of the grape (Vitis vinefera. L) pollen, Punjab Hort. Journal. 12 (2/3) : 101-110.

Moyazaki, S. Tashiro, Y., Kanazwa, K. and Matsumoto, H. (1985). Production and characteristics of polyploids derived from cultured shoot tips of taro (Colocasia esculenta) by treatment with colchicine. Bulletin of the faculty of Agriculture. Saga Univ. 59 : 37-45.

Nair, P.K.K. (1979). In : Nair, P.K.K. and Lawrenie, R. Advances in pollen spore research. Vol.14. p.1. Scholar Publishing house, India.

Nagorajan, C.R. Krishnamurthi, S. and Rao, V.N.M. (1965). In Stanley, R.G. and Linskens, H.F. (Eds) : Pollen Biology Biochemistry Management, p.81. Spnnger - Verlag Berlin Heidelberg New York.

Nissen, O. (1950). In : Stanley, R.G. and Linskens, H.F. (Eds) : Pollen Biology Biochemisty Management,p.28. Springer Verlag Berlin Heidelberg New York.

Nuckles, E.M. and Kuc, J. (1990). Use of a Haemocytometer-Laboratory Exercises in plant Pathology, APS Press.

Oberle, G.D. and Goertzen, K.L. (1952). In : Stanley, R.G. and Linskens, H.F. (Eds) : Pollen Biology Biochemistry Management, p.31, Springer-Verlag Berlin Heidelberg . New York.

Owizarzak, A. (1952). In : Stanley, R.G. and Linskens, H.F. (Eds): Pollen Biology Biochemistry Management,p.81. Springer-Verlag Berlin Heidelberg New York.

Pant, N.M., Sen. D.R., Tawar, P.N., Jagtap, R.A., Kale, V.R. and Hapase, D.G. (1989). Stomatal studies in 4 varieties of sugarcane (Saccharum officinarum L). Biovigyanum,15 (1) : 45-50.

Parameswar, N.S. and Venugopal, R. (1975). Preliminary studies on pollen germination in cardamom. Mysore Journal of Agrl. Science. 9 (1): 59-66.

Parfitt, D.E. and Ganeshan, S. (1989), Comparison of procedures for estimating viability of Prunus pollen. Hort.Science. 24 (2) : 354-356.

Parker, J.S., Jones, G.H., Edgar, L. and Whitehoue, C. (1989) The population cytogenetics of Crepis capillaris II The stability and inheritance of B chromosomes. Heredity. 63 : 19-27.

Person, H.M. and Harney, P.M. (1984). Pollen viability in Rosa. Hort.Science. 19 (5) : 710-711.

Pfitzer, P. (1957 a). Chromosome numbers of Araceae. Bot 2001. 8 : 1492.

Pfitzer, P. (1957 b) Chromosomenzahlen Von Araceen, Chromosoma .8 : 436-446.

Pfitzer, P. (1957 c). Konstanz and Variabilitatder chromosomen Zahl bei einigen Anthurien unter Besounderer Berucksich tigung der B chromosome. Chromosoma . 8 : 545-572.

Phol, F. (1937). In : Stanley R.G. and Linskens, H.F. (Eds): Pollen Biology Biochemistry Management, p.28. Springer-Verlag Berlin Heidelberg. New York.

** Porlingis, I H. and SFAkiotakis, E.M. (1968). Germination in vitro and storage of olive pollen Epistem. Epet. Thessalonika. 12 : 525-556.

**Pradescu, G., Dragenescu, E., Taranu, P., Papuc, A. and Viloin,M.(1985). Studies on pollen germinatin and viability in sour cherry. Cerectari privind germinatia viabilita tea polenului in visin Lucrari stintifice Institutul Agronomic Timisoara, Agronomie.20 : 123-132.

** Prolingh, I.X. (1956). Pollen germination studies in fruit trees. Ph.D thesis of Univ.Thessalonski. Coll. Agri. Lab. Hort. 32:

Rana, A., Bharadwaj, G. and Chawhan, S.V.S. (1989). Effect of some growth regulators in in vitro pollen germination in Impatiens balsamina. Plant Cell Incompatibility News letter. 21 : 62-66.

Randolph, L.F. (1941). Genetic characters of B chromosomes in maize. Genetics. 120 : 608-631.

Rao, A.N. and Chin. L.W. (1973). Pollen viability and germination in some orchid hybrids. Gardens Bulletin. 26 (2) : 245-257.

Rao, N.N., Ponnaiya, B.W.X. and Menon, P.M.(1980). Karyotype analysis in ten nontuberous Taxa of Solanum. L. Madras Agric. J. 67 (2) : 94-101.

Raven P.H. (1975). The basis of angiosperm phylogeny. Ann. Mo. Bot. Gard. 62 : 724-764.

Ravindran, P.N. (1977). In vitro germination and growth of cacao pollen. Journal of Plantation Crops. 5 (2) : 109-111.

Sandhu, A.S. Bains, S.S and Singh.S.N. (1988). Studies on the floral biology of Guava (Psidium guajava L) under Ludhiana conditions. II Time of anthesis, anther dehiscence, pollen studies, stigma receptivity and fruit set. The Punjab Horticultural Journal. 28 (3-4) : 172-178.

Satyadas, Jessy,.(1985). Karyomorphological studies on eight species and varieties of Anthurium with special reference to B chromosomes. M.Sc. thesis, University of Kerala, India.

Schlarbaum, S.E. and Tsuchiya, T. (1984). Cytotaxonomy and phylogeny in certain species of Taxodiaceae. Pl. Syst. Evol. 147 : 29-54.

Schwanitz, F. (1952). In : Stanley, R.G. and Linskens, H.F. (Eds): Pollen Biology Biochemistry Management, p.28-29, Springer-Verlag-Berlin Hieldelberg, New York.

Selvanathan, M. and Khanna, V.K. (1990). Mitotic and meiotic studies in different cultivars of Oryza sativa. Acta Botanica Indica. 18 (2) : 280-282.

Seth, J.N. (1962). Floral biological studies in Psidium II Anthesis and dehiscence of anthers, pollen studies stigma receptivity, fertilization and fruit setting Hort.advance. 6 : 110-136.

Sengupta, D.K. and Chettri, R. (1989). Cytological studies on two ornamental aroids growing in Eastern Himalaya. Environment and Ecology. 7 (4): 1025-1028.
1025-1028.

*** Sharma, A.K. and Bhatacharya, G.N.(1966). A taxonomic study on some taxa of Araceae. Genet. Iber. 18 : 1-26.

Sharma, A.K. and Bhattacharyya, U.C. (1961). Structure and behaviour of chromosomes in species of Anthurium with special reference to accessory chromosome. Proc. Natl. Inst. Sci. India (B). 27 : 317-327.

Sharma, A.K. and Das, N.K. (1954). Study of karyotypes and their alternations in Aroids. Agron. Lusit. 16 : 23-48.

Sharma, A.K and Mallick, R. (1965). Interrelationships and evolution of the tribe Aloineae as reflected in its cytology. J. Genet. 59 : 110-138.

Sharma, A.K. and Sharma, A (1959). Chromosomal alternation in relation to speciation. Bot.Rev. 25 : 514-544.

Sheffer, R.D. and Croat, T.B. (1983). Chromosome numbers in the genus Anthurium (Araceae) II. Ammer. J. Bot. 70 (6) : 558-571.

Sheffer, R.D. and Kamemoto,H. (1976). Cross compatibility in the genus Anthurium. J. Amer. Soc. Hort. Sci. 101 (6): 709-713.

Sheffer, R.D. and Kamemoto, H. (1976). Chromosome numbers in genus Anthurium. Amer. J. Bot. 63 : 74-81.

Sheffer, R.D. Threobald, W.L, and Kamemoto, H. (1980). Taxonomy of Anthurium scandens (Araceae). Amideana. 3 : 86-93.

Shim, K.K. Sub, B.K. and Han, B.K. (1988). A palynological study of Hibiscus syriacus, Hibiscus rosa-sinensis and Hibiscus mutabilis. J.of the Korean society for Horticultural Science. 29 (1): 38-45.

Shim, K.K., Sub, B.K, Han. B.K. and Park, S.H. (1989). A palynotaxonomic study of some Chrysanthemum Species. J. of Korean Society for Horticultural Science. 30 (3): 224-233.

Shindo, K. and Kamemoto, H. (1963). Karyotype analysis of some species of Phalaenopsis, Cytologia. 28 : 390-398.

Simmonds, N.W. (1954). Chromosome behaviour in some tropical plants. Heredity. 8 : 139-146.

Singh, B.D. (1990). Plant Breeding. Kalyani Publishers, Lunhiana. p.46.

Singh, H (1989). Studies on vegtative, floral and fruit charactrys of some local varieties of grape grown in Kinnau district of H.P. Ph.D, India.

Singh, J.D and Dhuria, H.S. (1960). Studies on floral biology of sweet lime (Citrus limettioides Tanaka). Indian J.Hort. 17 : 9-20.

Singh, R.N. (1954), Studies on the floral biology and subsequent development of fruits in mango (Mangifera indica L) varieties Dashehari and Langra. Indian J.Hort. 11 : 1-20.

Singh, S.N. (1960). Pollen analysis of subtropical peaches. Hort. Advance. 4 : 78-82.

Singh, S.N. (1961), Studies on the morphology and viability of the pollen grains of mango. Hort. Advance. 5 : 121-144.

Singh, S.N. (1962), Studies on the morphology and viability of pollen grains of Litchi. Hort. Advance. 6 : 28-53

Smith, D.M and Levin, D.A. (1967). Karyotypes of Eastern North American Phlox. Amer. J.Bot. 54 (3): 324-334.

Sreekumari, M.T. and Mathew, P.M. (1991). Karyotypically distinct morphotypes in Taro (Colocasia esculenta (L) Schott). Cytologia. 56 (3) : 399-402.

Stanley, R.G. and Linskens, H.F. (1974). (Eds): Pollen Biology Biochemistry Management. Springer Verlag Berlin Heidelberg New York p.307.

** Stebbins, G.L. (1940 b). Studies in the Cichorieae: Dubyaea and Soroseris, endemics of the Sino-Himalayan region. Mem. Torrey Bot. Club. 19 : 1-76.

Stebbins, G.L. (1958). Longevity, habitat and release of genetic variety in higher plants. Cold. Spr. Harb. Symb. Quant. Biol. 23 : 365-378.

Stebbins, L. (1971). Chromosomal Evolution in Higher Plants. Addison Wiley Pub. Co. Metro Park (Calif) p.1-122.

** Sun, R.Q., Yu, S.M., Chu, B.Y. and Yan, X.Q. (1986). A preliminary study on the physiological and ecological indices in rice breeding for high yield. Zheijiang Agrl. Science. 1 : 1-5.

Swanson, C.P. (1968). Cytology and Cytogenetics. Macmillans Co.Ltd. pp.596.

Swanson, C.P. Merz, T and Young, W. (1982). Cytogenetics (2nd Edn). Prentice Hall International Inc. London p.577.

** Tarasevich, V.F. (1989). Pollen grain ultra structure in the genus Anthurium (Araceae) in relation to its systematics. Botanicheskii Zhurnal. 74 (3) : 314-324.

** Tjio, J.H. and Hagberg, A.(1951), Cytological studies on some X-ray mutants of barley. Ant. Estac. Aula. Dei. 2 : 149-167.

Torres, A.M.(1968). The karyotypes of diploid cespitose zinnias: a method and analysis. Amer. J.Bot.55(5) : 582-589.

Tsuchiya, T. and Takada, M. (1962). Chromosome studies in five species of Araceae. CIS. 3: 36-38.

Vazhnitskaya, E.F. (1960). In : Stanley, R.G. and Linskens, H.F. (Eds) : Pollen Biology Biochemistry Management, p.81. Springer-Verlag Berlin Hiedelberg New York.

Wodehouse, R.P. (1935). Pollen grains. Mc Graw Hill, New York p.574.

** Zhan, Y.X., Wu, A.H. and Cheng, M. (1988). Karyotype analysis of 4 types of pea nut (Arachis hypogaea). Scientia Agricultura Sinica. 21 (1) : 61-67.

** Not seen

APPENDICES

A P P E N D I X - I

ABSTRACT OF ANOVA

Source	df	Mean Square						
		Mitotic index	TCL	Flower maturity period	Pollen size	Pollen production per anther	***Pollen sterility % by aceto carmine staining method	***Pollen sterility % by *in vitro* pollen germination method
Varieties	4	7.5750	36.5693*	53.3249*	2982912	189562**	18.6679	22.7031
Error	12	6.8083	1.5182	11.0250	2396800	11979.33	13.1192	12.9309

* Significant at 0.01 level

** Significant at 0.05 level

*** Pollen sterility % was analysed after transformation.

CHROMOSOME BEHAVIOUR AND POLLEN ANALYSIS IN *Anthurium* sp-

BY

BINDU. M. R.

ABSTRACT OF THE THESIS

SUBMITTED IN PARTIAL FULFILMENT OF

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VELLAYANI, THIRUVANANTHAPURAM

1992

A B S T R A C T

Chromosome behaviour and pollen characters had been studied in five commercially important varieties of Anthurium andreanum viz. Honeymoon Red, White (album), Lady Jane (Pink), Chillired and Pink. Some morphological characters were also recorded.

The varieties show distinct differences in morphological characters. They can be identified from one another even in the absence of their typical spadices.

All the varieties have a somatic chromosome number of 30+2B. The basic chromosome number is x = 15. Two B chromosomes are present irrespective of the varietal type. The B chromosomes are either acentric or telocentric and round or rod shaped. Their size is always smaller than the smallest A chromosome.

The karyotype of all the five varieties was analysed. On the basis of total chromosome length (TCL), average chromosome length (ACL) and arm ratio (r), the variety Pink appears to be most advanced. When the relative chromosome length (RCL) was taken in to consideration, the variety Chillired is found to be most advanced. With regard to the

character, chromosome asymmetry, Lady Jane appears to be most advanced. When the karyotype asymmetry as a whole is considered, all the varieties are falling in the '3B' category which represents a high position in evolution.

During meiosis, all the varieties exhibited a wide range of abnormalities like univalent formation, unequal seperation, presence of laggards, micronuclei etc. This points out the hybrid nature of the species.

All the varieties are found to be protogynous with distinct interphase. This is an adaptation for natural cross pollination. However, natural crossing is occuring only to a li m ited extent.

The pollen production capacity of the varieties varies significantly. The varieties Honeymoon Red and Pink produce the highest amount of pollen.

All the varieties have round pollen with a single germ pore. The pollen size among the varieties does not vary significantly. However, the variety Lady Jane has the largest pollen.

Pollen fertility is very low, when it was tested by acetocarmine staining and *in vitro* pollen germination

methods. The latter is found to be more reliable in estimating the pollen fertility.

All the varieties have paracytic type of stomata. But the varieties vary widely in their size and distribution.

Morphological variations, karyotypic differences, meiotic abnormalities, high pollen sterility and the wide variability in stomatal characters point out the basic hybrid nature of the species.