IMPROVEMENT OF *Anthurium andreanum* Lind BY *IN VIVO* AND *IN VITRO* METHODS

By
LEENA RAVIDAS

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture
Kerala Agricultural University

Department of Pomology and Floriculture
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR-680656
KERALA, INDIA

2003

DECLARATION

I hereby declare that this thesis entitled **"Improvement of *Anthurium andreanum* Lind by *in vivo* and *in vitro* methods"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Vellanikkara
4-8-2005

Leena Ravidas

CERTIFICATE

Certified that this thesis, entitled **"Improvement of *Anthurium andreanum* Lind by *in vivo* and *in vitro* methods"** is a record of research work done independently by **Mrs.Leena Ravidas** 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. P.K. Valsalakumari
Chairperson, Advisory Committee
Associate Professor
Department of Pomology & Floriculture
College of Horticulture
Vellanikkara

Vellanikkara
13.08.03

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Mrs.Leena Ravidas,** a candidate for the degree of **Doctor of Philosophy in Horticulture** with major in **Pomology and Floriculture,** agree that this thesis entitled **"Improvement of *Anthurium andreanum* Lind by *in vivo* and *in vitro* methods"** may be submitted by **Mrs.Leena Ravidas,** in partial fulfilment of the requirement for the degree.

4.8.03

Dr. P.K. Valsalakumari
Chairperson, Advisory Committee
Associate Professor
Department of Pomology & Floriculture
College of Horticulture
Vellanikkara

4/8/03

Dr. P.K.Rajeevan
Associate Professor and Head
Dept. of Pomology & Floriculture
College of Horticulture
Vellanikkara
(Member)

4/8/03

Dr. C.K.Geetha
Associate Professor
Dept. of Pomology & Floriculture
College of Horticulture
Vellanikkara
(Member)

4/8/03

Dr. P.V.Balachandran
Associate Director
RARS, Pattambi
(Member)

4.8.03

Dr.Achamma Oommen
Associate Professor
Department of Plant Breeding & Genetics
Vellanikkara
(Member)

EXTERNAL EXAMINER
N. KUMAR
PROFESSOR AND HEAD
DEPT OF FRUIT CROPS
TNAU - COIMBATORE

ACKNOWLEDGEMENT

I bow my head before the God Almighty for the blessings showered on me, which alone helped me to complete this project.

Words fail when I try to express my deep sense of gratitude and indebtedness to **Dr.P.K. Valsalakumari**, *Associate Professor, Department of Pomology and Floriculture and Chairperson of my advisory committee. I am always grateful to her for the constant encouragement, unreserved help, inspiring guidance, abiding patience, constructive ideas and above all the understanding and enthusiasm during the whole period of investigation and preparation of the thesis.*

With profound respect and esteem regards, I place my thanks to **Dr. P.K. Rajeevan**, *Associate Professor and Head, Department of Pomology and Floriculture and member of my advisory committee, for his valuable suggestions, never ending encouragement and proper guidance. His special attention and extra efforts in correcting the manuscript are gratefully acknowledged. I am also thankful to him for the beautiful photographs taken for the thesis.*

I am extremely indebted to **Dr.C.K. Geetha**, *Associate Professor, Department of Pomology and Floriculture and member of my advisory committee for the timely help, advices and kind concern throughout the course of research work.*

Heartfelt thanks are also due to **Dr.P.V.Balachandran**, *Associate Director, RARS, Pattambi and member of the advisory committee for his wholehearted co-operation and suggestions during the preparation of the thesis.*

I wish to express my sincere gratitude to **Dr.Achamma Oommen**, *Associate Professor, Department of Plant Breeding and Genetics for offering all possible help during this investigation.*

I remember with great gratitude the efforts of **Dr.K.V. Peter**, *Vice-Chancellor, K.A.U.for the help and guidance rendered to conduct laboratory analysis at Indian Institute of Spices Research, Calicut. I owe a lot to Dr.Chembakam and Smt.Anuradha of IISR, Calicut for extending their expertise in isozome studies.*

I am obliged much to Smt.N.V.Kamalam, Professor and Safety Officer and Dr.P.Sureshkumar, Assistant Professor, Radio Tracer Laboratory for their help during irradiation studies.

I thankfully acknowledge the help extended by Dr.V.K. Mallika, Professor C.C.R.P. during cytological studies.

I sincerely acknowledge the help and suggestions of each and every member of the Department of Pomology and Floriculture at different periods of my work.

The help received from Sri S. Krishnan, Associate Professor and Smt. Joicy in the statistical analysis of the data is gratefully acknowledged.

My thanks are also due to the labourers of the nursery, Department of Pomology and Floriculture, especially Smt. Valsala, Smt. Gouri, Smt. Koujumma, Smt. Pathumma and Smt. Elsy for their co-operation and assistance during field work.

I would like to thank my friends for their love, concern and encouragement especially Anu, Muthulakshmi, Shalini, Linnet, Geetha, Indira, Pushpalatha, Ajith and Simon.

The award of K.A.U. Senior Fellowship is gratefully acknowledged.

I sincerely thank Mr.Joy and family, J.M.J. Computer Centre, Thottappady, for the computer work (scanning and colour printing) and neat typing of the manuscript.

I could never forget the help rendered by my late mother in law who took great care and pain during the period of my work. I am deeply indebted to my loving husband, daughter, son, mother and sister, without whose boundless affection, warm blessings and encouragement this project would not have been fruitful.

LEENA RAVIDAS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

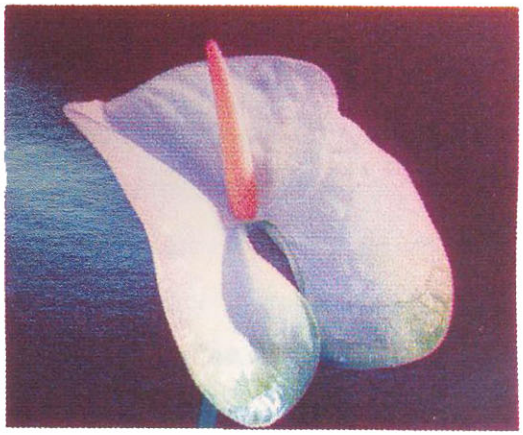
LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS

BA	- Benzyl adenine
CI	- Callus Index
2,4-D	- 2,4-Dichlorophenoxy acetic acid
EDTA	- Ethylene diamine tetra acetate
EW	- East West
GCV	- Genotypic coefficient of variation
Gy	- Gray
HCl	- Hydrochloric acid
H_2O_2	- Hydrogen peroxide
IAA	- Indole acetic acid
IBA	- Indole butyric acid
MSL	- Mean Sea Level
MS	- Murashige and Skoog's (1962)
NaOH	- Sodium hydroxide
NAA	- Naphthalene acetic acid
NS	- North South
PCV	- Phenotypic coefficient of variation
PRX	- Peroxidase
rg	- Genotypic correlation coefficient
rp	- Phenotypic correlation coefficient
SOD	- Super Oxide Dismutase

Introduction



INTRODUCTION

The genus *Anthurium*, with over 700 species (Sheffer and Croat, 1983), which are distributed worldwide, is the largest in the family Araceae. Of these species, not more than 50 are in cultivation and 10-15 are known to trade. The most popular and economically important flowering species of the genus are *Anthurium andreanum*, commonly known as painter's palette and *A. scherzerianum* known as flamingo flower. Several other species like *A. magnificum, A. digitatum, A. crystallinum, A. clarinervium* etc. are grown for their magnificient foliage. *A. amnicola* and *a. ornatum* are cultivated for both their foliage and flower.

The name anthurium is derived from the Greek 'anthos', flower and 'aura' tail, referring to the spadix. It is a native of tropical zone of Central and South America, from where it was brought to Europe. It was introduced to India via., England by coffee and tea planters, who wanted showy and exotic plants for their big bungalows. Anthurium cultivation is mainly concentrated in Hawaii, Holland and Mauritius. USA, Canada, Japan, Germany and other European countries import a lot of these flowers. The global trade of anthurium is valued at US $ 50 million and it occupies the 11^{th} position in the international market. Indian floriculturists can take up the cultivation of anthurium on a large scale as the demand is huge. This will also bring valuable foreign exchange to the country. Kerala is identified as one of the best places for growing anthurium, because of the congenial climatic conditions.

A. andreanum produces flowers all round the year, one flower from each leaf axil. The sequence of leaf, flower and new leaf is maintained through out the life of the plant. The plant is erect with lobed, heart shaped green leaves. The inflorescence is a combination of colourful modified leaf (spathe) and pencil like protrusion (spadix) borne on leafless stalk or peduncle (Bhatt and Desai, 1989). The true sessile and bisexual flowers are arranged on the spadix.

Today, hundreds of varieties are known in different colours. Bright red and bright orange colours have greatest demand all over the world, followed by white and

pink. Double coloured varieties are gaining more and more importance now and are also fetching higher price in the international market (Rajeevan *et al.*, 2002).

The qualities of good anthurium flowers are bright coloured, showy, heart shaped spathe with plenty of blisters and symmetrical overlapping of basal lobes; the spadix reclining to the spathe, shorter in length than the spathe, oriented at an angle less than 30° and an erect long flower stem about 5 times the length of the spathe.

Anthurium is propagated by seeds, suckers, cuttings or by *in vitro* multiplication. Variability is a problem in seed propagation. Availability of uniform planting material of elite varieties in large scale for commercial production is still the biggest limiting factor in anthurium cultivation (Gajanana and Subrahmanyam, 1999). Hence development of good varieties suitable for our climate and their quick multiplication for large scale production have to be given priority.

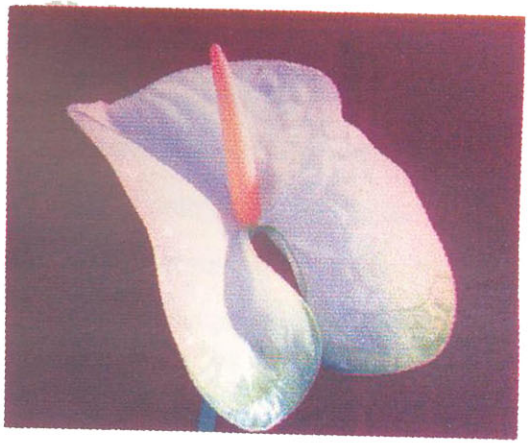
The present day cultivars of anthurium are mostly hybrids of different species involving mainly *A. andreanum* and *A. scherzerianum.* Hybridization followed by selection is the accepted method used for improving anthuriums (Kamemoto and Nakasone, 1955). Another method of crop improvement is mutation breeding through the use of ionising radiation.

In general, hybrid seedlings show good vigour and survival fitness and reach flowering in 24 to 30 months (Mercy and Dale, 1994). Zimmer (1990) has described a new method of developing *A. scherzerianum* varieties using *in vitro* seed culture. These facts point out the possibility of achieving crop improvement in *Anthurium* through hybridisation and quick multiplication through *in vitro* seed culture.

The present study was undertaken with the following objectives.

1. To evaluate the anthurium varieties for their vegetative and floral characters.
2. To evolve new varieties of *Anthurium andreanum.*
3. To standardise the age of seed for successful *in vitro* seed culture.
4. To evaluate the hybrid seedlings morphologically, cytologically and through isozyme analysis.

Review of Literature



2. REVIEW OF LITERATURE

Anthurium is gaining popularity as one of the most important commercial ornamental crops of the modern world. Among the different species, *A. andreanum* is an important cut flower much valued for the attractive long lasting spikes. Availability of planting material and marketing of flowers have been viewed as major problems of commercial cultivation of anthurium in Kerala. So the present study was conducted to produce new varieties of *Anthurium andreanum* through intervarietal and interspecific hybridisation. One of the major limitations in the hybridisation programme is the long time taken from seed set to maturity of the seed. This time lag could be substantially reduced if seedlings could be produced by the *in vitro* culture of seeds isolated from the immature berries. A brief review of the works relevant to the study is presented in this chapter.

2.1 CHARACTERIZATION

Detailed study of the morphological characteristics of parent varieties helps in understanding the variability that exists among them. It also helps in the identification and classification of varieties. A desirable anthurium plant should have short internodes, grow vigorously, produce more number of flowers, spathe should be heart shaped with symmetrical lobes and spadix should be reclining to spathe to facilitate packing. Dark colour with puckered surface is preferred in international market (Rajeevan and Valsalakumari, 2000).

2.1.1 Vegetative characters

2.1.1.1 Plant size

Plant height is used for distinguishing the different varieties of crop plants. Tisdale *et al.* (1985) reported that plant height can be used as an index of plant growth. Bindu and Mercy (1994) studied five varieties of anthurium and reported significant variation in plant height, ranging from 45 cm in the variety 'Lady Jane' to 85 cm in

the variety 'Pink'. Sindhu (1995) recorded the height of six other varieties which ranged from 43 cm to 70 cm.

Henny *et al.* (1988) described a new cultivar 'Southern Blush' obtained from interspecific hybridisation of a pink *A. andreanum* cultivar with *A. amnicola*, a dwarf species. The variety is intermediate in size between its parents. The leaves of 'Southern Blush' are about 25 cm long. The variety had a medium pink spathe with a slight lavender tint.

Anthurium scherzerianum variety 'Arabella' is described as more uniform and compact and is a medium sized plant with dark green, short leaves (Arndt, 1991).

Renu (1999) compared 10 varieties which showed significant variation in height ranging from 29.7 cm in 'Midori' to 70.9 cm in 'Pompon Red'.

Though height is a varietal character, it is significantly influenced by shade level, nutrient supply, growth regulators, as well as potting media.

2.1.1.2 Number of leaves/flowers (spikes) per year

Morphological studies conducted by Christensen (1971) showed that *A. andreanum* had a long juvenile phase followed by a generative phase in which flower buds are produced. *Anthurium andreanum* produces flowers all round the year, one flower from each leaf axil. The sequence of leaf, flower and new leaf is maintained throughout the life of the plant. On comparing the productivity and inflorescence quality of 120 individual anthurium plants, Steen and Vijverberg (1973) found that their productivity was highly variable ranging between 4 to 16 flowers over two years. A close correlation between the number of leaves and the number of flowers was also observed by Gajek and Schwarz (1980). The monthly pattern of leaf formation in *Anthurium* cultivars was analysis for four years by Klapwijk and Spek (1984) and they found that the average leaf number/m^2 glass house rose from 1.5 in March to 5 in June, thereafter declining until the following March.

On the basis of a study conducted on *A. andreanum* Lind cv. Ozaki, Higaki and Poole (1978) concluded that flower production decreased with age of the plant.

Mercy and Dale (1994) observed that anthurium produced only five to eight new leaves on a stem axis per year and five to eight spadices per year.

Sindhu (1995) has recorded that the number of spadices produced annually by an anthurium plant varied from four to eight. According to Rajeevan *et al.* (2002) the number of leaves/spikes per year varied from 4-9 in anthurium.

2.1.2 Floral characters

Anthurium plant flowers round the year producing 5 to 7 spadices per year which exhibit wide variability in colour, size, shape and texture of the spathe.

2.1.2.1 Floral morphology

The structure which is commonly called the anthurium flower is a combination of colourful modified leaf (spathe) and hundreds of small flowers on the pencil like protrusion called candle (spadix). Spadix and spathe are borne on a leaf less stalk or peduncle.

2.1.2.1.1 Spathe size

The size of spathe is a commercially important trait of anthurium flowers. Hijaki and Poole (1978) noticed increase in flower size with aging.

Henny *et al.* (1988) reported that the *Anthurium* cultivar 'Southern Blush' had an average spathe length of 7 cm and width of 5 cm.

In a study of five varieties of *A. andreanum*, Bindu and Mercy (1994) observed the largest spathe size for pink (10.4 x 9.7 cm) and the smallest for 'Lady Jane' (6.5 x 3.5 cm). In a similar study, Sindhu (1995) found that the varieties 'Pink' and 'Kalimpong Red' produced super large flowers and the smallest flowers were

produced in the variety 'White'. The variety 'Ruth Morat' syn 'Lady Ruth' had spathes larger than those of 'Lady Jane', with a mean width and length of 5.01 and 7.68 cm, respectively (Oglesby Plant Laboratory Inc., 1996). Henny (1999) recorded that the new variety 'Red Hot' had 6 to 7 cm long and 4 to 5 cm wide spathes. The spathe size of 10 varieties studied by Renu (1999) revealed variation in the spathe size ranging from 17.12 cm in 'Pompon Red' to 30.74 cm in 'Dragon's Tongue Red'. According to Rajeevan *et al.* (2002) the spathe size ranged from 7 cm in 'White Alba' to 17 cm in 'Pink' and 'Kalimpong Red'.

Mercy and Dale (1994) graded the anthurium flowers based on length + width measurements, as super large (30 cm and above), large (25-29 cm), medium (20-24 cm), small (15-20 cm), mini (12-14 cm) and micro (9-11 cm).

2.1.2.1.2 Length of spadix

The spadix (candle) lengths of five varieties were recorded by Bindu and Mercy (1994), which ranged from 4.0 cm to 9.5 cm. The candle was long and fleshy in ordinary varieties while it was shorter and more slender in highly bred hybrids and exotics (Mercy and Dale, 1994). Among the six *A. andreanum* varieties studied by Sindhu (1995) 'Kalimpong Red' had the highest spathe-candle ratio (2.86:1) and 'White' had the lowest ratio (2:1). Henny (1999) recorded that the miniature hybrid 'Red Hot' had a spathe size of 10 to 12 cm and a candle length of 3 to 4 cm.

2.1.2.1.3 Nature of spathe and spadix

Varying degrees of smoothness and blistering were reported in *A. andreanum* by Birdsey (1956). Arndt (1991) described the spathe of *A. scherzerianum* variety 'Arabella' as broad with free lobes and a shallow sinus having a recurving spadix.

Sindhu (1995) noticed that the variety 'Honeymoon Red' had smooth thick and glossy spathes without prominent veins while 'Pink' and 'White' had smooth, thin and lightly veined spathes. The ideal *Anthurium* spadix with an angle less than 45° was found in varieties 'Chilli Red', 'Kalimpong Orange' and 'Kalimpong Red'.

The differences in spathe colour, texture and candle colour were reported for ten varieties by Renu (1999) and for 21 varieties by Rajeevan *et al.* (2002).

2.1.2.1.4 Spathe colour

The five major spathe colours of *A. andreanum* are red, pink, orange, coral and white. These colours are determined by the concentration of two anthocyanins, cyanidin and pelargonidin.

The presence of 3-cyanidin glucoside and 1-pelargonidin glucoside in the spathes of *A. andreanum* was reported by Forsyth and Simmonds (1954).

Birdsey (1956) recorded that *A. andreanum* plants offered in the trade have a complete colour range from white to dark red. Lowry (1972) observed that the spathe of all the varieties of *A. andreanum* had the presence of both pelargonidin and cyanidin 3-rutinoside.

Iwata *et al.* (1979) studied the anthocyanins in the spathes of *A. andreanum* cultivars and they were identified to be cyanidin 3-rhamnosyl glucoside and pelargonidin 3-rhamnosyl glucoside. They also analysed the genetics of spathe colour and found that both pigments were present in the red cultivars and in pink cultivars. The orange and coral coloured contained only pelargonidin 3-rhamnosyl glucoside. In white varieties either pelargonidin pigment or both cyanidin and pelargonidin pigments are absent.

Maurer (1979), while describing the technique of cross-pollination in *A. scherzerianum*, discussed the presence of recessive characters, i.e., A = with anthocyanin and a = without anthocyanin, B = whole spathe coloured and b = spotted spathe. When the parents were Aa/Bb, the descendants were 9 red (AB), 3 red spots on white (Abb) and 4 white (aab and aabb) and the deficit in white plants was attributed to their lack of vigour.

Gajek and Schwarz (1980) identified the varieties 'Iga-gold' with shiny red spathe and a white candle with yellow tip and variety 'Ellrina' with light salmon spathe and a sulphur yellow spadix to be the best suited for green house cultivation.

Iwata *et al.* (1985) inferred that the spathe colour in *Anthurium* was determined by the relative concentration of anthocyanins; a predominance of cyanidin 3-rhamnosyl glucoside resulted in pink to dark red colours, whereas a predominance of pelargonidin 3-rhamnosyl glucoside resulted in coral to orange.

Kamemoto *et al.* (1988) concluded that two major genes M and O were responsible for the five major colours in *A. andreanum* red, orange, pink, coral and white. The gene M was found to control the production of cyanidin 3-rutinoside and gene O controlled that of pelargonidin 3-rutinoside. Red and pink resulted when both M and O genes were present and orange and coral resulted when only O gene was present. The double recessive mmoo produced white. The recessive oo was epistatic to M, and therefore white resulted when both were recessive (mmoo) or M was in combination with recessive oo (MMoo, Mmoo). Orange and white were found to be true breeding. The incremental effects of M appeared to be greater than that of O and therefore the intensity of colours decreased from MMOO, MMOo, MmOO, to MmOo. Orange is mmOO and coral is mmOo.

MMOO
MmOO } Red
MMOo

MmOo - Pink

mmOO - Orange

mmOo - Coral

MMoo
Mmoo } White
mmoo

Classification of colours of the important cultivars and new introductions in Hawaii was done by Criley (1989), according to the Royal Horticultural Society colour chart. Wannakrairoj and Kememoto (1990) in their study on inheritance of purple spathe in *Anthurium* proposed a scheme for the genetic control of purple spathe colour. A recessive allele 'p' modified the colour of anthocyanins controlled by M and O loci. They found that a spathe was purple when the genotype was M-O-pp. If P locus was dominant, M-O-PP was red, while mm-OO-PP was orange and mmO-PP was coral. The P allele has no effect on the oo (white) genotype.

M-O-pp	- Purple
M-O-PP	- Red
mm-OO-PP	- Orange
mm-Oo-PP	- Coral

Arndt (1991) described the *A. scherzerianum* variety 'Arabella' as having red spathe and candle.

Mercy and Dale (1994) reported that the colour of spathe fades gradually as flowers get older and after fertilization of candle, the spathe becomes green and photosynthetic. They also reported that the candle had a single colour red, pink or green in ordinary anthurium varieties and hybrids had yellow, white pink or red colours in two or more bands. Abdussamed (1999) observed the significant alteration in the anthocyanin content of *Anthurium* cv. 'Hawaiian Red' under different levels of growth regulator and nutrient treatments. Henny (1999) noticed that the new anthurium hybrid 'Red Hot' had spathes that were medium red at anthesis, which changed to a lighter red prior to senescence and candle was orange red apically and blending to red basally.

According to Nirmala *et al.* (1999) the relative concentrations of cyanidin and pelargonidin along with an unknown pigment effects the spathe colour in anthuriums.

2.1.2.2 Flowering pattern

Flowers of *Anthurium* are bisexual and are closely congested and arranged in a series of spirals on the spadix (Croat and Bunting, 1978). Flowers develop acropetally (Croat, 1980), an individual flower has 4 colourless perianth segments (tepals) arranged in a four sided configuration which envelop four stamens with 4 loculed anther, pistil is cylindrical and bicarpellary. The ovary is superior, bilocular with 1 or 2 ovules in pendulous placentation. As the pistil develops, a stout style exerts to expose a receptive stigma.

2.1.2.2.1 Female phase

Studies on five *A. andreanum* varieties by Bindu and Mercy (1994) revealed that, *A. andreanum* flowers are bisexual and protogynous and the female phase varied from three to twelve days in the five varieties. The gynoecium reached receptivity about 4-7 days after the opening of the spathe which is easily detected by a viscous and colourless exudate secreted by receptive stigma at this time which is sticky to touch and the receptive female phase lasted for three to seven days. Sindhu (1995) studied six varieties and observed that the days to initiation of female phase ranged within a period of 10 days, after opening of spathe.

2.1.2.2.2 Male phase

Croat (1980) observed that the initiation of stamen emergence appeared to be equal from all parts of the spadix or initial maturation and staminal exertion appeared for many flowers in the basal fourth, basal third or basal half of the candle and further development proceeded in a systematic manner. He also reported that usually one or both of the lateral stamens emerge first followed by the alternate pair with the interior stamen usually preceeding the posterior one.

Bindu and Mercy (1994) recorded that the anther exertion started from the base and proceeded regularly towards the apex and the duration of male phase ranged from 3 to 7 days in the five *A. andreanum* varieties studied by them. Mercy and Dale

(1994) reported that all anthers on a candle emerged in about 4-8 days. Sindhu (1995) concluded that the male phase may range from 3-8 days depending on the variety. Renu (1999) reported that the male phase ranged from 5.4 days in 'Mauritius Orange' to 10.4 days in 'Tropical Red', after observing 10 varieties.

According to Chen-JungBin *et al.* (1999) the pollen shedding habits varied greatly dependant on the variety. Most varieties shed pollen after their stigmas were no longer receptive. However, some varieties occasionally shed pollen soon after the spathe unfurled.

2.1.2.2.3 Interphase

Croat (1980) reported that in most *Anthurium* species, separation period for female and male phases was several days whereas in a few of them the time lag was so short that it was not certain whether the species involved were homogamous or protogynous. The interphase for the five varieties studied by Bindu and Mercy (1994) ranged from four to seven days. Mercy and Dale (1994) reported that the interphase lasted for about one week in general.

An interphase ranging from four to ten days was reported by Sindhu (1995) in *A. andreanum* varieties.

According to Renu (1999) the interphase may range from 4.8 to 10.2 days on an average.

2.1.2.2.4 Longevity of spadix and spathe

Paull (1982) recorded the visible changes accompanying the senescence of *Anthurium* flowers as spathe gloss loss, necrosis of spadix and greening of spathe and spadix. These changes were nonreversible process leading to death of spadix. Mercy and Dale (1994) reported that the life of an unfertilized spadix was about two months while that of a fertilized inflorescence was about 4-7 months. Senescence was marked by yellowing of peduncle followed by withering of spathe and candle.

The life of unfertilized spadix was observed by Sindhu (1995) and it ranged from 1½ months in 'Kalimpong Orange' to 3½ months in 'Honeymoon Red'. For fertilized spadices, this period ranged from 4½ - 8 months.

According to Renu (1999) the life of spathe varied from 2.5 months in 'Nitta Orange' to 3.7 months in 'Ceylon Red' in the case of unfertilized spadices, but for fertilized spadices the range was found to be higher, 3.8 to 7.5 months.

2.1.2.3 Pollen characters

Bhojwani and Bhatnagar (1974) referred to the study of external morphological features of mature pollen grains as palynology. This deals with the pre-tetrad and post tetrad stages, the latter including anthesis, pollen production, pollen morphology, pollen dissemination, pollination, pollen germination and fertilization (Srivastava, 1982).

2.1.2.3.1 Pollen morphology

Size as well as shape of pollen grains show a broad spectrum of variability. Pollen grain size is affected both by chemical treatment and mounting media. For reproducible size measurements, determinations have to be made under identical conditions (Stanley and Linskens, 1974).

Pollen morphology was a useful means of classifying plants into families, tribes, genera, species etc. He also stated that the pollen grains possessed a unique form and performed a special and vital function (Erdtman, 1952). The pollen morphology analysis has been used as an effective means to throw light on taxonomy, phytogeny and evolution of angiosperms (Nair, 1970).

Tarasevich (1989) studied pollen grains of 34 *Anthurium* species and 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.

According to Bindu (1992), the pollen grain size varied from 81.8 x 68.0 μ in 'Pink' to 87.2 x 86.4 μ in 'Lady Jane'.

2.1.2.3.2 Pollen viability

Appearance of the pollen alone, even at collection time, is not always a good index of viability, according to Stanley and Linskens (1974). The capacity of the pollen to germinate and grow is also to be assessed. They also emphasized the importance of pollen viability in hybridization and suggested various methods for testing the viability of pollen grains. To assess the viability of pollen grains, staining with different chemicals and dyes has been adopted.

Zirkle (1937) described a method for mounting pollen grains in acetocarmine. The pollen grains which stained well and looked plumb and normal were taken as viable and the unstained shriveled ones as non-viable. Mitu and Acatrinei (1974) reported that the germination of pollen grains was proportionate to pollen grain stainability.

Lalithambika (1978) reported that the pollen sterility of different species of *Anthurium* varied from 63 per cent (*A. cordatum*) to 96.5 per cent (*A. veitchii*). According to Sathyadas (1985) the pollen sterility varied from 67 per cent (*A. warocqueanum*) to 80 per cent (*A. ornatum*).

Bindu and Mercy (1994) reported that pollen grain fertility ranged from 20.4 per cent in 'Honeymoon Red' to 28.8 per cent in 'Pink' by acetocarmine staining method.

Renu (1999) studied the pollen fertility of seven varieties and observed the highest fertility (42%) for 'Liver Red' and the lowest (14%), for 'Lady Jane'.

2.1.2.3.3 Pollen production pattern

According to Singh (1990) the details of anthesis vary from one crop species to another and they are also affected by environmental factors such as humidity and temperature.

In *A. andreanum*, Sindhu (1995) observed that the inter phase was prolonged with the suppression of male phase from March to August.

According to Mercy and Dale (1994) anthesis occurs on sunny days between 8 to 10 am and on cloudy and rainy days anther dehiscence is delayed. The optimum temperature for anthesis in *A. andreanum* was 22°C night temperature and 25°C day temperature.

Study of pollen emergence pattern revealed significant differences among varieties (Renu, 1999). Pollen emergence was completely absent in 'Pompon Red', 'Nitta Orange' and 'Midori Green' during the period of study from August 1998 to July 1999. In general pollen production was high in the cooler months of October to December and was suppressed during the hot months of March to June.

According to Bindu and Mercy (1996) anthesis and anther dehiscence occurred between 8 am and 10 am, as they were favoured by moderately low temperature and high relative humidity. The pollen grains were more or less uniform in size, round in shape with a single germ pore. But the pollen fertility was low reflecting the hybrid nature of the species.

2.1.2.4 Post harvest studies

Varieties differ in vase life as well as the longevity in packing. Several pre harvest and post harvest factors also influence the longevity of cut flowers (Abdussamed, 1999). Senescence of flowers is associated with the plugging of stem vascular tissues accompanied by the loss in weight, visible changes including spathe gloss loss, necrosis of spadix, blueing of spathe, stem collapse and abscission of the spathe and spadix from the stem (Akamine, 1976). Various floral preservatives and growth regulators are increasingly used in anthurium and other cut flowers to extend their vase life by means of pulsing and holding.

According to Kamemoto (1962) the keeping quality of flowers increased as they developed and was maximum when $3/4^{th}$ of the length of spadix had changed colour. Large and medium sized flowers kept better than small and miniature ones.

According to Salvi (1997) and Rajeevan and Valsalakumari (2001), for getting maximum vase life, better stage of harvest is at 1/3rd flowers opened on the spadix. But Singh (1998) recommended harvesting at a stage when ¾th of stigma along the spadix became receptive.

The differences in the post harvest life of different cultivars were reported by Kalkaman (1983) and Salvi *et al.* (1997).

Anthurium flowers are packed in card board cartons of various sizes. The cartons are lined with polythene sheets and layers of newspapers. Flowers are packed with their spathe face down and their stems interwoven and moistened shredded newspaper is inserted to provide a cushion and to maintain humidity (Akamine, 1976).

According to Bhattacharjee (1977), the cut ends of each flower stem should be wrapped with cotton pad soaked with water and covered with wax paper and securely tied on the stem ends and the spadix dipped in melted paraffin to reduce moisture loss and first packed in polythene bags and thereafter placed in cartons. Flowers are fixed to the bottom of the box, separation papers placed between the layers.

Abdussamad (1999) suggested packing of flowers in cardboard boxes with ethylene scrubbers like $KMnO_4$ for better longevity.

2.2 VARIATION AND CORRELATION STUDIES

Variability, the differences or variations present among single species or different species, may be due to environment or genotype or both. In plant breeding programmes, an insight of the magnitude of variability present in a crop species is important as it provides the basis for effective selection.

Johnson *et al.* (1955) found it more useful to estimate heritability value together with genetic advance for predicting the expected progress to be achieved through selection.

Genetic analysis of some characters of *Anthurium andreanum* was done by Renu (1999). Variability studies indicated high phenotypic and genotypic coefficients of variation for the characters viz., plant height, position of candle, days to initiation of female phase, duration of female phase and spathe size. The characters with high heritability coupled with high genetic advance values were plant height, spathe size, spathe-candle ratio, position of candle, number of flowers per candle and days to initiation of female phase, indicating additive gene action, which revealed a high genetic potential for the improvement of this crop.

2.3 HYBRIDIZATION

2.3.1 Compatibility studies and improvement works

Pollination is the simple process of transferring the pollen from one flower to the stigmatic surface of another flower. Anthurium is pollinated by insects (Croat, 1980). According to him, the modes of flowering behaviour probably have a direct influence on pollination biology and evolution.

A. andreanum is an outbreeding species with protogynous flowers, the mechanism of protogyny prevents self fertilization, as the stigmatic surface becomes receptive about 7-10 days before the pollen is shed (Singh, 1992).

Controlled pollination in anthurium is accomplished by touching a fine brush to the pollen - laden spadix and transferring the pollen to the sticky receptive stigma of the other plant. After pollination and fertilization, the spadix begins to grow and takes a warty appearance.

According to Chen-JungBin *et al.* (1999) the cooler period from December to April was the best time for pollination when anthurium varieties shed their pollen. The transparent minigrip polyethylene bag proved a good envelope to encase anthurium flower in order to prevent undesired pollination. The bag was easy to handle and allowed the flower development to be viewed, thus aiding the assessment of when to pollinate.

Kamemoto and Nakasone (1955) suggested that hybridization and selection was the most common method for improving anthuriums. They identified that characters to be selected were productivity, flower colour, shape and texture, short internodes and suckering ability. Controlled hybridization indicated that neither white or red flower colour exhibited dominance and pink showed intermediate heterozygous condition.

A general mode of spathe colour inheritance in *A. andreanum* was suggested by several workers based on intraspecific and interspecific hybridization (Kamemoto and Nakasone, 1955, 1963; Kamemoto *et al.*, 1969; Sheffer and Kamemoto, 1977). Birdsey (1956) attributed much of the variation in blistering patterns of spathes of *A. andreanum* to hybridization of this species with *A. linderianum, A. ornatum* and *A. nymphaefolium*.

Selection has been widely used as a method to develop suitable cultivars in the major anthurium producing countries. Of 113 clones evaluated by Kamemoto and Nakasone (1963), 13 were recommended for commercial cut flower production. Two cultivars 'Uniwaii' (an exceptionally high yielding white) and 'Marian seefurth' (with a rose coloured spathe) were evolved by clonal selection. They also suggested that the inheritance of spathe colour was under the control of multiple alleles and modifying genes. The presence of both the orange and magenta pigments in the pink cultivar 'Marian seefurth', which arose from a cross between a white clone and a pink clone, substantiates the hypothesis that separate genes designated as M and O are responsible for the production of magenta and orange pigments respectively.

Kamemoto *et al.* (1969) described two seedling selections 'Anuenue' and 'Chameleon' for cut flower production and a compact clone 'Red Elf' suitable for pot growing.

Sheffer and Kamemoto (1976a) evaluated the interspecific cross compatibilities among 56 species of *Anthurium* and they concluded that interspecific

hybrids with *A. andreanum* and *A. scherzerianum* were not readily obtainable. But they got hybrids of *A. andreanum* with six other closely related species.

Sheffer and Kamemoto (1977) recorded good cross compatibility among *A. andreanum, A. concinnatum, A. hoffmani, A. linderianum, A. micromysterium, A. nymphaefolium* and *A. pinchinchae*. They developed some cultivars all of which successfully flowered.

Kaneko and Kamemoto (1978) reported the chromosome numbers 2n = 30 for *A. andreanum* var. 'Kaumana' and 2n = 30 + 2B for 'Uniwaii'. Meiotic configurations in pollen mother cells were similar for both, with the exception of 2B chromosomes in the latter. They concluded that meiotic irregularities suggested a hybrid origin for cultivated anthurium.

Kamemoto and Sheffer (1978) reported a new species hybrid from the cross, *Anthurium scherzerianum* x *A. windlingerii*. The hybrid had a greyish-orange spathe. Other characteristics such as the length and coil of the spadix and the length and position of leaf blade were intermediate between the highly contrasting characteristics of the parental species. Fertility in the hybrid was very good, which indicated the close taxonomic relationship of the two species.

Kaneko and Kamemoto (1979) found that the chromosome number of *Anthurium* sp. was 2n = 30+2B. They inferred that the appearance of offsprings with 2, 3 and 4B chromosomes on self pollination, indicated the transmission of B chromosomes through both pollen and egg.

Henny *et al.* (1988) described a new cultivar 'Southern Blush' obtained from interspecific hybridization of a large pink *A. andreanum* cultivar with *A. amnicola*, a dwarf species from Costa Rica. This cultivar was intermediate in size between its parents, spathes were 70 mm long and 50 mm wide and medium pink with a slight lavender tint.

Henny (1989), while studying the development, testing and release of new ornamental aroid cultivars, opined that the studies of factors affecting the flowering, pollination, seed set and genetics of ornamental tropical aroids made possible the development of new *Anthurium* hybrids suitable for use as indoor foliage plants. Hybrids selected for green house tests were asexually propagated from cuttings or by tissue culture.

Bindu and Mercy (1997) studied the karyotype of five commercial *Anthurium* varieties and concluded that all of them had a somatic chromosome number of 2n = 30+2B. They observed a wide range of meiotic abnormalities, morphological variation, karyotypic differences, high pollen sterility and stomatal characters attributed to the hybrid nature of the species. They concluded that *A. andreanum* is a secondary polyploid with a probable basic chromosome number of x = 6.

Attempts to transfer systemic resistance from *A. antioquiense* to the cultivated *A. andreanum* were done by Kuehnle *et al.* (1995) and they produced resistant F_1 hybrids. As the genetics of the available resistance was not properly understood, resistant varieties released soon became susceptible to blight.

Anais *et al.* (2000) identified a bacterial resistant clone of *Anthurium* (resistant to bacterial blight caused by *Xanthomonas campestris*) which can easily be crossed with the commercial cultivars, so that it will be possible to breed resistant varieties which meet the demands of both the export and local markets.

Cross compatibility among six *A. andreanum* varieties viz. 'Honeymoon Red' (HR), 'Chilli Red' (cR), 'Kalimpong Orange' (KO), 'Kalimpong Red' (KR), 'Pink' (P) and 'White' (W) were analysed by Sindhu (1995) who found that a large number of combinations were incompatible. Highly compatible crosses were HR x P and P x HR. The cross combinations, HR x CR, HR x KR, CR x W, KR x P, P x P and W x KR exhibited medium fruit set and high germination.

Oglesby Plant Laboratory Inc. (1996) described a variety 'Ruth Morat' syn 'Lady Ruth' as a derivative from the cross *A. antioqueiense* x Rotolante 1. This hybrid had spathes larger than those of 'Lady Jane', with a mean width and length of 50.1 and 76.8 mm, respectively.

A new variety 'Champion' developed from *A. andreanum* hybrids was released by Anthura (1997). This variety had small leaves and flowers with cupped white spathe held above the canopy and red spadix.

Henny (1999) described the new interspecific anthurium hybrid 'Red Hot' as highly suitable for pot planting because of its compact growth and production of numerous showy red spathes. 'Red Hot' originated from hybridization of *A. amnicola* Dressler, a dwarf species with small lavender spathes, with an unnamed selection of *A. andreanum* (accession code G-79) that had pink spathes. One of the resulting F_1 hybrids was again crossed with 'Lady Jane' from the progeny of which 'Red Hot' was selected. 'Lady Jane' according to Kamemoto and Kuehnle (1996) was an interspecific hybrid with *A. antioquiense* Engler in its background; however, its exact origin was unknown.

Anthura (2001) described an *Anthurium* variety 'Antikeles' syn. 'Pink Champion' derived from cv. 'Sweet heart Cherry' x 93-372-02 and selected for its characteristic pink-red flower.

Anthurium cv. 'Show Biz', an interspecific hybrid was described by Henny and Norman (2001). This hybrid produced numerous attractive light red spathes and had a compact branched growth habit.

Floral fragrance compounds of *A. armeniense*, *A. fragrantissimum*, *A. linderianum*, *A. ochranthum* and *A. roseospadix* and 3 hybrids of *A. armeniense* were studied by Kuanprasert *et al.* (1998) who identified 28 compounds. Their concentrations were assessed at the scent emitting pistillate stage of the spadix. The hybrid 'Tatsuta Pink Obake' emitted strong fragrance all day.

2.3.2 Seed set and development

After pollination and fertilization, the spadix begins to grow and takes on a warty appearance.

According to Mercy and Dale (1994), in a well fertilized candle, about 100-200 or even more berries are developed. A candle with developing fruits could be visually identified from the second month of fertilization, as it became swollen and fleshy with developing fruits embedded in it. In about eight weeks, the tip of the berries start projecting out like small pin heads. They also observed that in the commercial varieties of *A. andreanum,* each berry contained one or two seeds and the seeds matured in about 4-7½ months. Seeds remain enclosed within the thin fruit wall in a gelatinous pulp and if not harvested, remained attached to the candle for a few days more before they dried up and fell off the candle.

Zimmer (1986), while evaluating the development of *Anthurium* cultivars, observed that the berries contained two to three seeds and for ripening it took 5-12 months. He identified the absence of full fruit set in spadix and long ripening period as the problems in the development of anthurium cultivars.

Pierik *et al.* (1974a) opined that breeding of *A. andreanum* was handicapped by the long period from fertilization to ripening of seeds (6-7 months). Geier (1989) also recorded that the time required for seed maturity was about 6-7 months for *A. andreanum* and 10-12 months for *A. scherzerianum.*

Sindhu (1995) studied six varieties and found that the maximum average number of fruits was produced in the 'Pink' variety followed by 'Honeymoon Red'. The lowest number of fruits was produced by 'Kalimpong Red'. The cross 'Pink' x 'Honeymoon Red' produced maximum number of fruits (170) and the lowest number in 'Kalimpong Red' x 'Kalimpong Red' (2). The percentage of single seeds produced was more than the double seeds except in cross 'Kalimpong Red' x 'Honeymoon Red', where the percentage of double seeds was 63.

Based on their attempts to transfer resistance to bacterial pathogens from *A. antioquiense* to cultivated *A. andreanum*, Kuehhle *et al.* (1995) concluded that the production of horticulturally desirable varieties took many years since it is a perennial crop, with a long juvenile phase and slow seed germination.

Renu (1999) studied 10 varieties and found that the number of fruits per candle was highest in variety 'Pompon Red' and lowest in 'Lady Jane'. The percentage of fruit set was below 50 per cent for all the crosses except 'Pompon Red' x 'Liver Red'. The crosses involving 'Pompon Red' as female parent had the highest percentage of fruit set.

2.3.3 Seed germination

Bachthaler (1977) studied seed germination in *A. scherzerianum* hybrids and concluded that the fresh seeds germinated in light or darkness at 10-35°C with an optimum at 20-25°C in light, when germination occurred after 5-7 days. After drying and storing at 20°C for 24 hours germination under favourable conditions was 70-75 percentage.

In another study by Bachthaler (1978), seeds extracted from (1) green unripe, (2) reddish (half ripe), (3) red (ripe) or (4) reddish brown (overripe) berries were placed in petridishes on damp, sterile sand and kept at 25°C in 12 hours light (about 1200 lux). The first three groups showed 100 per cent germination, but group four only 42 per cent. Group two and three were the first to germinate and were the most suitable for commercial seed production.

While studying the storage of seeds of *A. scherzerianum* hybrids, Bachthaler (1979) observed that the best storage temperature was 10°C and after about six weeks, 60 per cent of the seeds germinated. Ninety five per cent of seeds from berries treated with thiram dust before storage, germinated after 12 weeks at 10°C and 60 per cent after 16 weeks.

Szendel *et al.* (1982) germinated the seeds of *A. andreanum* harvested at three maturity stages and those of *A. scherzerianum* at one maturity stage (light orange) on three substrates, at pH ranging from 4 to 8 in light or darkness at 18, 24 or 28°C. In *A. andreanum*, the best germination was obtained on a high peat substrates at pH 4-5 in light at 28°C using seeds harvested at an early maturity stage (yellow green to light orange).

According to Zimmer and Bahnemann (1982) *A. scherzerianum* seeds from different sources varied in their ability to germinate at low, sub-optimal temperatures. Optimum germination temperature was recorded to be 20-25°C, but some seeds germinated well at 10 or 15°C.

A new method of developing *A. scherzerianum* varieties using seeds cultured *in vitro* was described by Zimmer (1990). It involved selecting genotypes with a high regenerative ability, carefully adjusting the NH_4:NO_3 ratio of the liquid medium for regeneration and multiplication, maintaining clonal explants for up to about 2 years at 11-15°C and 300 lux light in twist off glass vessels and carefully selecting for horticulturally desirable traits, especially flower colour, flowering onset and cold requirements for flowering.

According to Mercy and Dale (1994) the hybrid seeds from crosses between ordinary hardy varieties had above 90 per cent germination and that their seedlings showed high survival fitness and vigour. Seeds produced in crosses between exotic varieties were smaller in size and poor in germination.

Among the six varieties studied by Sindhu (1995), the maximum average germination was observed in combination with the variety 'White' as the female parent (63.4 per cent) and the lowest germination in the variety 'Kalimpong Orange'.

Renu (1999), after studying 10 varieties, concluded that the number of days taken for seed germination varied from 3 to 12 days.

2.4 *IN VITRO* STUDIES

2.4.1 *In vitro* germination of seeds

Several studies have been conducted in the micropropagation of *Anthurium andreanum* and *A. scherzerianum*. Plantlet regeneration has been obtained via. callus from cultured embryos and explants of leaf lamina, petiole, inflorescence stalk, spathe and spadix, or without intervening callus from embryo and axillary bud explants. Pioneering studies were conducted by Pierik and collaborates (1974a, b). They succeeded in the induction of regeneration, first from embryo and seedling tissues and later from non-meristamatic parts of mature plants. A modified MS medium supplemented with a cytokinin (PBA) was used. Optimum growth of callus tissue was obtained at 25°C in darkness.

Mature embryos of hybrid seeds of *Anthurium andreanum* were used for producing axillary buds and adventitious shoots by direct culture of mature seeds on Nitsch media supplemented with 0.2 ppm of BAP and other additives (Rajasekharan *et al.*, 1994).

In vitro seed germination in *Anthurium andreanum* was also reported by Swaminathan (1986) and Randhawa (1990). Devinder Prakash *et al.* (2001) obtained regeneration of plantlets from petiole explants of *Anthurium andreanum* cv. 'Mauritius Orange'. They cultured selfed seeds of *A. andreanum* cv. 'Mauritius Orange' on agarified Nitsch medium and callus was induced on petioles excised from eight week old seedlings and cultured on MS medium supplemented with 1.0 or 0.5 mg l^{-1}, 2,4-D. Subculturing to MS basal medium produced green spots which subsequently developed into shoot primordia and eventually into shoots. Similarly, sterilized seeds of *A. parvispathum* were germinated *in vitro* and used as source of explants by Atta-Alla *et al.* (1998).

2.4.2 Seed culture from immature berries

It will take 6-8 months from opening of flower to maturity of the seed, in anthurium depending on the variety. This time lag could be substantially reduced if

seedlings could be produced by the *in vitro* culture of seeds isolated from the immature berries.

Cultures of immature embryos is infact successfully done in orchids, which is referred to as green pod culture. Immature embryos from a three month old capsule of the orchid *Acampe rigida* germinated within four weeks. Seed capsules of this species reached maturity approximately one year after pollination and mature seeds failed to germinate (Yam and Weatherhead, 1988).

Sobhana (2000) observed maximum seed germination with 90, 100 and 110 days old pods in Dendrobium orchids whereas it took 150 days for full maturity (pod broken in the field). Germination percentage was also very low at full maturity stage.

Post pollination investigations were carried out on *A. andreanum* cv. 'Kalapana' from 4 to 24 weeks (Matsumoto *et al.*, 1998). Anatomical features were correlated with the morphology of the spadix and the capacity of embryos to germinate *in vitro*. According to them the development from a single celled zygote to fully mature seed took 24 weeks. Differentiation of the shoot apex, cotyledon and protoderm occurred at 14 weeks. The embryo started to derive nutrition from the endosperm at this time and germination of cultured ovules reached 56 per cent. By 20 weeks, shoot apex had visible leaf primordia and the root apex was clearly defined. The cotyledon was well developed and 100 per cent germination occurred at 20 weeks and thereafter.

2.4.3 Seed sterilization

The seeds are cultured in completely aseptic condition. Hence the seeds are to be sterilized before inoculation into the medium. The most commonly used surface sterilant is sodium hypochlorite. For softer tissues, a dilution to lower strength may be needed; but anything below 0.5 per cent may prove ineffective. Concentrations ranging from 1 to 10 per cent (Kuo and Tssay, 1977) have been generally used.

Mercuric chloride is another commonly used surface sterilant. Sterilization with 0.1 per cent mercuric chloride for 10 minutes was reported in *Anthurium* explants (Anu, 1998). An initial treatment with Bavistin 0.1 per cent followed by wiping with alcohol 70 per cent is also reported.

Pod sterilization after dipping in alcohol followed by flaming was reported in *Dendrobium ovalum* by Pyati and Murthy (1995). Seeds directly transferred to the medium without exposure to outside germinated well and produced strong seedlings within 8 to 10 weeks (Rao and Avadhani, 1963).

According to Somaya *et al.* (1998), seeds of *A. andreanum* sterilized in 3 per cent NaOCl for 30 minutes produced the highest percentage germination, while sterilizing for 15 minutes resulted in the highest seedling height and leaf number.

Sobhana (2000) observed maximum survival percentage with the combination in which the pod was kept in mercuric chloride (0.1%) for one minute and flamed after a dip in 70 per cent alcohol.

2.4.4 Culture medium

Wide variety of tissue culture media has been reported. The choice depends on the plant species and intended use of the culture. The Murashige and Skoog (1962) medium, characterized by high concentrations of mineral salts has been widely used for general plant tissue culture and specifically for morphogenesis and plant regeneration.

Auxin and cytokinins are inevitable components of plant tissue culture media. BA has been the most effective cytokinin for meristem, shoot tip and bud cultures, followed by kinetin (Murashige, 1974). Several scientists have reported 2-isopentenyl adenine as the best cytokinin for multiple shoot induction and callus regeneration (Ettinger and Preece, 1985; Voyatzi and Voyatziz, 1989). Lo *et al.* (1980) reported that high cytokinin content was deleterious to the initiation and elongation of roots of both monocotyledonous and dicotyledonous plants.

Tissue culture in *A. andreanum* was first reported by Pierik *et al.* (1974a). Young actively growing plant part has been described as an active site for auxin biosynthesis. However in anthurium a low auxin has been reported to be suitable for callus formation and further growth and regeneration (Pierik *et al.*, 1975; Finnie and Van Staden, 1986).

Half strength MS medium was found to be a good basal medium for the somatic embryogenesis in anthurium (Kuehnle *et al.*, 1992). Sreelatha (1992) also found that modified MS with reduced major nutrient concentration was better for callus formation in anthurium.

Kuehnle and Sugii (1991) observed embryogenic callus of *A. andreanum* cultures on medium containing 2,4-D and BAP. Somatic embryogenesis was reported in *A. andreanum* by Kuehnle *et al.* (1992) on modified half strength MS medium supplemented with 1.0 to 4.0 mg l^{-1}, 2,4-D and 0.33 to 1.0 mg l^{-1} kinetin.

Lightbourn and Prasad (1990) reported *in vitro* techniques for rapid multiplication of *A. andreanum* cultivars 'Tropical Pink', 'Premium Red', 'White' and 'Tulip'. They obtained the best callus formation in cv 'Tulip' at 0.5 mg l^{-1} 2,4-D, whereas 'Tropical Pink' gave good results with 0.05-0.5 mg l^{-1} 2,4-D. Good shoot multiplication was obtained at 0.2-0.8 mg l^{-1} BA in all cultivars. Root formation was not affected by varying concentration of ammonium nitrate. However, larger leaves and more prolific leaf production occurred with increased ammonium nitrate concentrations.

Rajasekharan and Kumar (1994) produced somatic embryos of anthurium on modified Nitsch and Nitsch medium containing BAP, kinetin and 2,4-D.

Sreelatha (1992) opined that MS medium with $¼^{th}$ strength major nutrients was ideal for callus multiplication. Shoot regeneration and growth of the shoots were the best in MS medium with BA 0.5 mg l^{-1} and IAA 2.0 mg l^{-1}. She also reported that one forth strength of MS major nutrients with full strength of micro nutrients was ideal

for multiple shoot induction. In *A. andreanum*, 2,4-D 0.08 mg l^{-1} and BA 1.0 mg l^{-1} was ideal for callus initiation.

Somaya *et al.* (1998) obtained multiple shoots from shoot tips and nodes on MS medium supplemented with 1 mg l^{-1} BA. Seeds produced highest callus production with 2.0 mg l^{-1} 2,4-D while leaf, petiole, node and root explants gave the highest callus production with 0.1 mg l^{-1} 2,4-D + 1.0 mg l^{-1} BA. Rooting occurred easily with growth regulators but the addition of 0.25 mg l^{-1} NAA increased the quality and number of roots produced.

Malhotra *et al.* (1998) observed callus induction in three cultivars of *Anthurium andreanum* on Nitsch medium supplemented with BA 1 mg l^{-1}, 2,4-D 0.1 mg l^{-1} and ammonium nitrate 200 mg l^{-1}. Shoot formation occurred when the BA concentration was reduced to 0.5 mg l^{-1} and ammonium nitrate concentration was increased to 720 mg l^{-1}. Rooting occurred readily on Nitsch medium supplemented with IBA 1 mg l^{-1}, but percentage of rooting improved by addition of activated charcoal (0.04%) to the culture medium.

A four fold increase in the number of shoots produced was obtained in a multiplication medium containing 2 mg l^{-1} BA and 0.2 mg l^{-1} NAA. The shoots elongated to a length of 2.3 cm on medium containing 20 mg l^{-1} kinetin (Atta-Alla *et al.*, 1998).

Mahanta and Paswan (2001) conducted experiment to develop a commercial tissue culture technique for mass multiplication of *Anthurium andreanum* cv. 'Agnihothri'. They observed that MS basal medium supplemented with BA (0.8 mg l^{-1}), vitamin B_5 (0.5 mg l^{-1}), IAA (0.1 mg l^{-1}), polyvidone (200 mg l^{-1}) and coconut water (150 ml l^{-1}) was the best for multiple shoot production. The highest rooting percentage (80%) was observed in MS medium supplemented with IAA (1.0 mgl^{-1}).

Montes *et al.* (2000) obtained white callus mass from leaf explants of *A. cubense* under darkness, after subculture to a medium containing 4.7 µM Pectimorf and regeneration rate was up to 17 buds.

Rooting of *in vitro* regenerated plants need not always have to be carried out *in vitro* (Mc Cown and Amos, 1979). Direct rooting of root less shoots of anthurium is possible but is not recommended because it requires very long time, success is inconsistent and losses are considerable (Geier, 1987). Auxin is considered essential for root initiation. Among the auxins, NAA and IBA are widely used for root induction (Iida *et al.*, 1986). 80 per cent rooting was observed in MS basal media supplemented with IAA 1 mg l^{-1} (Mahanta and Paswan, 2001).

Though auxins are considered essential for rooting, several workers have reported better rooting on a medium free of plant growth substances (Nair *et al.*, 1984; Maria and Segura, 1989).

Sometimes root induction fails at high salt concentration regardless of the types of hormone present. Abundant rooting was observed when the salt concentration was reduced to one-half of the standard strength (Iida *et al.*, 1986; Omura *et al.*, 1987; Maria and Segura, 1989).

2.5 IRRADIATION STUDIES

Genetic diversity which is the backbone of crop improvement, may be introduced deliberately by employing ionizing radiations and chemical mutagents.

Tissue culture greatly facilitates the application of mutagens to tissues or cells and makes it easy to handle a large number of irradiated materials and also for the selection of the trait at tissue level (Nickel, 1973).

Ionising radiations can interact with cells to produce a genetic effect in the immediate vicinity of its ionization track (Muller, 1954).

2.6 PLANTING OUT AND HARDENING

Physical, chemical and biological properties of the potting media and the atmospheric conditions during post-transfer growth are important in the establishment of *in vitro* regenerated plantlets, which have been planted out.

Geier (1989) observed that anthurium plantlets could be established without losses in a peat / sand media.

According to Sreelatha (1992) sand was the best potting medium for planting out. Nutrient solutions when used for the irrigation of the plantlets had a negative influence on the survival of plantlets.

According to Rajasekharan *et al.* (1994) incorporating vermicompost to a suitable growing medium helped in reducing the mortality to less than five per cent.

Establishment and hardening of *in vitro* derived plantlets of *Anthurium andreanum* were examined by Ajithkumar and Nair (1996). According to them plantlets 2.5 to 3.0 cm long survived best and plantlets grown on a soilrite medium grow best for the first two months under *ex vitro* conditions. But they observed no clear relationship between type of containers and growth factors.

Mahanta and Paswan (2001) observed a survival rate of 60 per cent when *in vitro* raised plantlets were transferred to *in vivo* condition in plastic pots containing soilrite - perlite (10:1) mixture.

Jawaharlal *et al.* (2001) standardised growing media for *Anthurium andreanum* cv. 'Temptation'. According to them, cocopeat applied alone or in combination with leaf mould or FYM resulted in the highest number of branches and suckers per plant and cocopeat alone produced the highest flower number. Cocopeat alone or cocopeat + leaf mould produced the shortest preblooming period and increased inflorescence longevity.

2.7 CYTOLOGICAL STUDIES

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 diversity and complexity of genus *Anthurium* is evident both cytologically and morphologically. The chromosome numbers reported for some members of the genus *Anthurium* illustrates this point.

The somatic chromosome number of *Anthurium* vary from 20 (*A. gracile*) to 124 (*A. lucedum*). However the most common number is 30 (Sheffer and Kamemoto, 1976b).

Chromosome number for the species *A. andreanum* was reported as 2n = 30 by Gaiser (1927), Ito (1942), Sharma and Bhattacharya (1966), Sheffer and Croat (1983), Satyadas (1985), Sengupta and Chettri (1989). Presence of 0 to 2B chromosomes was first reported by Sharma and Bhattacharya (1966) in *A. andreanum* variety 'Rhodochlorum' and Lalithambika (1978) in the variety 'Roseum'. Satyadas (1985) reported one B chromosome in the variety 'Roseum'.

Chromosome number for the species *A. crystallinum* was reported as 2n = 30 by Gaiser (1927). The occurrence of B chromosomes has been reported in *A. crystallinum* as 2n = 40 + 0 - 2B (Lalithambika, 1978) and 2n = 45 + 0 - 4B (Satyadas, 1985). 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*.

The chromosome number 2n = 30 + 0 - 2B was reported by Gaiser (1927) and Satyadas (1985) in *A. ornatum*. According to Marutani *et al.* (1993), the reduction in pollen fertility estimated by pollen stainability suggested genetic divergence of species.

Mitotic behaviour of five varieties of *Anthurium andreanum* was studied by Bindu (1992) and Balachandran (1998) who had reported somatic chromosome number of 30 + 2B. The B chromosomes were seen in all the five varieties, namely, 'Honeymoon Red', 'White' (album), 'Lady Jane' (pink), 'Chilli Red' and 'Pink', irrespective of the time of sampling and tissues studied. The B chromosomes were seen to be deeply stained, smaller in size and were either round or rod shaped.

According to Bindu and Mercy (1997), *A. andreanum* appeared to be a highly evolved secondary polyploid with a probable basic chromosome number of x = 6.

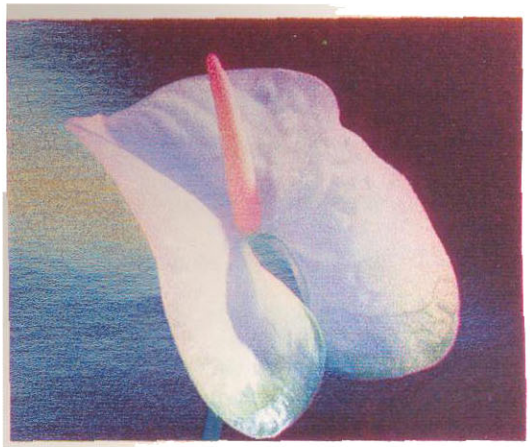
2.8 ISOZYME STUDIES

Isozyme analysis by electrophoresis is a well defined and effective method to detect genetic differences among individuals and is widely used as a supplementary tool along with morphological methods of plant classification. The use of gel electrophoresis of different isozyme systems was investigated to identify cultivars of *A. andreanum* (Kobayashi *et al.*, 1987). They were able to characterize the different varieties using combined data for peroxidase, malate dehydrogenase and phosphogluco isomerase.

Differences in banding pattern observed for leaf peroxidase was used in identification of rose hybrids (Yoneda *et al.*, 1993). In *Cereus peruviana* isocitrate dehydrogenase was considered as good marker for investigating possible genetic variation in plant population regenerated from calli cultures (Mangolin *et al.*, 1994). Jeong *et al.* (1996) suggested that variation in morphological characteriscs of *Lilium hansonii* can be studied using isozymes, esterase and peroxidase.

In *Cymbidium* orchids, genetic variation among cultivars was detected using isoenzymes. The identification potential increased with the number of isoenzymes used. *In vitro* derived protocorm like bodies could be used successfully for the isoenzyme analysis (Obara-Okeyo and Kako, 1997).

Materials and Methods



3. MATERIALS AND METHODS

The present investigation entitled 'Improvement of *Anthurium andreanum* Lind by *in vivo* and *in vitro* methods' was carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during the period from 1998 to 2001. Six commercially important varieties of *Anthurium andreanum* and three other species of *Anthurium* were used for the study with the objective of evolving varieties suitable for commercial cultivation with qualities preferred in the international market.

3.1 THE STUDY COMPRISED OF THE FOLLOWING MAJOR EXPERIMENTS

3.1.1 Characterisation of the selected *Anthurium* varieties

3.1.2 Interspecific and inter varietal hybridization

3.1.3 *In vitro* studies

1. Suitability of age of seed for successful *in vitro* seed culture
2. Standardisation of media for seed germination, callusing, shoot and root regeneration

3.1.4 Irradiation studies

1. Seed irradiation
2. *In vitro* irradiation

3.1.5 Evaluation of hybrids by morphological, cytological and isozyme studies

3.2 SPECIES AND VARIETIES SELECTED FOR THE STUDY

3.2.1 Male parents

Anthurium amnicola

A. ornatum

A. crystallinum

3.2.2 Female parents

A. andreanum var. 'Nitta'

A. andreanum var. 'Candy Queen'

A. andreanum var. 'Lima'

A. andreanum var. 'Red Dragon'

A. andreanum var. 'Eureka Red'

A. andreanum var. 'Agnihothri'

3.3 CLIMATE

Vellanikkara is located at an altitude of 22.25 m above MSL and between 10°31' N latitude and 76°10' E longitude. The area enjoys a typical tropical humid climate. The meteorological data during the cropping period is presented in Appendix I.

3.4 MANAGEMENT OF PLANTS

The experimental plants were grown in pots with the potting mixture containing coarse sand, well rotten cowdung clumps, coconut husk pieces (3 cm size) and earthern crocks (2:1:1:0.5) (Salvi *et al.*, 1998, 2001). All plants received uniform cultural practices. Organic manuring was done by the application of cowdung clumps, 20 g per plant and bone meal 10 g per plant once in a month To boost up growth, weekly sprays of NPK 20:20:40 at 0.25 per cent concentration were given (Abdussamed, 1999; Valsalakumari *et al.*, 2001). Watering was done by sprinklers provided in the shade houses twice daily (morning and afternoon) during summer months. During rainy season, plants were watered only on sunny days. Care was taken to provide good drainage. Plants were maintained under 80 per cent shade (Plate 1) and sprays of fungicides and insecticides were given as and when required.

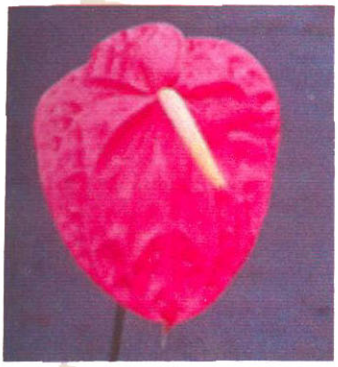
3.5 CHARACTERISATION

The varieties and species selected for the study were morphologically described (Plate 2). Observations were made on the following characters.

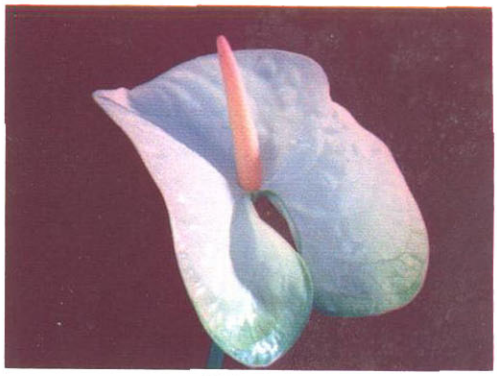
Plate 1. Overall view of the net house with 80 per cent shade

Agnihothri

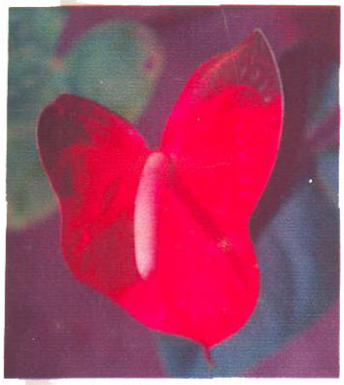
Nitta



Candy Queen



Lima



Red Dragon

A. amnicola

Eureka Red

Plate 2. *Anthurium andreanum* varieties and species used for the study

3.5.1 Vegetative characters

a. Plant height (a) (cm)

The height of the plants was measured from the collar region to the tip of the shoot portion and expressed in cm.

b. Plant height (b) (cm)

Height of the plant was measured from the collar region to the tip of the topmost leaf and expressed in cm.

c. Spread of the plant (cm)

Spread of the plant in east west and north south directions was measured and expressed in cm.

d. Number of leaves

The total number of leaves borne on the plant was counted and recorded.

e. Internodal length (cm)

The length of the stem in between the consecutive leaves was measured and expressed in cm.

f. Petiole length (cm)

Petiole length of the second leaf from the top was measured from the point of its emergence to the base of the leaf lamina and expressed in cm.

g. Leaf area (cm^2)

Length and width of the leaf was measured and area was computed using the formula suggested by Salvi *et al.* (1995).

Leaf area = l x b x 0.72

l = length of leaf

b = breadth of leaf

h. Number of suckers

The total number of suckers borne on the plant in a year was counted and recorded.

3.5.2 Floral characters

3.5.2.1 Floral morphology

a. Colour of spathe

Colour of spathe was recorded by visual observation.

b. Texture of spathe

Texture of the spathe was recorded by visual observation.

c. Length of spathe (cm)

Length of the spathe from the base to the tip was measured and expressed in cm.

d. Width of spathe (cm)

Width at the middle of the spathe was measured and expressed in cm.

e. Colour of spadix

Colour of spadix was recorded by visual observation.

f. Length of spadix (cm)

Length of spadix was measured from the base to the tip and expressed in cm.

g. Angle of orientation of spadix

Angle between the spathe and spadix was measured and recorded.

h. Relative length of spathe to spadix

This was calculated as the ratio of the length of spathe to the length of spadix.

i. Length of peduncle (cm)

Length of peduncle from its point of emergence to the point of attachment of the spathe was measured and expressed in cm.

j. Girth of peduncle at base (cm)

Girth of peduncle at the base was measured and expressed in cm.

k. Nature of peduncle

Nature of peduncle such as straight or bending was observed and recorded.

l. Longevity of spike on plant (days)

The number of days from the opening of the spathe to the loss of quality as indicated by spathe necrosis, spadix necrosis or discolouration of the spathe on the plant was recorded in the case of unfertilized spikes.

m. Number of leaves / spikes per year

The total number of spikes produced by the plant in an year was counted and recorded.

n. Duration from flower opening till ripening (days)

Number of days from the opening of the flower to the ripening of the seeds was recorded in the case of fertilized spike.

3.5.2.2 Flowering pattern

Individual flowers of *Anthurium andreanum* is bisexual but they show a clear protogynous nature. Anthesis occur in *Anthurium* between 8.00 and 9.00 am. Stigma receptivity is distinguished by the presence of stigmatic droplets or glistering of stigmas.

The following were the observations recorded.

a. Duration from spike emergence to unfurling (days)

Number of days taken from the inflorescence emergence to the unfurling of the spathe was recorded.

b. Duration from spathe unfurling to female phase (days)

Number of days taken from the unfurling of the spathe to the start of female phase was recorded.

c. Duration of female phase (days)

Number of days spadix remains in female phase was recorded. Female receptivity was identified by the presence of sticky stigmatic droplets and colour change of the spadix.

d. Duration of interphase (days)

Number of days taken from the end of female phase to the start of pollen emergence was recorded.

e. Duration of male phase (days)

Number of days from the beginning of emergence of pollen on the spadix to the end of pollen emergence was recorded.

Data from five spadices were recorded for each variety and the mean was calculated.

3.5.2.3 Pollen characters

3.5.2.3.1 Pollen morphology - size and shape

The pollen grains from freshly dehisced anthers were collected after anthesis, dispersed on a drop of acetocarmine : glycerine mixture (1:1) and mounted on a microscopic slide and observed under microscope. Diameter of ten normal, plumpy, well shaped and well stained pollen grains from each variety was measured at

random using a standard ocular micrometer under microscope. The mean diameter was expressed in microns from which the size was worked out. The shape of the pollen grain was studied under the high power magnification.

3.5.2.3.2 Pollen fertility

The fertility of pollen grains was estimated by acetocarmine staining technique.

Pollen grains were collected during the male phase from all the six varieties and species and stained with 1:1 glycerine acetocarmine (2%). Pollen fertility was estimated by counting fertile and sterile pollen grains separately. Pollen grains which stained well, looked plumpy, well filled and well shaped were considered as fertile. Unstained, small or shriveled or misshaped pollen grains were counted as sterile (Zirkle, 1937). The observations were made in five different microscopic fields. This was repeated using three such slides of pollen in each variety and species. Pollen fertility was worked out as follows.

$$\text{Percentage of fertile pollen} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.5.2.3.3 Pollen production pattern

The pollen production pattern of the different varieties and species under study was recorded by visual observations of the spadices of the plants for 2 years from May 1999 to April 2001.

3.5.2.4 Post harvest characters

For this uniform normal size flowers were used. Flowers were cut in the morning at $1/3^{rd}$ maturity stage and placed in distilled water (Salvi, 1997) and observations were recorded on the following characters.

3.5.2.4.1 Vase studies

a. Fresh weight of the spike (g)

Weight of the spike was taken soon after harvesting and expressed in g.

b. Days to colour fading

Number of days from the date of harvest to the colour fading or discolouration of the spathe was recorded.

c. Days to spathe necrosis

Number of days from the date of harvest to the spathe necrosis was recorded.

d. Days to spadix necrosis

Number of days taken from the date of harvest to the necrosis of spadix was recorded.

e. Weight loss (g)

Initial and final weight of the spike was measured at the beginning and at the end of the experiment, respectively, and weight loss was computed by working out the difference between initial and final weights.

f. Water uptake (ml)

The spike was placed in a conical flask containing measured quantity of water. The quantity of water left in the flask after the removal of spike on the last day in vase was also measured. The difference gave the water uptake, which was expressed in ml.

3.5.2.4.2 Packing studies

Flowers were cut at 1/3rd maturity stage and packed with moistened cotton covered with polythene strip at the cut end. They were than packed in cardboard cartons of size 2.5 x 1.5 x 0.5 ft^3 with holes on opposite sides. The following observations were recorded.

a. Days to colour fading of spathe

Number of days from the date of packing to the colour fading of the spathe was recorded.

b. Days to spathe necrosis

Number of days from the date of packing to the spathe necrosis was recorded.

c. Days to spadix necrosis

Number of days from the date of packing to the necrosis of the spadix was recorded.

d. Weight loss

Fresh weight of the spike and final weight after the termination of the life of spike was measured and the difference was calculated and expressed in g.

3.6 HYBRIDISATION

Flowers for hybridisation were marked at the time of spathe unfurling. Individual flowers of *Anthurium andreanum* is bisexual, but they show a clear protogynous nature. So no emasculation was needed. The spadices of the selected female parents were protected using polythene cover, before the commencement of the female phase, to prevent unwanted pollination. When the female phase started as indicated by the maturity of the lower flowers, pollen was collected from the male parent and applied on to the spadix of the female parent, which was done in the morning hours. Repeated pollinations were done over a period of 5 to 8 days and the spadix was kept bagged until the male phase started. Post pollination changes were observed in each of the pollinated flowers. Hybridisation in all the possible combinations using these varieties and species were carried out and compatibility was analysed.

A. andreanum var. 'Nitta' x 'Nitta'

'Nitta' x 'Lima'

'Nitta' x 'Candy Queen'

'Nitta' x 'Eureka Red'

'Nitta' x 'Agnihothri'

'Nitta' x *A. amnicola*

'Nitta' x *A. ornatum*

'Nitta' x *A. crystallinum*

A. andreanum var. 'Candy Queen' x 'Candy Queen'

'Candy Queen' x 'Nitta'

'Candy Queen' x 'Lima'

'Candy Queen' x 'Eureka Red'

'Candy Queen' x 'Red Dragon'

'Candy Queen' x 'Agnihothri'

'Candy Queen' x *A. amnicola*

'Candy Queen' x *A. ornatum*

'Candy Queen' x *A. cystallinum*

A. andreanum var. 'Lima' x 'Lima'

'Lima' x 'Nitta'

'Lima' x 'Candy Queen'

'Lima' x 'Eureka Red'

'Lima' x 'Red Dragon'

'Lima' x 'Agnihothri'

'Lima' x *A. amnicola*

'Lima' x *A. ornatum*

'Lima' x *A. crystallinum*

A. andreanum var. 'Eureka Red' x 'Eureka Red'

'Eureka Red' x 'Nitta'

'Eureka Red' x 'Lima'

'Eureka Red' x 'Candy Queen'

'Eureka Red' x 'Red Dragon'

'Eureka Red' x 'Agnihothri'

'Eureka Red' x *A. amnicola*

'Eureka Red' x *A. ornatum*

'Eureka Red' x *A. crystallinum*

A. andreanum var. 'Red Dragon' x 'Red Dragon'

'Red Dragon' x 'Nitta'

'Red Dragon' x 'Lima'

'Red Dragon' x 'Candy Queen'

'Red Dragon' x 'Eureka Red'

'Red Dragon' x 'Agnihothri'

'Red Dragon' x *A. amnicola*

'Red Dragon' x *A. ornatum*

'Red Dragon' x *A. crystallinum*

A. andreanum var. 'Agnihothri' x 'Agnihothri'

'Agnihothri' x 'Nitta'

'Agnihothri' x 'Lima'

'Agnihothri' x 'Candy Queen'

'Agnihothri' x 'Eureka Red'

'Agnihothri' x *A. amnicola*

'Agnihothri' x *A. ornatum*

'Agnihothri' x *A. crystallinum*

Observations were recorded as follows.

a. Percentage of spadices setting berries

Successfully fertilized inflorescences that remained healthy with peduncles strong and green were noted and their percentage was calculated as

$$\frac{\text{The number of spadices setting berries}}{\text{Number of spadices pollinated}} \times 100$$

b. Number of fruits per spadix

The number of fruits in each successful pollination was counted and recorded.

c. Percentage of fruit set per spadix

The percentage was calculated as

$$\frac{\text{Number of flowers showing fruit set}}{\text{Total number of flowers in the spadix}} \times 100$$

d. Number of seeds per berry

The number of seeds in each ripe berry was recorded.

3.7 *IN VITRO* STUDIES

3.7.1 Standardisation of the maturity of seed for successful seed culture *in vitro*

Seeds were isolated from berries at different stages of maturity starting from 70 days till ripening at an interval of 10 days to standardise the correct maturity stage for *in vitro* culturing. This was done in two crosses involving 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon'.

Media used: MS and Nitsch media

Observations were recorded on the percentage of seeds germinated and days taken for initiation of germination.

3.7.2 Standardisation of media

The basal media used in the present study were full strength MS (Murashige and Skoog, 1962), ½ strength MS (50% concentration of inorganic salts),

¼ strength MS (25% concentration of inorganic salts), Nitsch medium (Nitsch and Nitsch, 1969). The composition of the different media are given in Appendices II and III.

3.7.2.1 Media preparation

MS medium was prepared according to the standard procedure given by Gamborg and Shyluck (1981). Stock solution was prepared by dissolving required quantities of major and minor nutrients in distilled water and was stored under refrigerated conditions. The chemicals used for the preparation of the media were of analytical grade from British Drug House (BAH), Sisco Research Laboratory (SRL), Merck or Sigma.

Specific quantities of stock solutions of chemicals were pipetted out into a 1000 ml standard flask. To this the required quantity of sucrose, inositol and growth regulators were added and the volume was made up with double glass distilled water. Then the pH of the solution was checked and adjusted to 5.8 using one normal NaOH/HCl. To get the solid medium, agar was added and final volume of the medium was made up to 1000 ml. Agar was dissolved in the medium by heating. Then the medium was poured hot into clean and dry culture vessels. They were plugged with non-absorbent cotton and sterilized at a pressure of 1.1 kg/cm^2 at 121°C for 20-30 minutes.

After sterilization, the culture tubes were stored in an air conditioned culture room for further use.

3.7.2.2 Inoculation process

Inoculation was carried out under strict aseptic conditions in a laminar air flow cabinet. Sterilized forceps, petridishes, surgical blades and blotting paper were used.

3.7.2.3 *Culture conditions*

The cultures were incubated in a culture room where the temperature was maintained at 27±2°C.

3.7.2.4 *Preparation of seeds for inoculation*

Berries were collected from the spadix and rinsed with tap water, teepol and distilled water. Seeds were extracted from the berries inside a laminar air flow cabinet. The cabinet was irradiated with UV rays for 20 minutes prior to working.

The working table was also thoroughly wiped with absolute ethyl alcohol. Sterilized forceps, petri dishes and surgical blades were used.

3.7.2.5 *Standardisation of surface sterilisation*

Different surface sterilisation treatments adopted are given in Table 1.

Table 1. Surface sterilants, their concentrations and duration of treatment for *in vitro* seed culture in *A. andreanum*

Sl.No.	Surface sterilants	Concentration (%)	Duration (minutes)
1	Silver nitrate	0.1	20
2	Silver nitrate	0.1	10
3	Bavistin + Mercuric chloride	0.1 0.1	30 10
4	Mercuric chloride	0.1	10
5	Mercuric chloride	0.2	10
6	Mercuric chloride + flaming	0.1	10

In all the treatments, the explants were submerged in the sterilant for the required period with frequent agitation.

3.7.2.6 *Media for seed germination and development*

The seeds, after sterilization, were washed with distilled water four times, wiped dry and inoculated with a sterile forceps into the culture media. The culture vessels were then kept in the culture room.

Different media tried for seed germination are

Basal MS

½ MS

¼ MS

Nitsch

MS + 0.5 mg/l BA

MS + 1 mg/l BA

½ MS + 0.5 mg/l BA

½ MS + 1 mg/l BA

Observations recorded

a. Days taken for germination

Number of days taken from the inoculation of the seed to the germination was recorded.

b. Germination percentage

Percentage of germination was calculated as

$$\frac{\text{Number of seeds germinated}}{\text{Number of seeds inoculated}} \times 100$$

c. Days taken for first leaf formation

Number of days taken from inoculation to the appearance of first leaf was recorded.

d. Days taken for callus development

Number of days taken from inoculation to the initiation of callus was recorded.

e. Days taken for multiple shoot formation

Number of days taken from inoculation to the multiple shoot emergence was recorded.

3.7.2.7 *Refinement of the medium with growth hormones*

The germinated seeds were subcultured to various media and their effect on the growth of the seedlings was studied. Different combinations of auxins (IAA, IBA, NAA, 2,4-D) and cytokinins (kinetin, BA) were used for the study, each at different concentrations ranging from 0.5 to 8.0 ppm.

The following observations were recorded

a. Percentage of cultures showing callus

Percentage of cultures showing callus was calculated as

$$\frac{\text{Number of cultures showing callus initiation}}{\text{Number of cultures inoculated}} \times 100$$

b. Growth score

Growth of the callus was assessed based on a visual rating. Score 1 was given to the smallest and score 4 was given to the largest and the mean was given as growth score.

c. Callus index

Callus index was computed by multiplying percentage of explants initiating callus with the growth score.

d. Percentage of cultures showing shoot regeneration

The percentage was calculated as

$$\frac{\text{Number of cultures showing shoot regeneration}}{\text{Number of cultures inoculated}} \times 100$$

e. Number of shoots formed

Number of shoots formed after 4 weeks and 8 weeks were counted and recorded.

f. Length of longest shoot (cm)

Length of the longest shoot was measured and expressed in cm.

g. Number of roots formed

Number of roots formed after 8 weeks were counted and recorded.

h. Number of days taken to root formation

Number of days taken from inoculation of culture to rooting medium to the initiation of roots was recorded.

i. Percentage of cultures showing root formation

This was calculated as

$$\frac{\text{Number of cultures showing rooting}}{\text{Number of cultures inoculated}} \times 100$$

3.8 IRRADIATION STUDIES

The experiment was done using the seeds obtained from the cross 'Lima' x 'Eureka Red'. Gamma rays were used to irradiate the hybrid seeds and the *in vitro* cultures. The seeds were exposed to various doses of gamma radiation, from 2 Gy to 20 Gy. *In vitro* cultures were irradiated at callus initiation stage and shoot regeneration stage. The cultures were irradiated using gamma rays from 0.5 Gy to 10 Gy. Immediately after irradiation, the materials were transferred to a fresh medium. Comparative evaluations were made during the development of irradiated and non irradiated cultures.

Following observations were recorded.

3.8.1 Irradiation of seeds

a. Germination percentage

Germination percentage was calculated as

$$\frac{\text{Number of seeds germinated}}{\text{Number of seeds inoculated}} \times 100$$

b. Days taken for germination

Number of days taken from inoculation of seeds to germination was recorded.

c. Days taken for leaf formation

Number of days taken from inoculation of seeds to the formation of leaf was recorded.

d. Size of the leaf (mm)

Size of the first leaf was measured and expressed in mm.

3.8.2 Irradiation at callus initiation stage

a. Percentage of cultures showing shoot regeneration

This was calculated as

$$\frac{\text{Number of cultures showing shoot regeneration}}{\text{Number of cultures inoculated}} \times 100$$

b. Number of shoots

Number of shoots formed after two months was counted and recorded.

c. Number of leaves

Number of leaves produced after two months was counted and recorded.

d. Number of roots

Number of roots formed was recorded after two months.

3.8.3 Irradiation at multiple shoot regeneration stage

a. Percentage of cultures showing growth

The percentage was calculated as

$$\frac{\text{Number of cultures showing growth}}{\text{Number of cultures inoculated}} \times 100$$

b. Number of shoots

Number of shoots was counted eight weeks after inoculation and recorded.

c. Length of longest shoot (mm)

Length of longest shoot was measured after eight weeks and expressed in mm.

3.9 PLANTING OUT AND HARDENING

Plantlets with at least 2 to 3 leaves were carefully removed from the containers, washed thoroughly to remove the adhering agar and then immersed in a fungicide solution (Bavistin 0.05%) for 10 minutes. The plantlets were then transplanted to different media in pots. Required humidity was maintained around the plants. Field establishment of each hybrid was recorded.

3.9.1 Potting media

The following potting media were used for the study

a) Sand alone
b) Vermiculite alone
c) Cocopeat
d) Sand + Vermiculite (1:1)
e) Sand + Cocopeat + Husk pieces (1:1:1)
f) Sand + Cowdung treated with trichoderma + Husk pieces (1:1:1)

Observations were recorded on the number of plantlets survived after 30 days of planting out and percentage was calculated.

3.9.2 Containers

The following containers were used for the study.

a. White plastic tea cup (holes made on sides and bottom)

b. Plastic thumb pot

c. Clay pot (tea cup size)

d. Polythene cover with holes (tea cup size)

Observations were recorded on the number of plantlets survived after 30 days of planting out and percentage was calculated.

3.10 EVALUATION OF HYBRIDS

The hybrids were maintained in the field by giving regular dose of fertilizers and plant protection chemicals.

Fertilizer NPK (30:10:10) @ 0.2 per cent was given twice a week to supply the required nutrients.

3.10.1 Morphological studies

The growth performance of the plantlets was observed for a period of one year after planting. Observations were recorded on plant height, number of leaves, length of largest leaf and number of suckers.

Flowering of hybrids

When the hybrids started flowering, observations were recorded on days taken from planting to flowering, days taken from spike emergence to unfurling, length of flower stalk, length of the spathe, width of the spathe, length of spadix, angle of orientation of spadix to spathe and colour of the spathe and spadix.

3.10.2 Cytological studies

Root tips (2 to 3 mm in length) were excised (between 11.30 am and 12.30 pm) from hybrids, pretreated with 0.002 M 8-hydroxy quinoline for four hours (at 10°C) and fixed in Carnoy's fluid (3:1:1 ethanol : acetic acid : chloroform) for 24 hours. The root tips were then hydrolysed for 15 minutes in IN HCl at 60°C, squashed in two per cent acetocarmine and observed for numerical changes in chromosomes.

3.10.3 Isozyme studies

Different hybrids as well as their parents were analysed for activities of enzymes, namely, peroxidase, esterase, catalase and Super Oxide Dismutase (SOD).

Extraction

The extraction was carried out as per Sadasivam and Manickam (1992). Leaf samples were used for the study. The extraction buffer had the following composition.

Tris HCl	0.05 M (adjusted to pH 7.4)
Cystrine	0.1 per cent
Ascorbic acid	0.1 per cent
Sucrose	17 per cent

Plant material weighing three grams was crushed in 1.5 ml of Tris HCl extraction buffer in a chilled mortar and pestle.

The homogenates were filtered through a cheese cloth into centrifugation tubes and centrifuged at 20,000 rpm for 20 minutes at 4°C. The cheese supernatant liquid was collected in eppendorf tubes.

Electrophoretic run

The procedure of Sadasivam and Manickam (1992) was followed for electrophoretic run of the samples.

Running buffer-stock solution

A stock solution was prepared by mixing 6.00 g Tris and 15.02 g Glycine. The pH of the solution was adjusted to 8.90 with 1 M Tris. Out of this solution 500 ml was diluted to 1000 ml.

Composition of the different reagents and their respective concentrations in the spacer and separation gels is presented in Table 2.

Table 2. Composition of reagents in the spacer and separation gels

Sl.No.	Reagent	Composition	Quantity (g)	pH
1	Stock A (100 ml)	1.50 M Tris	18.171	8.9
2	Stock B (100 ml)	1.00 M Tris	12.140	6.7
3	Stock C (100 ml)	4.20 M Acrylamide 0.065 M bisacrylamide	1.000	
4	Stock D*	10 per cent APS	0.200	
5	TEMED			

* Prepared at the time of mixing

Composition of different solutions for the preparation of one gel slab is given in Table 3.

Table 3. Composition of different solutions for the preparation of one gel slab

Stock solution	Spacer gel 4 per cent (ml)	Separation gel (10%) ml
C	2.50	13.30
A	-	10.00
B	5.00	-
Distilled water	5.60	14.30
D	0.20	0.40
TEMED	0.01	0.02

Preparation of the gel

The polyacrylamide gel used for resolving the protein and isozymes consisted of a four per cent spacer gel poured over a ten per cent separation gel. Two gel plates were adhered together with a grease coated Teflon spaces of 1 mm thickens. The separation gel was prepared by pouring the mixture in the gel casting set, leaving 2.5 cm from the top. After the polymerization, the mixture for spacer gel was poured

over this and immediately a comb was inserted into the spacer gel. Care was taken to avoid bubbles in the gel while pouring the gel mixtures. After polymerization the comb was removed for electrophoretic run.

Electrophoresis

The polymerized gel was fixed in an electrophoretic tank. Fifty millilitre each of the sample was loaded in the wells. The upper and lower tank were filled with running buffer. The gel was run at 20 mA. About 6-7 hours were taken for completing the electrophoretic run at low (5°C) temperature. After completion of running, the gel was transferred to the staining and substrate solutions respectively.

Staining of gel

The staining procedure of Vallejos (1983) was followed for peroxidase and esterase. For catalase staining, the method followed by Haris and Hopkinson (1976) was used.

Staining procedure for peroxidase

The following reagents were used.

0.2 M Acetate buffer pH 5.6	- 200 ml
Benzidine	- 0.2 g
H_2O_2 3 per cent	- 0.80 ml
Water	- 200 ml

Fresh staining solution was prepared each time. Acetate buffer and benzidine were mixed, heated to boil, cooled, filtered and then hydrogen peroxide was added. The gels were immersed in staining solution till the brown bands of peroxidase appeared and destained in seven per cent acetic acid. As the bands faded on standing for long time, photographs were taken on the same day of staining. The zymogram was sketched and Rm values were calculated.

Staining procedure for SOD

Reagents used were

Na EDTA	- 7.5 mg
Riboflavin	- 4 mg
NBT	- 10 mg
Buffer	- 5 ml
Distilled water	- 100 ml

The reagents were dissolved in distilled water and filtered. The gel was kept in this solution at 37°C for 20 minutes. Then the solution was drained off and seven per cent acetic acid was added. The electrophorogram was photographed sketched and the Rm values calculated.

Staining procedure for esterase

Reagents used

Sodium hydrogen phosphate	- 2.80 g
Disodium hydrogen phosphate	- 1.10 g
Fast blue RR salt	- 0.12 g
1-naphthyl acetate	- 0.08 g
EDTA	- 0.006 g
Distilled water	- 200 ml

Sodium hydrogen phosphate and disodium hydrogen phosphate were dissolved in 200 ml of water in which EDTA, Fast blue and 1-naphthyl acetate (dissolved in 4 ml acetone) were added and filtered. The gel was then incubated in the staining solution at 37°C till light brown bands appeared. Then the solution was drained off and seven per cent acetic acid was added. The electrophorogram was photographed sketched and the Rm values calculated.

Staining procedure for catalase

Reagents used

Potassium ferric cyanide	- 1 g
Ferric chloride	- 1 g
Distilled water	- 200 ml

The above chemicals were dissolved in water to prepare staining solution (Haris and Hopkinson, 1976).

The gel was placed in a dish resting on a bed of ice and chilled for 10 minutes. The gel was covered with a 0.01 per cent solution of hydrogen peroxide and kept under dark at room temperature for 20 minutes. The gel was then rinsed with two changes of distilled water. It was then covered with staining solution and agitated slowly for 1 or 2 minutes. The gel was then rinsed with hydrogen peroxide and then stored in 7 per cent acetic acid.

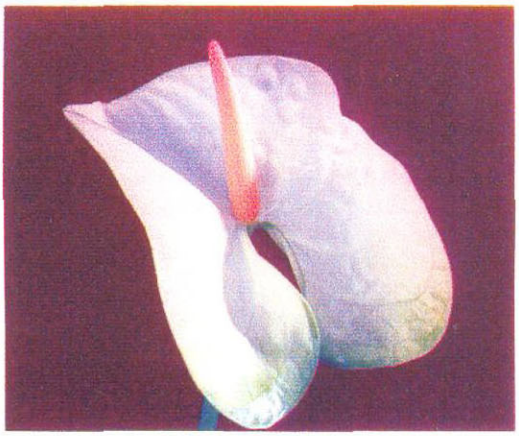
3.11 STATISTICAL ANALYSIS

The data recorded on the various experiments were subjected to statistical analysis by applying the technique of analysis of variance (ANOVA) for completely randomized design (CRD) by following the method of Panse and Sukhatme (1985).

The morphological and floral characters were analysed and data processed for the analysis of variance, genotypic and phenotypic coefficients of variation and heritability, following the method compiled by Singh and Choudhary (1977). Computations were carried out using the software GENSTAT (developed at the College of Horticulture, Kerala Agricultural University).

The genotypic and phenotypic correlation coefficients were worked out to study the extent of association between the characters adopting the method suggested by Johnson *et al.* (1955). Observations from all the six varieties for the 10 characters namely plant height, flower stalk length, length of spathe, width of spathe, length of spadix, number of suckers, longevity of spike on plant, interval of flower production, duration of female phase and duration of male phase were used for the study.

Results



4. RESULTS

The results of the present investigation entitled 'Improvement of *Anthurium andreanum* by *in vivo* and *in vitro* methods' are presented in this chapter under the following heads.

4.1. Characterisation of *A. andreanum* varieties

4.1.1 Vegetative characters

4.1.2 Floral characters

4.1.2.1 Floral morphology

4.1.2.2 Flowering pattern

4.1.2.3 Pollen characters

4.1.2.4 Post harvest characters

4.1.3 Variation of characters with age

4.1.4 Seasonal variation in flowering behaviour

4.2 Variation and correlation studies

4.3 Hybridisation and compatibility studies

4.4 *In vitro* studies

4.4.1 Standardisation of maturity of seed for seed culture

4.4.2 Standardisation of media

4.5 Irradiation studies

4.5.1 Seed irradiation

4.5.2 *In vitro* irradiation

4.6 Planting out and hardening

4.7 Evaluation of hybrids

4.7.1 Morphological studies

4.7.2 Cytological studies

4.7.3 Isozyme studies

4.1 CHARACTERISATION OF *A. ANDREANUM* VARIETIES

Morphological description of the varieties

Nitta

The plant has 13.56 cm height and 32.00 cm x 34.33 cm spread at flowering stage. The spathe is 14.78 cm long and 10.22 cm wide. The variety 'Nitta' exhibits bright orange cup shaped spathe with yellow spadix. The spadix is 6.89 cm long and inclined to the spathe at an angle of 65°. Flower stalk (75.56 cm) is straight and sturdy. It produces 7 flowers/ leaves per year.

Candy Queen

This variety is short (6.33 cm height) with 46.78 cm x 46.22 cm spread. The spathe is 17.67 cm long and 13.33 cm wide. The spathe is peach coloured with yellow spadix inclined to the spathe at an angle of 40° Length of spadix is 8.56 cm. The flower stalk is weak, 75.33 cm long and drooping. It produces 7-8 flowers/leaves per year.

Lima

The plant height is 19.22 cm with long internode of 3.67 cm. The mean spread is 36.00 cm x 36.56 cm. Length of spathe is 16.11 cm and width is 13.33 cm. This is a white variety with spathe having greenish colour at the base of the lobes. The spadix is yellow and has an angle of inclination of 55° with the spathe. The spadix is long (9.11 cm). Peduncle is straight, and 58.22 cm long. The variety produces 7-8 flowers/leaves per year.

Red Dragon

The plant is short (4.22 cm height) with a spread of 32.11 cm x 33.00 cm. Spathe is of medium size (12.33 cm length and 8.00 cm width). This is an obake variety with red and green spathe. Spadix is short (6.22 cm length) and inclined at an angle of 55° to the spathe. Peduncle is straight and has 62.00 cm length. It produces 3-4 suckers and produce 7-8 flowers/leaves per year.

Eureka Red

The plant is short (5.33 cm height) and has short straight peduncle. The spread of the plant is 35.11 cm x 35.89 cm. Spathe is tilted towards the peduncle. Spathe has a length of 11.89 cm and width of 8.44 cm. The variety has red spathe and yellow spadix. The spadix is 6.78 cm long and inclined to the spathe at an angle of 60°. The peduncle is short (46.89 cm). Plant produces 8-9 flowers/leaves per year.

Agnihothri

Plant is short (4.22 cm height) with a spread of 37.89 cm x 37.89 cm. Spathe has a length of 14.44 and width of 13:11 cm. The length of spadix is 8.11 cm. The spathe is with an attractive deep pink colour and spadix is yellow. The angle of inclination of the spadix is 60°. Peduncle length is 56.44 cm. It produces 6-7 flowers/leaves per year.

4.1.1 Vegetative characters

Vegetative characters of the *Anthurium* varieties used in the study are presented in Table 4.

4.1.1.1 Plant height (a)

The varieties differed significantly in plant height. Among the different varieties used for hybridization, the shortest varieties were 'Red Dragon' and 'Agnihothri' (4.22 cm, each) which were on par with the variety 'Eureka Red' (5.33 cm) and 'Candy Queen' (6.33 cm). The variety 'Lima' was the tallest (19.22 cm) which was followed by 'Nitta' (13.56 cm).

4.1.1.2 Spread of the plant

Spread of the plant was maximum in the variety 'Candy Queen' both in east west (46.78 cm) and north south directions (46.22 cm), which was significantly

superior to all others. The lowest spread was in the variety 'Red Dragon' (32.11 cm east west and 33.00 cm north south).

4.1.1.3 Internodal length

Internodal length was found to be maximum in the varieties 'Lima' (3.67 cm) and 'Nitta' (3.11 cm). Both were significantly superior to all other varieties. Shortest internodes were recorded in the varieties 'Red Dragon' (1.72 cm) and 'Candy Queen' (1.78 cm).

4.1.1.4 Girth of the stem

Girth of the stem was maximum (9.78 cm) in the variety 'Agnihothri' which was on par with the variety 'Eureka Red' (9.17 cm) and 'Candy Queen' (8.78). Stem girth was low in the varieties 'Lima' (7.11 cm) and 'Nitta' (6.39 cm).

4.1.1.5 Number of leaves

In the case of number of leaves, the variety 'Red Dragon', showed highest number of leaves (10.33) which was found to be on par with the variety 'Nitta' which had 9.33 leaves. The variety 'Lima' had the least number of leaves (6.00).

4.1.1.6 Leaf area

Leaf area (681.3 cm^2) was highest in the variety 'Candy Queen' which was on par with 'Agnihothri' (579.4 cm^2) and 'Lima' (556.8 cm^2). Leaf area was lesser in the varieties 'Eureka Red' (377.7 cm^2) and 'Red Dragon' (306.3 cm^2).

4.1.1.7 Length of petiole

Significant difference was observed among the varieties regarding the length of petiole. The longest petiole was observed in the variety 'Nitta' (80.44 cm) which was significantly superior to others. The variety 'Red Dragon' had the shortest petiole (42.56 cm).

4.1.1.8 Number of suckers

The variety 'Red Dragon' produced largest number of suckers (3.22) which was significantly superior to others. Sucker production was least in the varieties 'Lima' and 'Eureka Red' (1.22).

4.1.1.9 Annual production of leaves/spikes

The results revealed that the production of each leaf was followed by the production of a spike from the leaf axil. The varieties differed in the production of leaves/spikes per year. The variety 'Eureka Red' produced 8.9 spikes/leaves per year and 'Red Dragon' produced 8.0 spikes/leaves per year. The production of leaves or spikes was the least in the variety 'Nitta' (6.5 per year).

4.1.2 Floral characters

The commercial flower of *Anthurium andreanum* consists of a relatively insignificant inflorescence called spadix which is subtended by a colourful modified leaf called spathe. The spadix or candle consists of plenty of small flowers on a pencil like protrusion, where the bisexual flowers are arranged in a series of spirals. Both spadix and spathe are borne on a leaf less stalk or peduncle.

4.1.2.1 Floral morphology

Data on the floral morphology of the different varieties are presented in Table 5 and Fig.1.

4.1.2.1.1 Spathe size

The largest spathe was produced by the variety, 'Candy Queen' which showed 17.67 cm length and 13.33 cm width for the spathe, followed by the variety 'Lima' (16.11 cm length and 13.33 cm width). The varieties 'Red Dragon' and 'Eureka Red' had the smallest spathes (12.33 cm x 8.00 cm and 11.89 cm x 8.44 cm, respectively).

Table 4. Vegetative characters of *Anthurium andreanum* varieties

Sl. No.	Variety	Plant height (a) (cm)	Spread of the plant		Internodal length (cm)	Girth of the stem (cm)	No. of leaves	Leaf area (cm^2)	Length of petiole (cm)	No. of suckers	No. of leaves/ spikes produced per year
			EW (cm)	NS (cm)							
1	Nitta	13.56	32.00	34.33	3.11	6.39	9.33	511.2	80.44	1.33	6.5
2	Candy Queen	6.33	46.78	46.22	1.78	8.78	6.22	681.3	59.11	1.33	7.3
3	Lima	19.22	36.00	36.56	3.67	7.11	6.00	556.8	47.22	1.22	7.6
4	Red Dragon	4.22	32.11	33.00	1.72	8.28	10.33	306.3	42.56	3.22	8.0
5	Eureka Red	5.33	35.11	35.89	2.17	9.17	8.00	377.7	50.00	1.22	8.9
6	Agnihothri	4.22	37.89	37.89	2.28	9.78	7.44	579.4	54.67	2.33	6.6
	CD (0.05)	2.918	4.292	4.354	0.657	1.005	1.433	126.6	7.962	0.651	0.76
	SEm ±	1.026	1.510	1.531	0.230	0.354	0.504	33.174	2.800	0.229	0.243

4.1.2.1.2 Length of spadix

The varieties 'Lima' (9.11 cm), 'Candy Queen' (8.56 cm) and 'Agnihothri' (8.11 cm) had the longest spadices. Short spadices were exhibited by the varieties 'Red Dragon', 'Eureka Red' and 'Nitta' (6.22, 6.78 and 6.89 cm, respectively).

4.1.2.1.3 Angle of orientation of spadix to spathe

The least angle of orientation of spadix to spathe was noticed in 'Candy Queen' (40°) and it was widest in the variety 'Nitta' (65°).

4.1.2.1.4 Relative length of spathe to spadix

This was calculated as the ratio of the length of spathe to the length of spadix. The relative length of spathe to spadix was found to be highest in the variety 'Nitta' (2.15) followed by 'Candy Queen' (2.06) and it was lowest in the variety 'Eureka Red' (1.75).

4.1.2.1.5 Length of peduncle

The varieties differed significantly in the length of peduncle. Longest peduncle was observed in the varieties 'Nitta' (75.56 cm) and 'Candy Queen' (75.33). The peduncle was shortest in 'Eureka Red' (46.89 cm).

4.1.2.1.6 Girth of peduncle at the base

The variety Eureka Red had maximum girth (2.4 cm) which was found to be on par with the variety 'Nitta' (2.3 cm). Girth was the lowest in 'Lima', 'Red Dragon' and 'Agnihothri' (2.00 cm).

4.1.2.2 Flowering pattern

The individual anthurium flower is a small segment on the spadix. Flowers developed acropetally on the spadix. An individual flower had 4 perianth segments (tepals) arranged in a four sided configuration which enveloped four stamens with four

Table 5. Floral characters of *Anthurium andreanum* varieties

Variety	Length of spathe (cm)	Width of spathe (cm)	Length of spadix (cm)	Length of peduncle (cm)	Girth of peduncle (cm)	Nature of peduncle	Angle of orientation of spadix to spathe	Relative length of spathe to spadix	Colour of spathe	Colour of spadix	Nature of spathe
Nitta	14.78	10.22	6.89	75.56	2.30	Straight	65°	2.15	Orange	Yellow	Glossy medium blistered thin
Candy Queen	17.67	13.33	8.56	75.33	2.20	Bending	40°	2.06	Peach	Yellow	Thin, glossy and blistered
Lima	16.11	13.33	9.11	58.22	2.00	Straight	55°	1.76	White (green colouration at the base)	Yellow	Thick smooth
Red Dragon	12.33	8.00	6.22	62.00	2.00	Straight	55°	1.98	Red (green colouration at the base)	Yellow	Medium thick smooth
Eureka Red	11.89	8.44	6.78	46.89	2.40	Straight (spathe tilted towards peduncle)	60°	1.75	Red	Yellow	Medium thick smooth
Agnihothri	14.44	13.11	8.11	56.44	2.00	Straight	60°	1.79	Deep pink	Yellow	Thin deeply blistered glossy
CD (0.05)	1.406	1.331	1.094	7.022	0.117						
SEm ±	0.495	0.468	0.384	2.470	0.030						

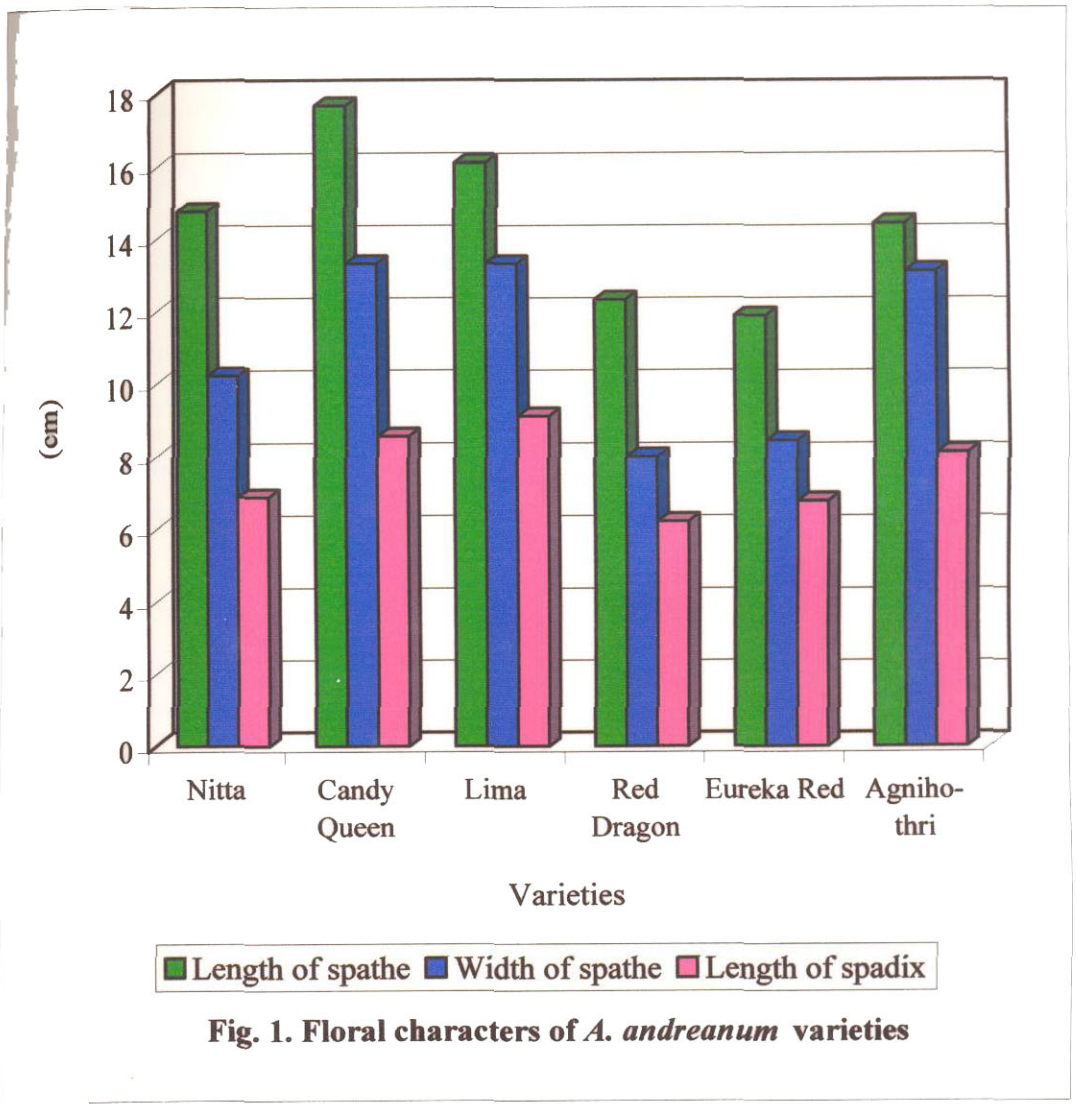


Fig. 1. Floral characters of *A. andreanum* varieties

loculed anther. Pistil was cylindrical and two-carpelled. As the pistil developed, a stout style exerted to expose a receptive stigma which is damp and shiny.

All the varieties studied were found to be protogynous and attained female fertility within 7 days of unfurling of spāthe. The interphase between female phase and male phase in different varieties varied from 3 to 20 days, preventing self fertilization.

The flowering pattern of selected varieties are presented in Table 6 and Fig.2. All the characters showed significant differences.

4.1.2.2.1 Duration from spathe emergence to unfurling

The duration from spathe emergence to unfurling was longest in the variety 'Agnihothri' (25.33 days) which was on par with the varieties 'Eureka Red' and 'Nitta'. The varieties 'Candy Queen' (19.00 days) and 'Lima' (19.00 days) showed shortest duration from spike emergence to unfurling.

4.1.2.2.2 Duration from spathe unfurling to female phase

In case of variety 'Nitta', the female phase started on the same or within one or two days of the unfurling of the spathe. The duration on an average was 2.67 days in the varieties 'Candy Queen' and 'Eureka Red'. The variety 'Red Dragon' took more time to start female phase (6.00 days).

4.1.2.2.3 Duration of female phase

Female receptivity was identified by the presence of honey dew or stigma droplets. Longest female phase was observed in the variety 'Eureka Red' (20.33 days) which was significantly superior to all other varieties. The duration was shortest in the variety 'Red Dragon' (12.00 days).

4.1.2.2.4 Duration of male phase

Male phase was identified by the appearance of stamens on the spadix. No pollen emergence was observed in the variety 'Candy Queen' during the period of

observation. The longest duration of male phase was noticed in the variety 'Red Dragon' (20.67 days) and shortest in the variety 'Lima' (9.33 days).

4.1.2.2.5 Duration of interphase

A clear cut interphase between female and male phases was exhibited by all the varieties studied. Interphase was longest in 'Lima' (20.00 days) followed by 'Nitta' (16.00 days). Shortest interphase was observed in 'Red Dragon' (3.67 days).

4.1.2.2.6 Longevity of the spike on the plant

The varieties differed significantly regarding the longevity of spike. Maximum longevity (90.00 days) was observed in the variety 'Lima' which was significantly superior to all other varieties, followed by 'Eureka Red' (71.67 days). Longevity was minimum in the variety 'Agnihothri' (43.00 days).

4.1.2.2.7 Duration from spathe unfurling to seed maturity

The varieties differed significantly regarding the duration to attain seed maturity also. The shortest period to attain full maturity of seed was observed in the variety 'Red Dragon' (145.00 days). The longest period was taken by the variety 'Agnihothri' which took 208.3 days to attain full maturity of seed.

4.1.2.3 Pollen characters

Pollen characters of the male parents, viz., 'Nitta', 'Candy Queen', 'Lima', 'Red Dragon', 'Eureka Red', 'Agnihothri', *A. crystallinum*, *A. ornatum*, *A. amnicola* are given in Table 7.

4.1.2.3.1 Pollen morphology - size and shape

The average size of pollen grains of the different parents showed significant variation. Pollen grains were the largest (25:4μ) in the variety 'Agnihothri'. It was followed by *Anthurium crystallinum*, 'Lima' and Eureka Red. Smallest pollen

Table 6. Flowering pattern of *A. andreanum* varieties

Variety	Duration from spathe emergence to unfurling (days)	Duration from spathe unfurling to female phase (days)		Duration of female phase (days)	Duration of male phase (days)	Duration of inter phase (days)	Longevity of spikes on the plant (days)	Duration from spathe unfurling to seed maturity (days)	Remarks
		Range	Mean						
Nitta	23.33	0 - 2	1.67	13.33	11.33	16.00	70.00	170.00	
Candy Queen	19.00	2-4	2.67	15.00	-	-	59.33	160.00	No male phase seen
Lima	19.00	4-7	5.67	12.33	9.33	20.00	90.00	170.00	
Red Dragon	19.33	2-7	6.00	12.00	20.67	3.67	61.33	145.00	
Eureka Red	24.67	2-4	2.67	20.33	12.33	5.67	71.67	165.00	
Agnihothri	25.33	2-7	5.67	12.33	12.67	5.00	43.00	208.30	
CD (0.05)	2.551		2.965	5.304	5.617	4.505	9.215	11.67	
SEm ±	0.828		0.963	1.722	1.782	1.430	2.991	3.788	

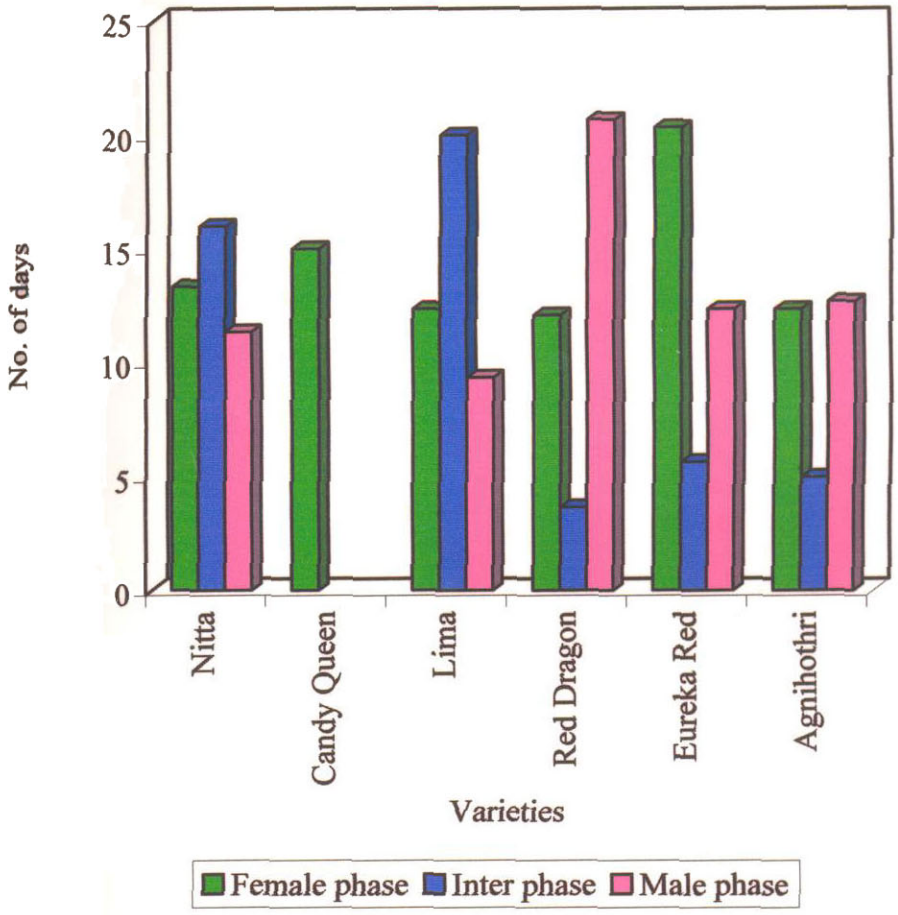


Fig. 2. Flowering pattern of *A. andreanum* varieties

grains were produced by the variety 'Red Dragon' and *Anthurium amnicola* (16.6μ). All the parents under the present investigation had more or less round pollen.

4.1.2.3.2 Pollen fertility

Pollen fertility was studied by acetocarmine staining method (Plate 3). It was found to be the highest (69.8%) in *Anthurium crystallinum* followed by 'Agnihothri' (64.6%). No pollen emergence was recorded in the variety 'Candy Queen' and in *A. ornatum*. Lowest pollen fertility was observed in 'Lima' (16.6%).

4.1.2.3.3 Pollen production pattern

Pollen production pattern during the period of two years from May 1999 to April 2001 was analysed (Table 8). During the year 1999, pollen production started from May and in the months of June, July and August there was maximum pollen production and no pollen emergence was recorded for the period from October 1999 to June 2000.

During 2000, pollen emergence started late in July and extended to October in most of the varieties.

Pollen production was the highest in the year 1999 and it was low in 2000 for the varieties, except 'Nitta' in which the pollen production was low in both the years. Pollen production was highest during July, August and September. No pollen emergence was noticed in the variety 'Candy Queen' and in *A. ornatum* during the period of study.

4.1.2.4 Post harvest characters

4.1.2.4.1 Vase studies

Flowers were harvested at one third maturity stage ($1/3^{rd}$ of the female flowers on the spadix opened, indicated by colour change on the spadix) in the

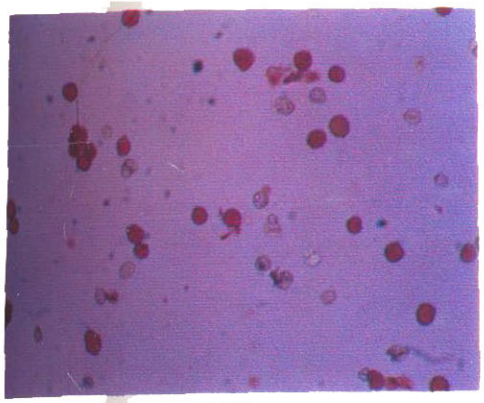
Table 7. Pollen characters of *Anthurium andreanum* varieties and *Anthurium* species

Varieties/species	Pollen size (diameter) (μ)	Pollen fertility (%)	Remarks on pollen availability
Nitta	17.0	19.0	Low availability
Candy Queen	-	-	No pollen emergence noted
Lima	21.4	16.6	Good availability depending on climate
Red Dragon	16.6	50.2	Good availability
Eureka Red	20.2	21.8	Good availability
Agnihothri	25.4	64.6	Good availability
A. crystallinum	22.2	69.8	Good availability
A. ornatum	-	-	No pollen emergence noted
A. amnicola	16.6	33.2	Good availability
CD (0.05)	2.94	3.51	
SEm ±	1.02	1.22	

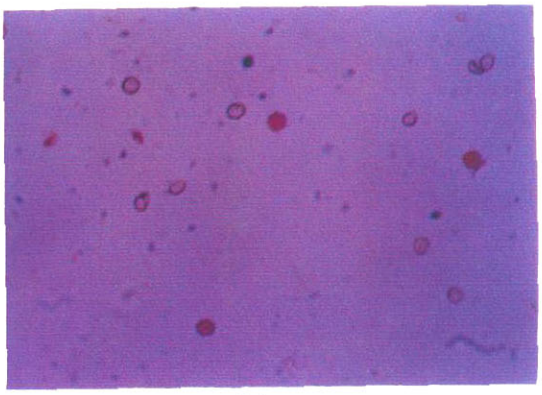
Table 8. Pollen emergence pattern of the male parents from May 1999 to April 2001.

Varieties/ Months		Nitta	Candy Queen	Lima	Red Dragon	Eureka Red	Agni-hothri	*A. crysta-llinum*	*A. orna-tum*	*A. amni-cola*	Average temperature (°C)		Relative humidity (%)	
											Max.	Min.	Mor-ning	After-noon
1999	May	-	-	++	++	++	-	*	*	*	30.7	24.7	92	72
	June	+	-	++	++	++	++	*	*	*	29.4	23.0	94	75
	July	+	-	++	++	++	++	*	*	*	28.4	23.0	96	82
	August	+	-	++	++	++	+	*	*	*	29.8	22.9	94	73
	September	-	-	+	+	+	+	*	*	*	31.6	23.4	89	63
	October	-	-	-	-	-	-	*	*	*	30.5	23.2	94	75
	November	-	-	-	-	-	-	*	*	*	31.4	22.7	81	57
	December	-	-	-	-	-	-	*	*	*	30.7	22.7	72	48
2000	January	-	-	-	-	-	-	*	*	*	32.9	23.2	76	43
	February	-	-	-	-	-	-	*	*	*	33.3	22.8	85	52
	March	-	-	-	-	-	-	*	*	*	35.6	23.9	87	46
	April	-	-	-	-	-	-	*	*	*	34.0	24.6	89	59
	May	-	-	-	-	-	-	*	*	*	33.7	24.4	88	56
	June	-	-	-	-	-	-	*	*	*	29.6	22.8	94	77
	July	+	-	+	+	+	+	+	-	-	28.8	21.9	93	76
	August	+	-	+	+	+	+	+	-	+	29.1	22.6	94	79
	September	+	-	+	+	+	+	++	-	++	30.7	23.0	91	70
	October	+	-	+	+	+	+	++	-	++	30.7	22.7	91	68
	November	-	-	+	+	-	-	++	-	++	33.3	23.1	77	54
	December	-	-	-	-	-	-	+	-	+	30.4	22.0	70	48
2001	January	-	-	-	-	-	-	+	-	+	32.6	23.2	71	41
	February	-	-	-	-	-	-	-	-	+	34.5	22.9	86	47
	March	-	-	-	-	-	-	-	-	-	34.9	24.0	84	54
	April	-	-	-	-	-	-	-	-	-	34.2	24.4	82	58

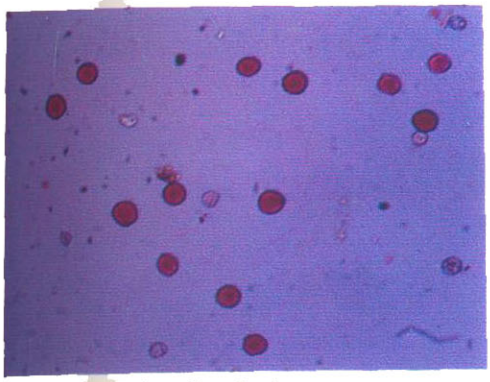
- No pollen emergence; + Low; ++ High; * Observations not taken



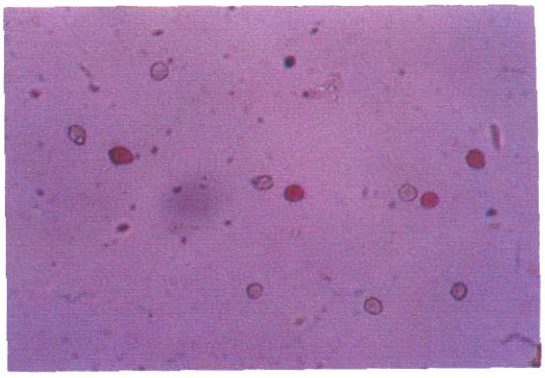
A. crystallinum



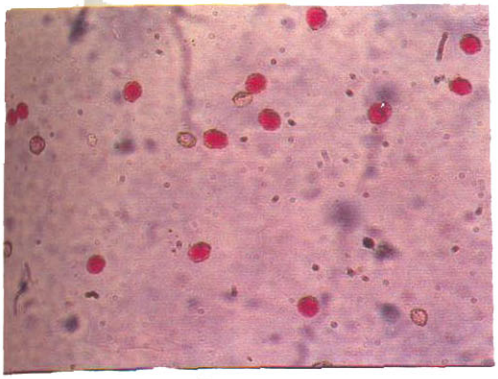
Lima



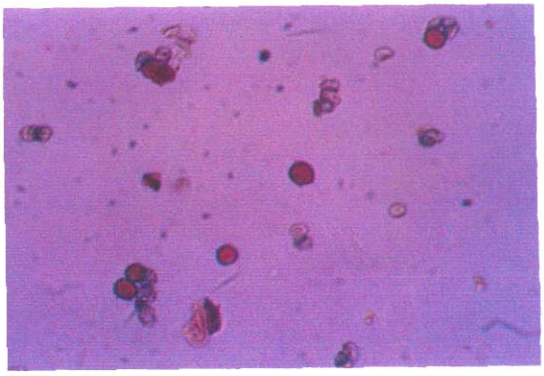
Agnihothri



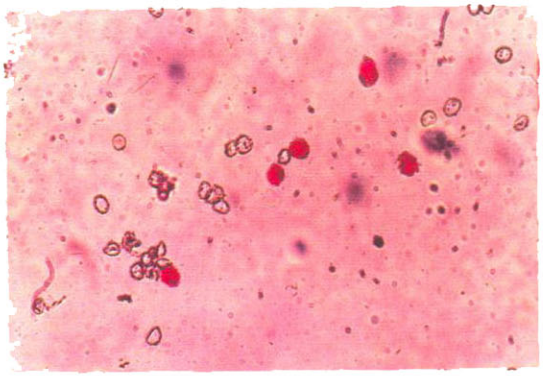
Eureka Red



Red Dragon



Nitta



A. amnicola

Plate 3. Pollen studies

morning and kept in distilled water for studying the post harvest characters. The results of the experiment are presented in Table 9 and Fig.3.

4.1.2.4.1.1 Fresh weight of the spike

The varieties differed significantly with respect to fresh weight of spike. Fresh weight was the highest in the variety 'Agnihothri' (22.89 g) which was followed by the variety 'Lima' (22.08 g). Fresh weight of the spike was the lowest for the variety 'Red Dragon' (13.92 g).

4.1.2.4.1.2 Days taken for colour fading

No colour fading was observed in the white variety 'Lima'. Early fading was noticed in the variety 'Agnihothri' (5.33 days) followed by 'Candy Queen' (9 days). The spathes retained colour for longer period in the varieties 'Eureka Red' (17.67 days) and 'Red Dragon' (15.67 days), which were significantly superior to all other varieties.

4.1.2.4.1.3 Days to spathe necrosis

Significant difference was noticed in the time taken for spathe necrosis, among the different varieties tried. Longest period for necrosis of spathe was observed in the variety 'Eureka Red' (18.00 days) which was found to be on par with 'Red Dragon' (17.67 days) and 'Lima' (17.33 days). The time taken for spathe necrosis was the lowest in the variety 'Candy Queen' (10.33 days).

4.1.2.4.1.4 Days to spadix necrosis

Late spadix necrosis was seen in the variety Eureka Red (16.33 days) which was found to be on par with the varieties 'Red Dragon' and 'Lima' (14.67 days, each) and they were significantly superior to all the other varieties. Early necrosis was noticed in the inflorescence of 'Agnihothri' (9.67 days).

4.1.2.4.1.5 Weight loss

The minimum weight loss (2.08 g) was observed in the variety 'Agnihothri', which was significantly different from others. The maximum (3.84 g) was recorded in 'Eureka Red'.

4.1.2.4.1.6 Water uptake

With respect to water uptake, the varieties did not show any significant difference.

4.1.2.4.2 Packing studies

Spikes of uniform maturity and length were used for the study. They were packed in boxes of size 60 cm x 37 cm x 10 cm, lined with polythene. Holes were made on the sides of the boxes for better ventilation. The results are presented in the Table 10 and Fig.4.

4.1.2.4.2.1 Days to colour fading of the spathe

The varieties differed significantly regarding the days taken to colour fading after packing. The variety 'Eureka Red' took 15 days for colour fading which was on par with 'Red Dragon' (13.33 days) whereas the variety 'Agnihothri', showed earlier colour fading (7.33 days). No colour change was observed in 'Lima'.

4.1.2.4.2.2 Days to spathe necrosis

Longest period for necrosis of the spathe was taken by the variety 'Eureka Red' (15.00 days) which was on par with the varieties 'Red Dragon' and 'Lima'. The variety 'Agnihothri' showed necrosis in 8.5 days, which was the shortest time.

4.1.2.4.2.3 Days to spadix necrosis

Days taken for spadix necrosis was the longest in the variety 'Eureka Red' (15.17 days) which was on par with the variety 'Red Dragon' (13.50 days). Shortest period was taken for spadix necrosis by the variety 'Agnihothri' (8.17 days).

Table 9. Post harvest characters of *Anthurium andreanum* varieties in vase

Varieties	Fresh weight (g)	Days to colour fading	Days to spathe necrosis	Days to spadix necrosis	Weight loss (g)	Water uptake (ml)	Remarks
Nitta	17.73	10.33	12.67	10.33	2.63	6.33	
Candy Queen	18.08	9.00	10.33	11.67	2.99	10.67	
Lima	22.08	-	17.33	14.67	3.48	11.00	No colour fading observed
Red Dragon	13.92	15.67	17.67	14.67	3.09	8.67	
Eureka Red	16.98	17.67	18.00	16.33	3.84	10.67	
Agnihothri	22.89	5.33	14.33	9.67	2.08	6.33	
CD (0.05)	4.29	3.58	1.88	2.18	1.05	NS	
SEm ±	1.393	1.135	0.608	0.707	0.340	1.680	

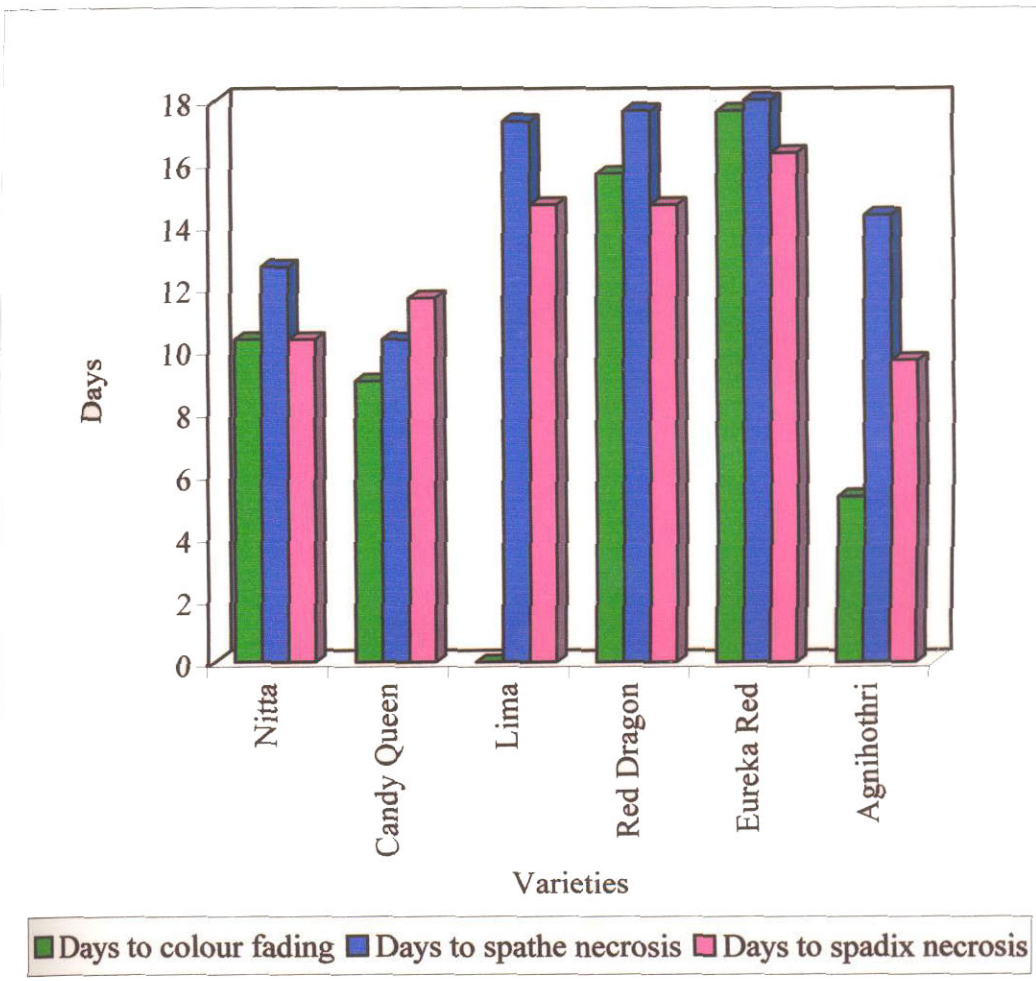


Fig. 3. Vase life of *A. andreanum* varieties

4.1.2.4.2.4 Weight loss

Maximum weight loss was observed in the variety 'Agnihothri' (8.04 g) and minimum in the variety 'Candy Queen' (4.29 g).

4.1.3 Variation in spathe length and plant height of *A. andreanum* varieties with age

Data pertaining to the variation in length of spathe and plant height of *Anthurium andreanum* varieties are presented in Table 11 and Figs.5, 6, 7, 8, 9 and 10.

Nitta

The variety 'Nitta' exhibited an increase in length of the spathe from 9.44 cm in the 1st month to 14.33 cm in the 12th month. During that period the plant height was increased from 75.67 cm to 107.55 cm. The flower size increased with age and during the last 6 months, it remained more or less same with no significant variation.

Candy Queen

Significant variation in flower size was observed in this variety. Spathe length was increased from 11.33 to 17.22 cm by 12 months. During the last 9 months, there was no significant variation. The plant height was increased from 66.00 cm to 97.3 cm. The maximum size for the flowers observed was 17.67 cm and minimum was 11.33 cm.

Lima

Minimum flower size (spathe length) was 13.55 cm (1st month) and maximum was 17.44 cm (11th month). The height of the plant was increased from 72.66 cm to 105.55 cm. With the increase in age, the spathe size was also increased.

Table 10. Effect of packing on longevity of *A. andreanum* varieties

Varieties	Days to colour fading	Days to spathe necrosis	Days to spadix necrosis	Weight loss (g)
Nitta	10.17	10.83	10.00	5.74
Candy Queen	7.67	9.67	10.83	4.29
Lima	-	13.17	10.00	5.92
Red Dragon	13.33	13.83	13.50	6.76
Eureka Red	15.00	15.00	15.17	4.61
Agnihothri	7.33	8.50	8.17	8.04
CD (0.05)	2.291	2.209	1.695	1.622
SEm ±	0.787	0.765	0.586	0.562

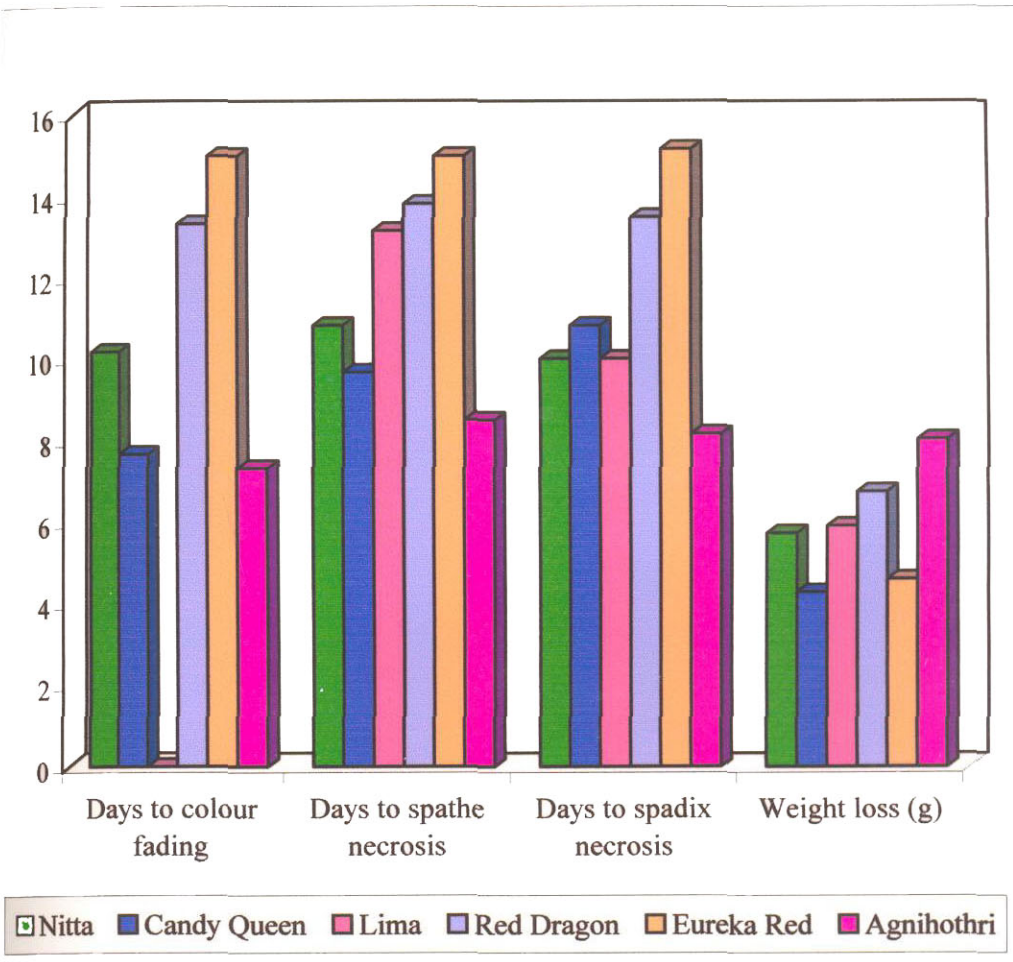


Fig. 4. Effect of packing on longevity of *A. andreanum* varieties

Red Dragon

The plant height increased from 56.66 cm to 91.00 cm during the period of study. With the increase in height, the size of spathe was also increased (from 10.88 cm to 21.66 cm). Significant variation in spathe size was observed. But the very large flowers of this variety were not attractive as the spathe changed its shape and colour. Biggest flowers were of 21.66 cm length, produced during the 12th month.

Eureka Red

This variety also exhibited a similar character like that of 'Red Dragon'. The spathe length increased from 10.55 cm in the 1st month to 19.22 cm in the last month. The height of the plant increased from 58.6 cm to 89.22 cm. During the last 3 months, the flower size was very high which were significantly superior to the others.

Agnihothri

The plant height was increased from 66.88 cm to 95.89 cm. With the increase on plant height, the spathe size was also increased (12.55 cm during the 1st month to 17.44 cm during the 12th month). The spathe size was significantly superior during the last four months and the highest value was observed during the last month (17.44 cm).

4.1.4 Seasonal variation in flowering behaviour of *A. andreanum* varieties

Seasonal variation in flowering behaviour of *A. andreanum* varieties were studied from May 1999 to April 2000. The data are presented in Table 12. Meteorological data is presented in Appendix I.

Nitta

The variety 'Nitta' produced highest number of flowers (2.0) during the hot months i.e., February, March and April. Least number of flowers were obtained (1.3) during the months from November to January. The variety produced 6.5 flowers by

Table 11. Variation in spathe length and plant height of *A. andreanum* varieties with age

Month/Varieties	Nitta		Candy queen		Lima		Red Dragon		Eureka Red		Agnihothri	
	Plant height (b) (cm)	Spathe length (cm)	Plant height (cm)	Spathe length (cm)	Plant height (cm)	Spathe length (cm)	Plant height (cm)	Spathe length (cm)	Plant height (cm)	Spathe length (cm)	Plant height (cm)	Spathe length (cm)
May, 1999	75.67	9.44	66.00	11.33	72.66	13.55	56.56	10.88	58.60	10.55	66.88	12.55
June, 1999	76.22	9.44	66.11	11.88	76.44	13.66	61.67	10.88	62.67	11.78	68.55	12.89
July, 1999	86.33	13.66	68.55	15.11	81.67	15.77	62.22	11.60	65.78	11.33	70.33	14.33
August, 1999	86.77	13.77	74.88	15.33	86.22	15.22	68.00	11.33	69.89	13.00	72.77	14.00
September, 1999	91.44	12.88	75.33	16.11	90.22	15.33	71.22	15.66	73.11	12.56	74.66	15.11
October, 1999	94.66	12.56	76.88	16.44	91.66	15.44	74.56	13.22	73.67	11.33	75.77	14.44
November, 1999	96.55	14.78	90.11	16.89	95.55	16.11	79.11	12.33	77.67	11.89	80.88	14.44
December, 1999	100.55	14.88	90.11	16.44	96.77	16.22	78.67	15.11	81.00	13.89	85.11	15.11
January, 2000	102.33	14.66	92.11	17.67	100.00	15.33	84.11	19.55	83.44	14.86	87.56	17.22
February, 2000	105.11	15.88	93.55	16.88	101.88	16.67	84.78	21.00	85.56	17.11	90.11	17.33
March, 2000	107.55	15.44	93.55	17.22	102.33	17.44	86.67	21.00	87.33	17.33	93.44	16.67
April, 2000	107.55	14.33	97.30	17.22	105.55	16.11	91.00	21.66	89.22	19.22	95.89	17.44
CD (0.05)	11.16	1.67	13.63	2.31	9.75	1.79	11.65	3.69	8.83	2.06	9.89	2.16
SEm±	4.06	0.59	4.92	0.82	3.48	0.63	4.15	1.31	3.15	0.73	3.53	0.77

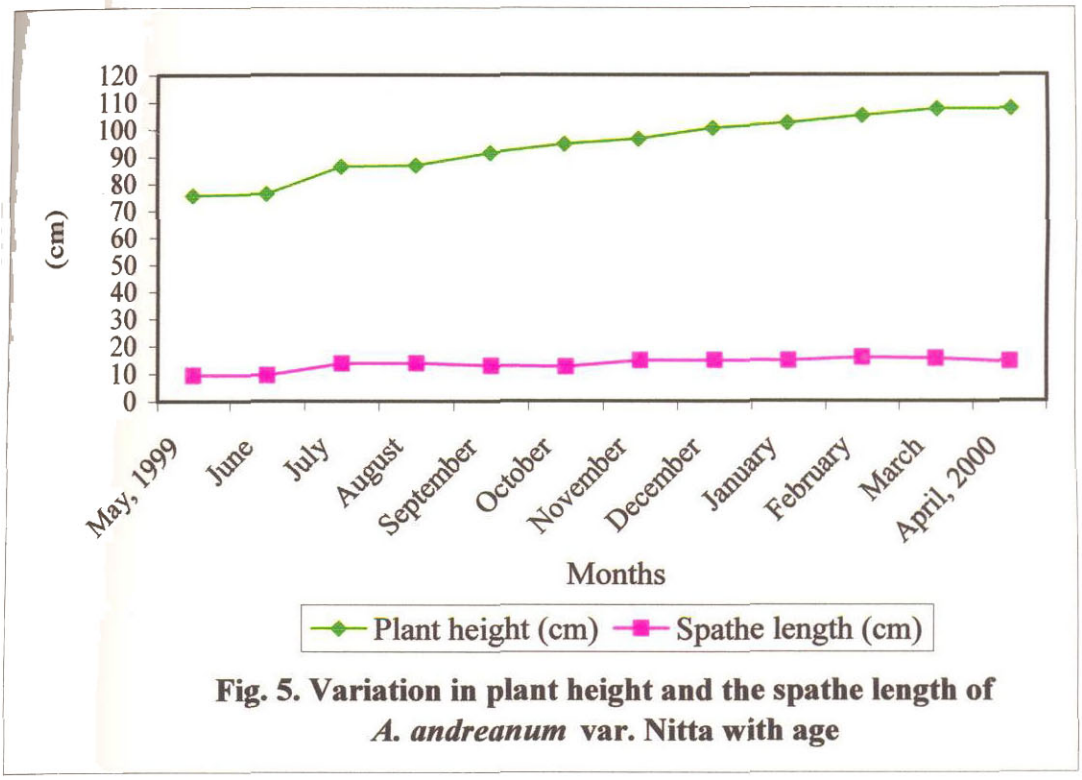


Fig. 5. Variation in plant height and the spathe length of *A. andreanum* var. Nitta with age

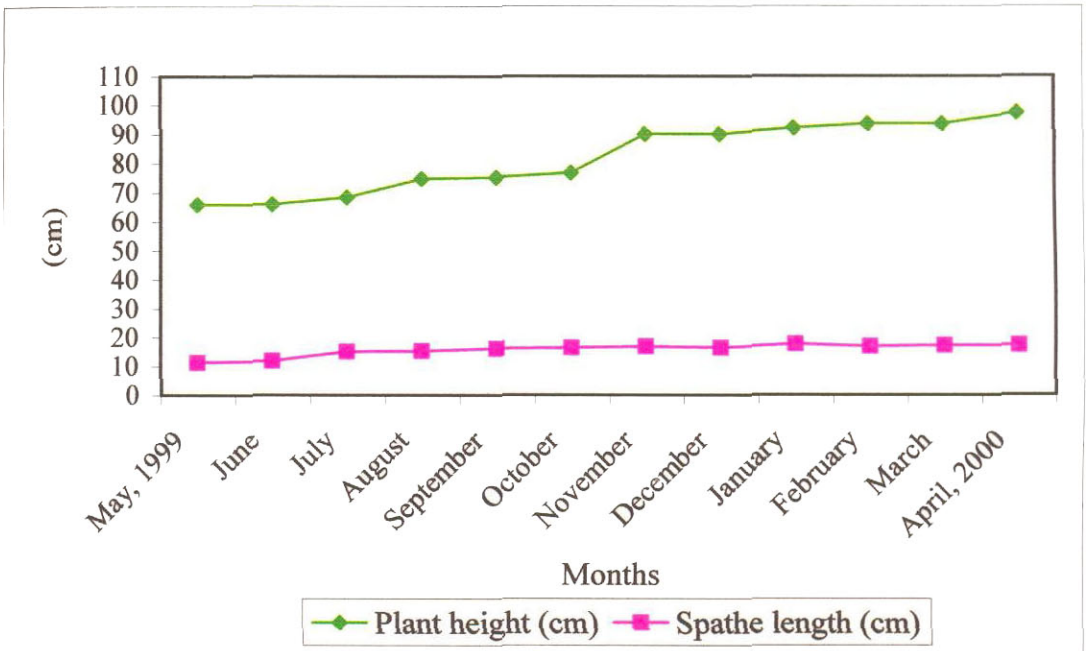


Fig. 6. Variation in plant height and the spathe length of *A. andreanum* var. Candy Queen with age

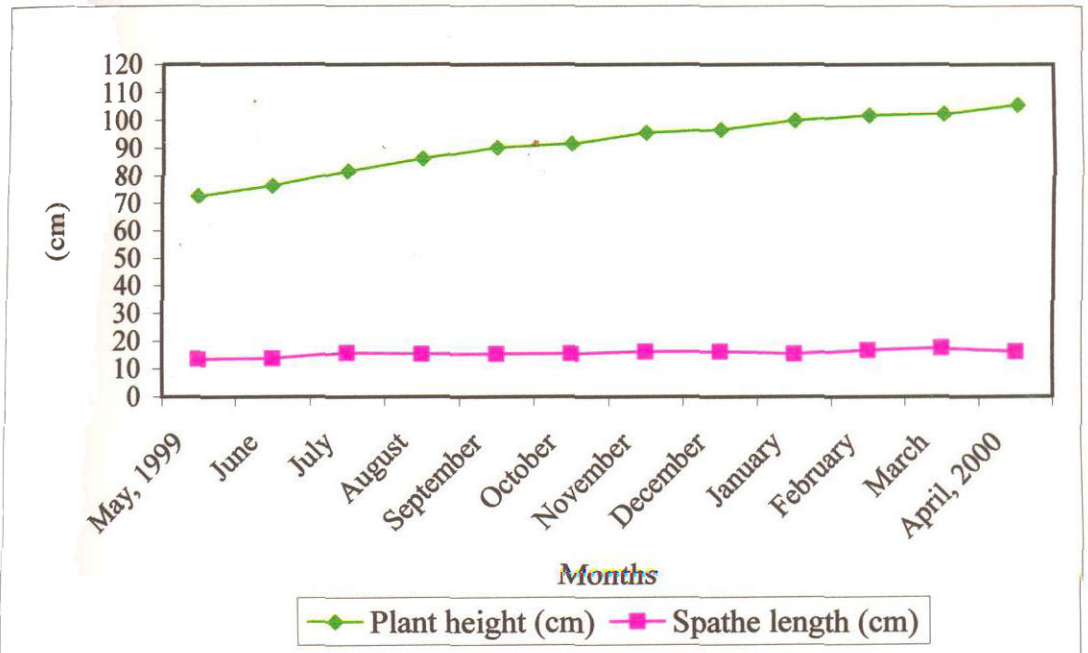


Fig. 7. Variation in plant height and the spathe length of *A. andreanum* var. Lima with age

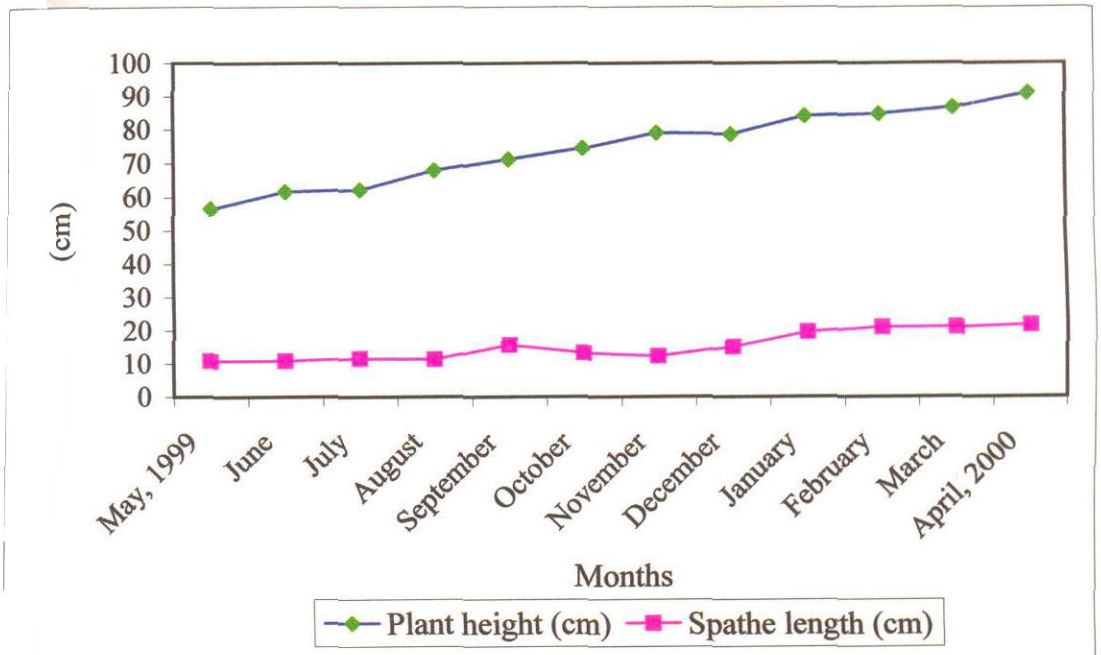


Fig. 8. Variation in plant height and the spathe length of *A. andreanum* var. Red Dragon with age

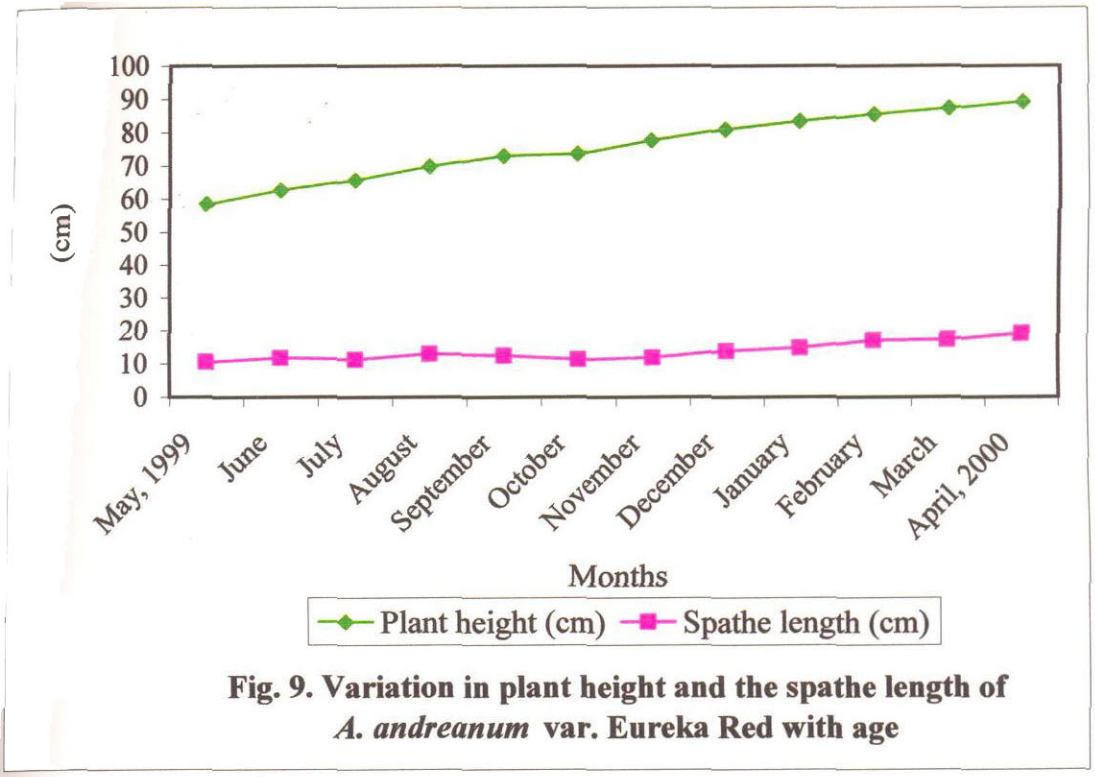


Fig. 9. Variation in plant height and the spathe length of *A. andreanum* var. Eureka Red with age

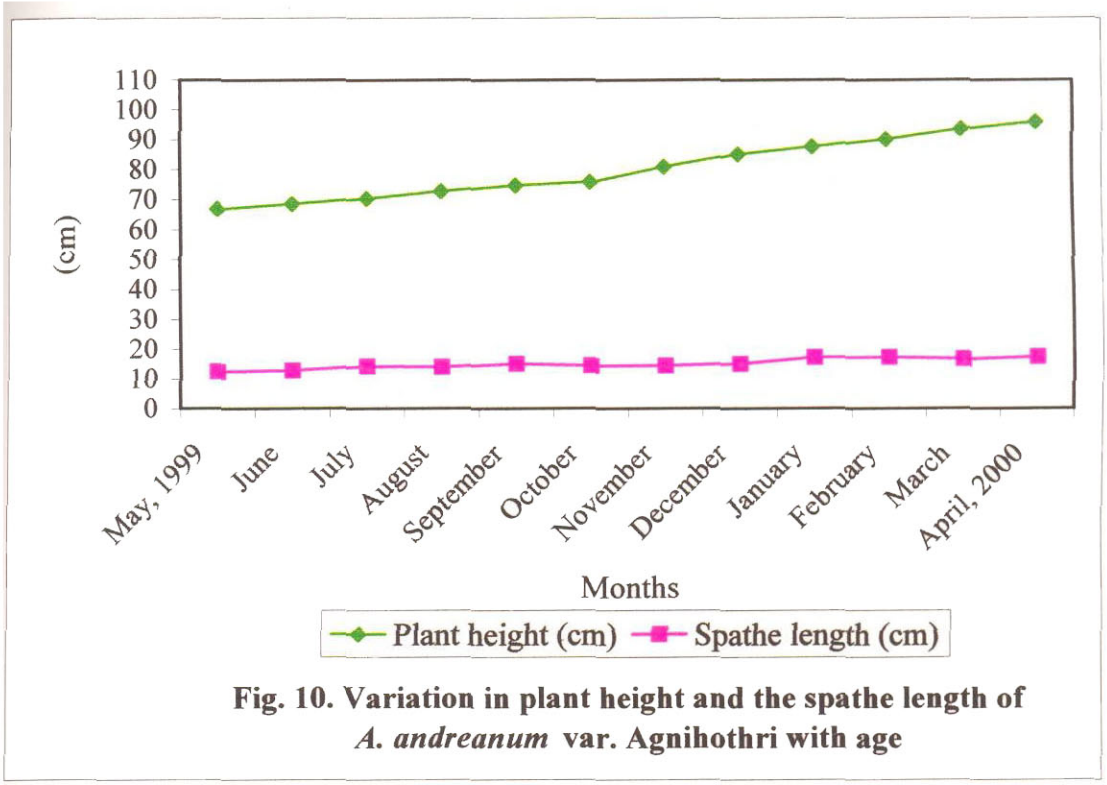


Fig. 10. Variation in plant height and the spathe length of *A. andreanum* var. Agnihothri with age

one year. The flower production interval was maximum (63.67 days) during November to January. Relative humidity was lowest during this period, i.e., November (81%), December (72%) and January (76%) in the morning.

Candy Queen

This variety produced more number of flowers during the months from August to October (2.3 flowers). Flower production was least (1.5) during the periods November to January and from January to April. The interval of flower production was long during November to January (54 days). The variety produced 7.3 flowers per year.

Lima

Highest flower production was recorded during summer months (February to April) in the variety 'Lima' (2.3 flowers by 3 months) and it produced less number of flowers (1.3) during the period November to January. The flower production interval was maximum (62.67 days) during November to January.

Red Dragon

This variety produced more number of flowers during the rainy season from May to July and from August to October (2.3 flowers each). Least flower production was noticed during the period from November to January (1.3). Flower production interval was also highest during this period (75.67 days).

Eureka Red

Flower production was more during February to April (2.6 flowers) in this variety. Lowest production (2.0) was recorded during the season from May to July and November to January. The interval of flower production was highest (58.00 days) during November to January.

Table 12. Seasonal variation in flowering behaviour of *A. andreanum* varieties

Months of the year	Nitta		Candy queen		Lima		Red Dragon		Eureka Red		Agnihothri	
	Number of flowers produced (mean)	Interval of flower production (mean) days	Number of flowers produced (mean)	Interval of flower production (mean) days	Number of flowers produced (mean)	Interval of flower production (mean) days	Number of flowers produced (mean)	Interval of flower production (mean) days	Number of flowers produced (mean)	Interval of flower production (mean) days	Number of flowers produced (mean)	Interval of flower production (mean) days
May to July	1.6	62.00	2.0	47.67	2.0	45.00	2.3	49.67	2.0	54.33	1.3	56.33
August to October	1.6	60.00	2.3	51.67	2.0	61.00	2.3	54.00	2.3	40.00	2.0	38.00
November to January	1.3	63.67	1.5	54.00	1.3	62.67	1.3	75.67	2.0	58.00	1.0	89.33
February to April	2.0	56.33	1.5	53.00	2.3	42.00	2.1	47.67	2.6	42.67	2.3	55.00
Total flowers	6.5		7.3		7.6		8.0		8.9		6.6	

The observations were taken during the period May 1999 to April 2000

Agnihothri

This variety, produced more flowers during the months from February to April (2.3 flowers) and it produced only 1.0 flower during the months from November to January. The flower production interval was also highest during this period (89.33 days).

4.2 VARIATION AND CORRELATION STUDIES

Data pertaining to the variation and correlation studies are presented in Tables 13, 14 and 15 and in Fig.11.

4.2.1 Coefficients of variation

In general, PCV was slightly higher than GCV in most of the characters (Table 13). Both PCV and GCV were highest for plant height (PCV = 70.57 and GCV = 69.96), followed by male phase (PCV = 63.82 and GCV = 58.39). The PCV and GCV were equal for the characters flower stalk length (18.05) and length of spathe (15.15). Lowest coefficients of variations were observed for length of spathe (PCV and GCV = 15.15) and for interval of flower production (PCV = 16.82 and GCV = 11.68).

4.2.2 Heritability

The characters which showed highest heritability values were flower stalk length (99.21%) and plant height (97.43%). The other characters which showed high heritability were longevity of spike on plant (89.74%), width of spathe (85.61%), male phase (84.00%) and length of spathe (81.86%). Heritability was lowest (25.82%) for the number of suckers (Table 13).

4.2.3 Genetic advance

Expected genetic advance was highest for longevity of spike on the plant (30.21) followed by flower stalk length (23.20) (Table 13). The lowest genetic

Table 13. Range, Mean, PCV, GCV and heritability for 10 characters in *Anthurium*

Sl. No.	Characters	Range	Mean	PCV (%)	GCV (%)	Heritability (%)	Genetic advance
1	Plant height	4.22-19.22	8.82	70.57	69.96	97.43	12.59
2	Flower stalk length	46.89-75.56	62.41	18.05	18.05	99.21	23.20
3	Length of spathe	11.89-17.67	14.54	15.15	15.15	81.86	4.54
4	Width of spathe	8.00-13.33	11.07	24.04	21.91	85.61	4.55
5	Length of spathe	6.22- 9.11	7.61	17.39	13.69	49.34	1.69
6	Number of suckers	1.22- 3.22	1.76	46.44	46.44	25.82	1.70
7	Longevity of spike on plant	43.00-90.00	65.89	24.44	23.32	89.74	30.21
8	Interval of flower production	41.00-60.67	50.00	16.82	11.68	53.77	8.36
9	Female phase	12.00-20.33	14.22	28.11	18.94	44.75	3.74
10	Male phase	0.00-20.67	11.06	63.82	58.39	84.00	12.16

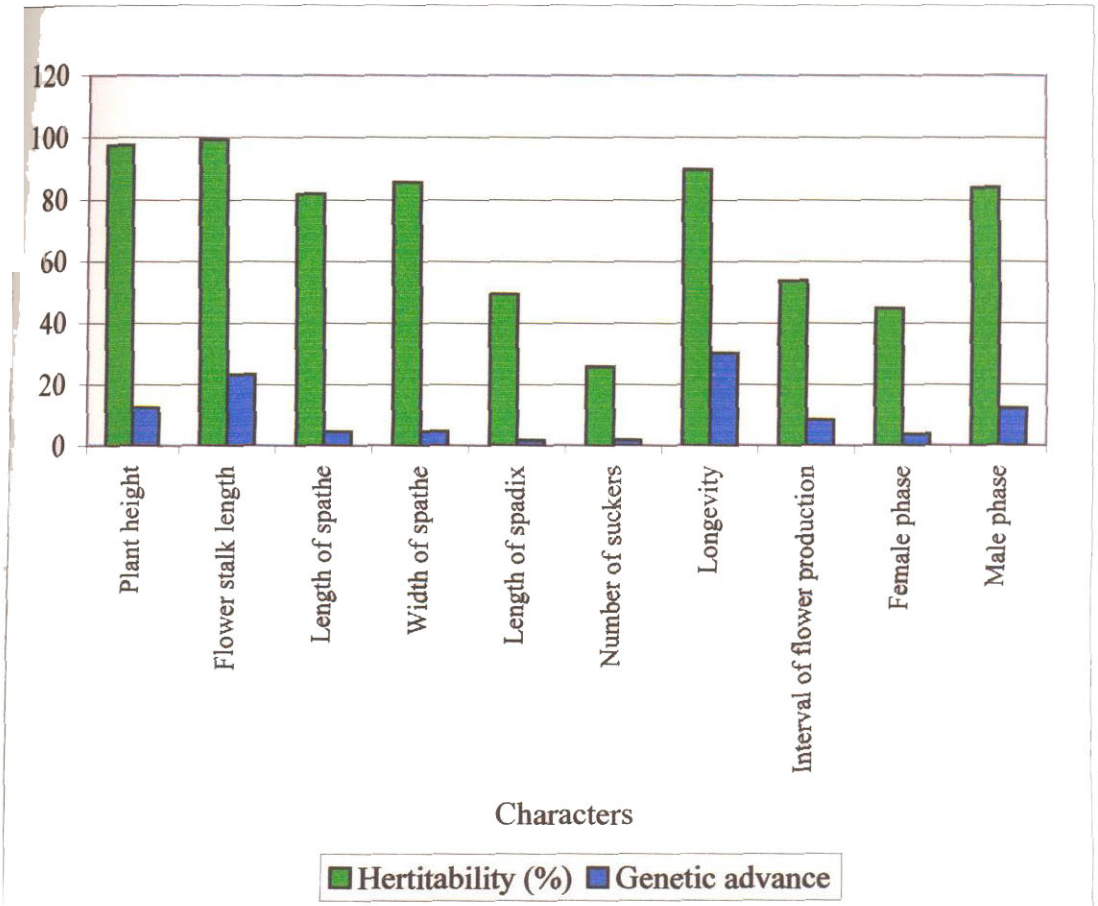


Fig. 11. Heritability and genetic advance of ten characters of *A. andreanum*

advance was recorded for length of spadix (1.69) followed by number of suckers (1.70).

4.2.4 Correlation studies

The genotypic and phenotypic correlations of various morphological and floral characters are presented in Tables 14 and 15, respectively.

4.2.4.1 Plant height

The character having significant correlation with plant height was longevity of spike on plant (r_p = 0.772, r_g =.0.835). The trait exhibited high positive but non significant correlations with the length of spathe, width of spathe and length of spadix and flower stalk length.

4.2.4.2 Flower stalk length

At genotypic level, this character exhibited significant positive correlation with length of spathe (r_g = 0.643). At phenotypic level also this character showed high positive but non significant correlation with length of spathe r_p = 0.642).

4.2.4.3 Length of spathe

The trait showed significant positive phenotypic and genotypic correlations with width of spathe (r_p) = 0.818, r_g = 0.897) and length of spadix (r_p = 0.711, r_g = 0.904). Significant negative correlation was observed with male phase at phenotypic and genotypic levels (r_p = -0.799, r_g = -0.873). This character exhibited non significant high positive correlation with the flower stalk length and plant height.

4.2.4.4 Width of spathe

Significant high positive correlations were exhibited by this character with the length of spadix (r_p = 0.697, r_g = 1.130) and length of spathe (r_p = 0.818, r_g = 0.897), both at phenotypic and genotypic levels. Significant negative phenotypic and

genotypic correlation was exhibited with the duration of male phase (r_p = -0.657, r_g = -0.730).

4.2.4.5 Length of spadix

Length of spadix showed significantly high positive correlation with the length of spathe (r_p = 0.711, r_g = 0.904) and width of spathe (r_p = 0.697, r_g = 1.130) both at phenotypic and genotypic levels. At genotypic level this character exhibited significant negative correlation with the duration of male phase (r_g = -0.810).

4.2.4.6 Number of suckers

The trait exhibited negative correlations with all the characters except duration of male phase. Maximum positive phenotypic as well as genotypic correlations were shown with the duration of male phase (r_p = 0.680, r_g = 0.744).

4.2.4.7 Longevity of spike on the plant

Significantly high positive correlations were exhibited by this trait with the character plant height, both at phenotypic and genotypic levels (r_p = 0.772, r_g = 0.835).

4.2.4.8 Interval of flower production

This character showed positive correlation with the female phase at phenotypic level (r_p = 0.153) and negative correlation at genotypic level (-0.093). Both at phenotypic and genotypic levels, the trait showed positive correlation with duration of male phase (r_p = 0.103, r_g = 0.156).

4.2.4.9 Duration of female phase

This character exhibited negative correlation in all characters except longevity of spike on plant (r_p = 0.160, r_g = 0.125) and interval of flower production (r_p = 0.153).

Table 14. Genotypic correlation coefficients (r_g) among different characters in *Anthurium andreanum* varieties

Character	Plant height	Flower stalk length	Length of spathe	Width of spathe	Length of spadix	Number of suckers	Longevity of spike on plant	Interval of flower production	Duration of female phase	Duration of male phase
	1	2	3	4	5	6	7	8	9	10
1	-									
2	0.202	-								
3	0.435	0.643*	-							
4	0.380	0.281	0.897**	-						
5	0.530	0.118	0.904**	1.130**	-					
6	-0.551	-0.098	-0.456	-0.372	-0.511	-				
7	0.835**	-0.096	0.091	-0.080	0.202	-0.549	-			
8	-0.249	0.348	-0.230	-0.233	-0.633	-0.014	-0.546	-		
9	-0.329	-0.504	-0.399	-0.449	-0.445	-0.590	0.125	-0.093	-	
10	-0.235	-0.455	-0.873**	-0.730**	-0.810**	0.744**	-0.047	0.156	-0.226	-

* Significant at 5 per cent level

** Significant at 1 per cent

Table 15. Phenotypic correlation coefficients (r_p) among different characters in *Anthurium andreanum* varieties

Character	Plant height	Flower stalk length	Length of spathe	Width of spathe	Length of spadix	Number of suckers	Longevity of spike on plant	Interval of flower production	Duration of female phase	Duration of male phase
	1	2	3	4	5	6	7	8	9	10
1	-									
2	0.200	-								
3	0.431	0.642	-							
4	0.319	0.256	0.818*	-						
5	0.398	0.093	0.711*	0.697*	-					
6	-0.547	-0.098	-0.456	-0.339	-0.402	-				
7	0.772*	-0.091	0.087	-0.025	0.178	-0.524	-			
8	-0.135	0.242	-0.159	-0.192	-0.262	-0.010	-0.336	-		
9	-0.240	-0.339	-0.269	-0.285	-0.028	-0.397	0.160	0.153	-	
10	-0.190	-0.416	-0.799*	-0.657*	-0.558	0.680	-0.135	0.103	-0.193	-

* Significant at 5 per cent level

4.2.4.10 Duration of male phase

This character exhibited negative correlations with most of the characters, except number of suckers (r_p = 0.680, r_g = 0.744) which showed high positive correlations both at phenotypic and genotypic levels and interval of flower production (r_p = 0.103, r_g = 0.156). Highest significant negative correlations were shown with the characters length of spathe (r_p = -0.1799, r_g = -0.873) and width of spathe (r_p = -0.657, r_g = -0.730) both at phenotypic and genotypic levels. At genotypic level, the trait exhibited significant negative correlation with the length of spadix (r_g = -0.810).

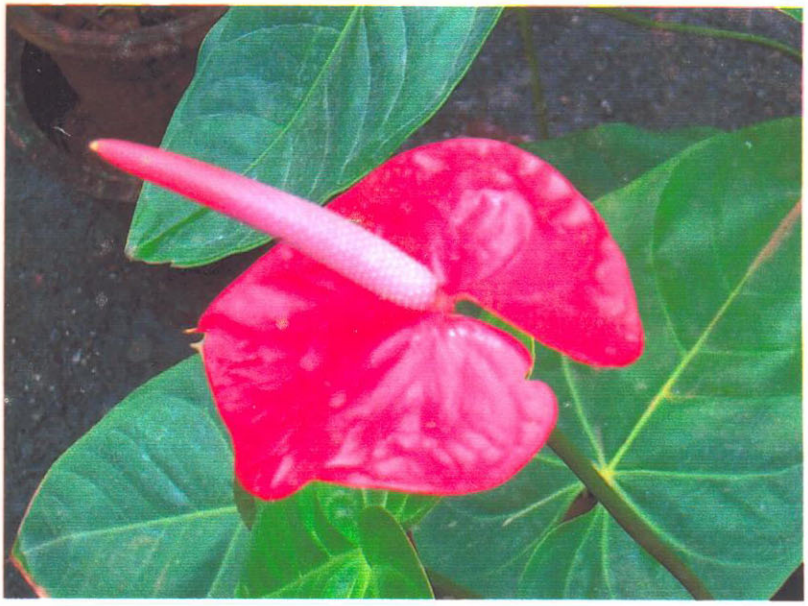
4.3 HYBRIDIZATION AND COMPATIBILITY STUDIES

Cross pollinations in all possible combinations were done, depending on the availability of receptive spadices and fresh pollen (Plate 4 and 5). This was done from May 1999 to April 2000.

The compatibility chart of the varieties and species is presented as Table 16. A total of 387 cross pollinations were done which included 42 of the 54 possible combinations (including selfs). The response of different varieties to crossing was different. Self pollination in all the varieties was found unsuccessful.

'Lima' had the highest cross compatibility followed by 'Candy Queen' and 'Red Dragon'. Maximum incompatible crosses were observed in 'Agnihothri', which was found compatible only with the variety 'Lima'. The only interspecific cross which set seed was 'Red Dragon' x *A. amnicola,* which later failed to germinate.

The percentage of seed set, seed set per spadix, seed germination and field survival are presented in Tables 17, 18, 19 and 20. Fruit set was 100 per cent in cross 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon'. Eighty six per cent fruit set was obtained in the cross 'Lima' x 'Red Dragon'. Percentage of fruit set per spadix was maximum (52.5%) in the cross 'Candy Queen' x 'Lima'. Hundred percentage germination of seeds was obtained in the cross 'Candy Queen' x 'Lima' and 98 per cent in 'Lima' x 'Red Dragon' and 'Lima' x 'Eureka Red'. Success in field



Female phase

Male phase

Plate 4. Female phase and male phase of flowers

Flowers covered with polythene bag for pollination

Seed set one month after pollination

Two months after pollination

Three months after pollination

Four months after pollination

Six months after pollination

Plate 5. Pollination and different stages of seed set

Table 16. Self and cross compatibility in *Anthurium andreanum* varieties and *Anthurium* species

Varieties Male / Female	Nitta	Lima	Red Dragon	Eureka Red	Agnihothri	*A. crystallinum*	*A. amnicola*
Nitta	Sx	C	Cx	Cx**	Cx	Cx*	Cx*
Candy Queen	Cx**	C	C	Cx	C	Cx	Cx
Lima	C	Sx	C	C	C	Cx	Cx
Red Dragon	C	C	Sx	C	Cx	Cx	Cx**
Eureka Red	Cx	C	C	Sx	Cx	Cx	Cx
Agnihothri	Cx	Cx***	Cx	Cx**	Sx	Cx	Cx

C - Cross compatible
Cx - Cross incompatible
S - Self compatible
Sx - Self incompatible
* - Set but later rot
** - Set but the seeds failed to germinate
*** - Seedlings failed to establish

Table 17. Percentage of fruit set in self and cross combinations

Varieties Male / Female	Nitta	Lima	Red Dragon	Eureka Red	Agnihothri	*A. crystallinum*	*A. amnicola*
Nitta	0	33	0	25	0	0	0
Candy Queen	33	50	33	0	25	0	0
Lima	50	0	86	100	50	0	0
Red Dragon	50	33	0	44	0	0	14
Eureka Red	0	66	100	0	0	0	0
Agnihothri	0	11	0	20	0	0	0

Table 18. Percentage of fruit set per spadix in self and cross combinations

Varieties Male / Female	Nitta	Lima	Red Dragon	Eureka Red	Agnihothri	*A. crystallinum*	*A. amnicola*
Nitta	-	14	-	4	-	-	-
Candy Queen	1.3	52.5	37	-	1	-	-
Lima	16	-	22	22	8	-	-
Red Dragon	5	4	-	8	-	-	1
Eureka Red	-	20	26	-	-	-	-
Agnihothri	-	15	-	20	-	-	-

Table 19. Germination of hybrid seeds (%) from self and cross combinations

Varieties Male / Female	Nitta	Lima	Red Dragon	Eureka Red	Agnihothri	*A. crystallinum*	*A. amnicola*
Nitta	-	40	-	0	-	-	-
Candy Queen	0	100	92	-	20	-	-
Lima	76	-	98	98	95	-	-
Red Dragon	50	50	-	58	-	-	0
Eureka Red	-	96	95	-	-	-	-
Agnihothri	-	52	-	0	-	-	-

Table 20. Field establishment of hybrids (%) from different crosses

Varieties Male / Female	Nitta	Lima	Red Dragon	Eureka Red	Agnihothri	*A. crystallinum*	*A. amnicola*
Nitta	-	22	-	0	-	-	-
Candy Queen	0	60	76	-	20	-	-
Lima	68	-	58	50	18	-	-
Red Dragon	25	30	-	22	-	-	0
Eureka Red	-	50	76	-	-	-	-
Agnihothri	-	0	-	0	-	-	-

establishment was more (76%) in the cross 'Candy Queen' x 'Red Dragon' and 'Eureka Red' x 'Red Dragon'.

4.3.1 Details of pollinations on 'Nitta'

Data pertaining to the results of all the crosses made on 'Nitta' are presented in Table 21. In 'Nitta', the highest percentage of fruit set was observed for the cross 'Nitta' x 'Lima' (33%) which was the only successful cross followed by 'Nitta' x 'Eureka Red' (25%). Other combinations as well as selfing gave no successful fruit set. Spadices started rotting after three months when crossed with *A. crystallinum* and *A. amnicola*. Percentage of fruit set per spadix was also high in the cross 'Nitta' x 'Lima' (14%). In the cross 'Nitta' x 'Lima', the days taken for fruit maturity on an average ranged from 170 to 180 days. All berries showed single seed. Only the seeds of the cross 'Nitta' x 'Lima' germinated with a germination percentage of 40. Seeds took 14.5 days for germination.

4.3.2 Details of pollinations on 'Candy Queen'

The details of crosses made are presented in Table 22. Among the crosses made on 'Candy Queen', the highest percentage of fruit set was recorded with 'Lima' (50%) and the lowest with 'Agnihothri' (25%). Interspecific combinations were unsuccessful. The average percentage of fruit set per spadix was also high in the cross 'Candy Queen' x 'Lima' (52.5%). Early fruit maturity was recorded with the cross 'Candy Queen' x 'Nitta' (135 days) whereas the maturity time was the longest (155 days) in the cross 'Candy Queen' x 'Lima'. The crosses 'Candy Queen' x 'Nitta' and 'Candy Queen' x 'Agnihothri' produced berries with single seeds. Crosses 'Candy Queen' x 'Lima' and 'Candy Queen' x 'Red Dragon' produced a few berries with double seeds. Eventhough seedset was observed in the cross 'Candy Queen' x 'Nitta', seeds did not germinate. All the seeds germinated from the cross 'Candy Queen' x 'Lima'.

Table 21. Details of crosses made on *Anthurium andreanum* variety 'Nitta'

Sl. No.	Male parent	Seed set (%)	Seed set per spadix (%)	Duration of fruit maturity		Rate of single seeds (%)	Rate of double seeds (%)	Time for germination mean (days)	Germination rate (%)
				Range (days)	Mean (days)				
1	Nitta	-	-	-	-	-	-	-	-
2	Lima	33	14	170-180	175	100	-	14.5	40.00
3	Red Dragon	-	-	-	-	-	-	-	-
4	Eureka Red	25	4	170	170	100	-	-	-
5	Agnihothri	-	-	-	-	-	-	-	-
6	*A. crystallinum*	-	-	-	-	-	-	-	-
7	*A. aminicola*	-	-	-	-	-	-	-	-

Table 22. Details of crosses made on *Anthurium andreanum* variety 'Candy Queen'

Sl. No.	Male parent	Seed set (%)	Seed set per spadix (%)	Duration of fruit maturity		Rate of single seeds (%)	Rate of double seeds (%)	Time for germination mean (days)	Germination rate (%)
				Range (days)	Mean (days)				
1	Nitta	33.00	1.30	135	135	100	-	-	0
2	Lima	50.00	52.50	150-160	155	79	21	8.5	100
3	Red Dragon	33.00	37.00	150	150	96	4	10.6	92
4	Eureka Red	-	-	-	-	-	-	-	-
5	Agnihothri	25.00	1.00	145	145	100	-	8.4	20
6	*A. crystallinum*	-	-	-	-	-	-	-	-
7	*A. aminicola*	-	-	-	-	-	-	-	-

4.3.3 Details of pollinations on 'Lima'

Data on the results of crosses made on 'Lima' are presented in Table 23. Hundred percentage fruit set was recorded for 'Lima' x 'Eureka Red'. The interspecific crosses as well as selfing were found unsuccessful. Among the successful crosses, the highest percentage of fruit set per spadix was observed in 2 crosses 'Lima' x 'Red Dragon' and 'Lima' x 'Eureka Red'. Double seeded berries were observed in all the crosses and it was maximum in the cross 'Lima' x 'Nitta' (8%). Ninety eight per cent germination was observed in 2 crosses 'Lima' x 'Red Dragon' and 'Lima' x 'Eureka Red'. Early germination was observed in the cross 'Lima' x 'Agnihothri' which took only 7 days.

4.3.4 Details of pollinations on 'Red Dragon'

Data on the results of all crosses made on 'Red Dragon' are presented in Table 24. Here maximum percentage of fruit set was recorded in 'Red Dragon' x 'Nitta' (50%) and lowest in the cross 'Red Dragon' x *Anthurium amnicola* (14%). The average fruit set per spadix was very low in this cross. It was the lowest in the cross 'Red Dragon' x *A. amnicola* (1%) and highest (8%) in 'Red Dragon' x 'Eureka Red'. Single seed was observed in all berries. Germination percentage was highest (58%) in the cross 'Red Dragon' x 'Eureka Red' and it took 26.0 days to germinate. Though the cross 'Red Dragon' x *A. amnicola* set seeds, the seeds failed to germinate.

4.3.5 Details of pollinations on 'Eureka Red'

Among the crosses of 'Eureka Red', only two were found successful (Table 25). Hundred percentage fruit set was recorded in the cross 'Eureka Red' x 'Red Dragon'. This cross showed maximum percentage of fruit (berry) set per spadix. The cross 'Eureka Red' x 'Lima' produced 66 per cent fruit set.

In both the crosses, some of the berries showed 2 seeds. Two per cent of berries showed 2 seeds in the cross 'Eureka Red' x 'Red Dragon'. Germination percentage was high (96%) in 'Eureka Red' x 'Lima'.

Table 23. Details of crosses made on *Anthurium andreanum* variety 'Lima'

Sl. No.	Male parent	Seed set (%)	Seed set per spadix (%)	Duration of fruit maturity		Rate of single seeds (%)	Rate of double seeds (%)	Time for germination mean (days)	Germination rate (%)
				Range (days)	Mean (days)				
1	Lima	-	-	-	-	-	-	-	-
2	Nitta	50	16	170-190	180	92	8	14.0	76
3	Red Dragon	86	22	180	180	93	7	9.5	98
4	Eureka Red	100	22	160-180	170	93	7	7.6	98
5	Agnihothri	50	8	180	180	97	3	7.0	95
6	*A. crystallinum*	-	-	-	-	-	-	-	-
7	*A. aminicola*	-	-	-	-	-	-	-	-

Table 24. Details of crosses made on *Anthurium andreanum* variety 'Red Dragon'

Sl. No.	Male parent	Seed set (%)	Seed set per spadix (%)	Duration of fruit maturity		Rate of single seeds (%)	Rate of double seeds (%)	Time for germination mean (days)	Germination rate (%)
				Range (days)	Mean (days)				
1	Red Dragon	-	-	-	-	-	-	-	-
2	Nitta	50	5	150-160	155	100	-	20.0	50
3	Lima	33	4	150	150	100	-	22.5	50
4	Eureka Red	44	8	140-150	145	100	-	26.0	58
5	Agnihothri	-	-	-	-	-	-	-	-
6	*A. crystallinum*	-	-	-	-	-	-	-	-
7	*A. aminicola*	14	1	150	150	100	-	-	0

Table 25. Details of crosses made on *Anthurium andreanum* variety 'Eureka Red'

Sl. No.	Male parent	Seed set (%)	Seed set per spadix (%)	Duration of fruit maturity		Rate of single seeds (%)	Rate of double seeds (%)	Time for germination mean (days)	Germination rate (%)
				Range (days)	Mean (days)				
1	Eureka Red	-	-	-	-	-	-	-	-
2	Nitta	-	-	-	-	-	-	-	-
3	Lima	66	20	160-170	165	99	1	8.0	96
4	Red Dragon	100	26	160-170	165	98	2	7.6	95
5	Agnihothri	-	-	-	-	-	-	-	-
6	*A. crystallinum*	-	-	-	-	-	-	-	-
7	*A. aminicola*	-	-	-	-	-	-	-	-

4.3.6 Details of pollinations on 'Agnihothri'

The details of crosses are presented in Table 26. In 'Agnihothri', 2 crosses recorded seed set, 'Agnihothri' x 'Lima' and 'Agnihothri' x 'Eureka Red'. In general seed set percentage was very low and it was the highest in the cross 'Agnihothri' x 'Eureka Red' (20%), but the seeds of this cross failed to germinate. The seeds of the cross 'Agnihothri' x 'Lima' showed 52 per cent germination, but failed to survive.

4.3.7 Compatibility parameters based on the performance of the varieties and species as pollen parents

Compatibility of species and varieties as male parents was analysed and the data are presented in Table 27. Highest percentage of fruit set was observed when 'Eureka Red' was used as the male parent (44.9) followed by 'Red Dragon' (33.3). No fruit set was recorded with *A. crystallinum*, and only 0.02 per cent was observed with *A. amnicola.*

The percentage of berries set per spadix was highest when 'Red Dragon' was used as the male parent (28.3%) followed by 'Lima' (21.1%) and it was lowest with *A. amnicola* (1.0%).

Very high germination (95%) was observed when 'Red Dragon' was used as male parent, followed by 'Lima' (67.6%) and no germination occurred with *A. amnicola.*

4.3.8 Comparison of compatibility performance of the *A. andreanum* varieties and species as female and male parents

The compatibility was analysed based on the seedset percentage, percentage of seedset per spadix and percentage of seed germination which were converted into a linear scale for easy computation. The percentage of seedset which ranged from 0-100 per cent, were divided into four compatibility classes as, high (100-76%)-A, medium (75-26%)-B, low (25-1%)-C and nil (0%)-D. The percentage of seedset per spadix ranged from 1 to 52.5. These values were classified as high (above

Table 26. Details of crosses made on *Anthurium andreanum* variety 'Agnihothri'

Sl. No.	Male parent	Seed set (%)	Seed set per spadix (%)	Duration of fruit maturity		Rate of single seeds (%)	Rate of double seeds (%)	Time for germination mean (days)	Germination rate (%)
				Range (days)	Mean (days)				
1	Agnihothri	-	-	-	-	-	-	-	-
2	Nitta	-	-	-	-	-	-	-	-
3	Lima	11	15	200-210	205	95	5	24.5	52
4	Red Dragon	-	-	-	-	-	-	-	-
5	Eureka Red	20	5	210	210	96	4	-	0
6	*A. crystallinum*	-	-	-	-	-	-	-	-
7	*A. aminicola*	-	-	-	-	-	-	-	-

Table 27. Compatibility parameters based on the performance of *Anthurium andreanum* varieties and species as pollen parents

Varieties/ Species	Spadix bearing seeds (%)	Berries/ spadix (%)	Seed germination (%)	Pollen fertility (%)	Pollen size (μ)	Remarks on pollen availability
Nitta	30.1	7.43	63	19	17.0	
Candy Queen	-	-	-	-	-	No pollen emergence noticed
Lima	32.5	21.10	67.6	16.6	21.4	
Red Dragon	**33.3**	**28.30**	**95.0**	**50.2**	**16.6**	
Eureka Red	44.9	9.75	52.3	21.8	20.2	
Agnihothri	14.8	4.50	57.5	64.6	25.4	
A. ornatum	-	-	-	-	-	No pollen emergence noticed
A. crystallinum	0	0	0	69.8	22.2	
A. aminicola	0.02	1	0	33.2	16.6	

40%)-A, medium (39-20%)-B, low (19-1%)-C and nil (0%)-D. The percentage of seed germination, which ranged from 0 to 100 per cent was classified as high (above 50%)-A, medium (49-20%)-B, low (19-1%)-C and nil (0%)-D. A score of 3 points was assigned to the class A, 2 for class B, 1 for class C and 0 for class D. The total score of the 42 combinations that ranged from 0 to 9 was analysed to get an idea of the cross compatibility relations among the varieties under study.

4.3.8.1 Comparison of female parents

Among the 42 combinations tried, 25 were found to be completely incompatible with a score of 0 (Table 28). Out of the 17 successful combinations, the crosses with highest compatibility score of 8 were 'Candy Queen' x 'Lima', 'Lima' x 'Red Dragon', 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon'. The next highest compatibility score of 7 was shown by the combination 'Candy Queen' x 'Red Dragon' and 'Eureka Red' x 'Lima. Among the successful crosses, the combinations involving 'Nitta' x 'Eureka Red' and 'Agnihothri' x 'Eureka Red' showed lower compatibility score (2). Higher compatibility scores were obtained for 'Candy Queen' and 'Lima'. The combinations 'Nitta' x 'Eureka Red', 'Candy Queen' x 'Nitta', 'Agnihothri' x 'Eureka Red' and 'Red Dragon' x *A. amnicola* produced seed set but the seeds from these failed to germinate, resulting in very low compatibility score.

In general, considering all the cross combinations involving the six female parents, Lima appeared to be the most compatible, showing the best performance with a total compatibility score of 28 with 4 successful crosses. The variety was found to be compatible with all the other varieties of *A. andreanum*. Self pollination and interspecific crosses were found incompatible. The variety 'Candy Queen' appear to be the next best female parent with a total score of 22 with 4 successful crosses. All the intervarietal crosses except with that of 'Eureka Red' was found to produce seeds. But with 'Nitta', the seeds failed to germinate. The varieties 'Red Dragon' had a total compatibility score of 20 with 3 surviving crosses. The variety 'Eureka Red' had score 15, with 2 successful crosses. The varieties 'Nitta' and 'Agnihothri' showed lowest compatibility score of 7 from a single surviving cross with the variety 'Lima'. With

Table 28. Compatibility score on the basis of the performance of *A. andreanum* varieties as female parents.

Sl. No.	Combinations	Seed set (%)	Seed set per spadix (%)	Seed germination (%)	Total score	Varietal score
1.	Nitta x Nitta	D	-	-	0	
2.	Nitta x Lima	B	C	B	5	
3.	Nitta x Red Dragon	D	-	-	0	
4.	Nitta x Eureka Red	C	C	D	2	
5.	Nitta x Agnihothri	D	-	-	0	
6.	Nitta x *A. crystallinum*	D	-	-	0	
7.	Nitta x *A. amnicola*	D	-	-	0	7
8.	Candy queen x Nitta	B	C	D	3	
9.	Candy queen x Lima	B	A	A	8	
10.	Candy queen x Red Dragon	B	B	A	7	
11.	Candy queen x Eureka Red	D	-	-	0	
12.	Candy queen x Agnihothri	C	C	B	4	
13.	Candy queen x *A. crystallinum*	D	-	-	0	
14.	Candy queen x *A. amnicola*	D	-	-	0	22
15.	Lima x Lima	D	-	-	0	
16.	Lima x Nitta	B	C	A	6	
17.	Lima x Red Dragon	A	B	A	8	
18.	Lima x Eureka Red	A	B	A	8	
19.	Lima x Agnihothri	B	C	A	6	
20.	Lima x *A. crystallinum*	D	-	-	0	
21.	Lima x *A. amnicola*	D	-	-	0	28

Contd.

Table 28. continued

Sl. No.	Combinations	Seed set (%)	Seed set per spadix (%)	Seed germination (%)	Total score	Varietal score
22.	Red dragon x Red dragon	D	-	-	0	
23.	Red dragon x Nitta	B	C	A	6	
24.	Red dragon x Lima	B	C	A	6	
25.	Red dragon x Eureka Red	B	C	A	6	
26.	Red dragon x Agnihothri	D	-	-	0	
27.	Red dragon x *A. crystallinum*	D	-	-	0	
28	Red dragon x *A. amnicola*	C	C	-	2	20
29.	Eureka Red x Eureka Red	D	-	-	0	
30.	Eureka Red x Nitta	D	-	-	0	
31.	Eureka Red x Lima	B	B	A	7	
32.	Eureka Red x Red dragon	A	B	A	8	
33.	Eureka Red x Agnihothri	D	-	-	0	
34.	Eureka Red x *A. crystallinum*	D	-	-	0	
35.	Eureka Red x *A. amnicola*	D	-	-	0	15
36.	Agnihothri x Agnihothri	D	-	-	0	
37.	Agnihothri x Nitta	D	-	-	0	
38.	Agnihothri x Lima	C	C	A	5	
39.	Agnihothri x Red dragon	D	-	-	0	
40.	Agnihothri x Eureka Red	C	C	D	2	
41.	Agnihothri x *A. crystallinum*	D	-	-	0	
42.	Agnihothri x *A. amnicola*	D	-	-	0	7

A - 3 points; B - 2 points; C - 1 point and D - 0 point

the variety, 'Eureka Red', both the varieties produced seed set but the seeds did not germinate. The only interspecific cross that found to set seeds was 'Red Dragon' x *A. amnicola* but seeds failed to germinate.

4.3.8.2 Comparison of male parents

Compatibility score of 7 male parents were analysed. It revealed that the compatibility reactions of the varieties and species were different from the compatibility reactions of those as female parents (Table 29). The highest score of 8 was exhibited by the combinations 'Candy Queen' x 'Lima', 'Lima' x 'Red Dragon', 'Eureka Red' x 'Red Dragon', 'Lima' x 'Eureka Red'. The male parents involved in these crosses namely 'Lima', 'Red Dragon' and 'Eureka Red' were the best male parents among the varieties and species studied, with high compatibility score. Among these, the best male parent was found to be 'Lima' with a total compatibility score of 31 from 5 successful cross combinations followed by the variety 'Red Dragon' with a score of 23 from 3 successful combinations. 'Eureka Red' showed the compatibility score of 18. Compatibility score of 0 was obtained for the *Anthurium crystallenum* and for *A. amnicola*, two that set seeds with the *A. andreanum* variety 'Red Dragon'. But the seed set percentage was very low and the seeds failed to germinate.

4.3.8.3 Comparison of male and female parents

The male and female parents were compared with respect to their compatibility score and the data are presented in Table 30 and Fig.12. The variety 'Lima' was the best performer as female as well as male parent with the total score of 28 and 31, respectively. 'Candy Queen' performed well as a good female parent with a score of 22, but it produced no pollen during the period of study and could not be included in the experiment as a male parent. The varieties 'Red Dragon' and 'Eureka Red' performed well both as female parents (scores 20 and 15, respectively) and male parents (scores 23 and 18, respectively). The variety 'Nitta' scored 15 as male parent but as female parent the score was 7. Considering the overall performance, the

Table 29. Compatibility score on the basis of the performance of *A. andreanum* varieties and species as male parents.

Sl. No.	Combinations	Seed set (%)	Seed set per spadix (%)	Seed germination (%)	Total score	Varietal score
1.	Nitta x Nitta	D	-	-	0	
2.	Candy queen x Nitta	B	C	D	3	
3.	Lima x Nitta	B	C	A	6	
4.	Red Dragon x Nitta	B	C	A	6	
5.	Eureka Red x Nitta	D	-	-	0	
6.	Agnihothri x Nitta	D	-	-	0	5
7.	Nitta x Lima	B	C	B	5	
8.	Candy queen x Lima	B	A	A	8	
9.	Lima x Lima	D	-	-	0	
10.	Red Dragon x Lima	B	C	A	6	
11.	Eureka Red x Lima	B	B	A	7	
12.	Agnihothri x Lima	C	C	A	5	31
13.	Nitta x Red Dragon	D	-	-	0	
14.	Candy queen x Red Dragon	B	B	A	7	
15.	Lima x Red Dragon	A	B	A	8	
16.	Red Dragon x Red Dragon	D	-	-	0	
17.	Eureka Red x Red Dragon	A	B	A	8	
18.	Agnihothri x Red Dragon	D	-	-	0	23
19.	Nitta x Eureka Red	C	C	D	2	
20.	Candy queen x Eureka Red	D	-	-	0	
21.	Lima x Eureka Red	A	B	A	8	
22.	Red Dragon x Eureka Red	B	C	A	6	
23.	Eureka Red x Eureka Red	D	-	-	0	
24.	Agnihothri x Eureka Red	C	C	D	2	18

Contd.

Table 29. continued

Sl. No.	Combinations	Seed set (%)	Seed set per spadix (%)	Seed germination (%)	Total score	Varietal score
25.	Nitta x Agnihothri	D	-	-	0	
26.	Candy queen x Agnihothri	C	C	B	4	
27.	Lima x Agnihothri	B	C	A	6	
28	Red Dragon x Agnihothri	D	-	-	0	
29.	Eureka Red x Agnihothri	D	-	-	0	
30.	Agnihothri x Agnihothri	D	-	-	0	10
31.	Nitta x *A. crystallinum*	D	-	-	0	
32.	Candy queen x *A. crystallinum*	D	-	-	0	
33.	Lima x *A. crystallinum*	D	-	-	0	
34.	Red Dragon x *A. crystallinum*	D	-	-	0	
35.	Eureka Red x *A. crystallinum*	D	-	-	0	
36.	Agnihothri x *A. crystallinum*	D	-	-	0	0
37.	Nitta x *A. amnicola*	D	-	-	0	
38.	Candy queen x *A. amnicola*	D	-	-	0	
39.	Lima x *A. amnicola*	D	-	-	0	
40.	Red Dragon x *A. amnicola*	C	C	-	2	
41.	Eureka Red x *A. amnicola*	D	-	-	0	
42.	Agnihothri x *A. amnicola*	D	-	-	0	2

A - 3 points; B - 2 points; C - 1 point and D - 0 point

Table 30. Comparison of compatibility performance of the *A. andreanum* varieties and species as female and male parents

Female parent			Male parent		
Variety	Score	Rank	Variety	Score	Rank
Lima	28	I	Lima	31	I
Candy queen	22	II	Red Dragon	23	II
Red Dragon	20	III	Eureka Red	18	III
Eureka Red	15	IV	Nitta	15	IV
Nitta	7	V	Agnihothri	10	V
Agnihothri	7	V	*A. amnicola*	2	VI
			A. crystallinum	0	VII

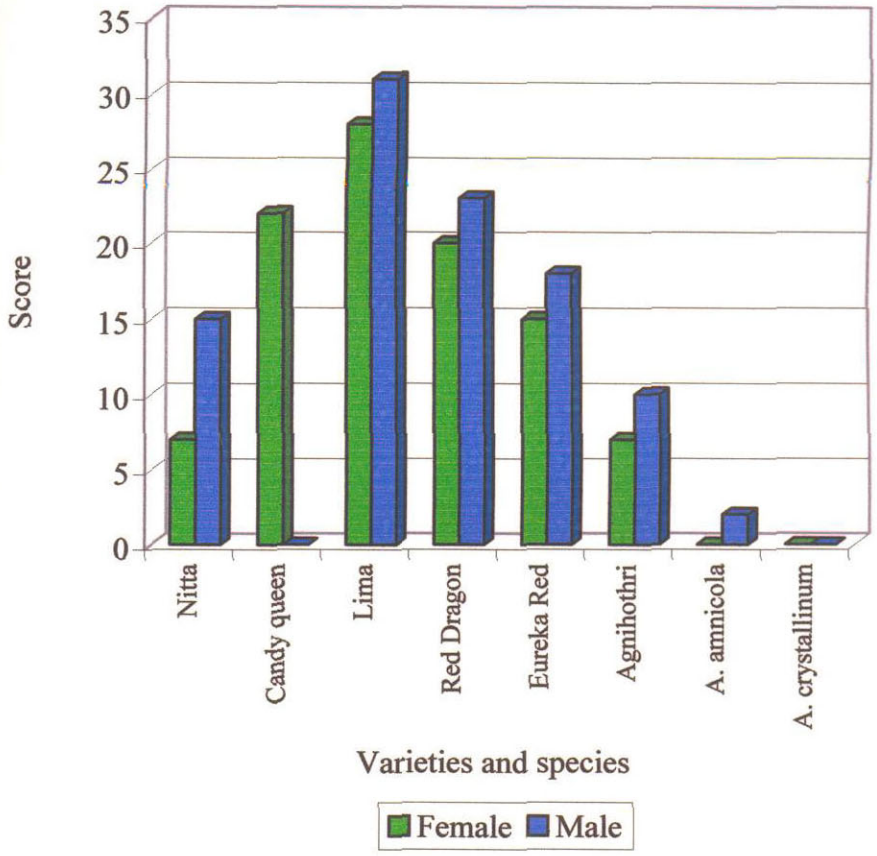


Fig. 12. Compatibility performance of *A. andreanum* varieties and species as female and male parents

varieties 'Lima', 'Red Dragon' and 'Eureka Red' can be considered as excellent performers.

4.4 *IN VITRO* STUDIES

4.4.1 Standardisation of maturity of seed for *in vitro* seed culture

For this study, seeds of 70 days maturity onwards were taken and cultured in MS and Nitsch media. Seeds from the cross 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon' were selected for the study. The results are presented in Table 31.

4.4.1.1 Germination percentage of seeds

Germination started from 90 days of maturity onwards in the cross 'Lima' x 'Eureka Red', whereas in the cross 'Eureka Red' x 'Red Dragon' germination started from 80 days of maturity. In the cross 'Lima' x 'Eureka Red', the germination percentage increased from 90 days (7%) to 120 days of maturity (100%). From 120 days onwards, the germination percentage remained same, irrespective of the age of seeds (Table 31).

In the cross, 'Eureka Red' x 'Red Dragon', germination started from 80 days of maturity and the germination percentage increased with age and reached 100 per cent at 130 days of maturity, thereafter it remained the same.

4.4.1.2 Number of days taken for germination

Significant difference was found in the number of days taken for germination among the different ages of seeds of 'Lima' x Eureka Red.

Longest time (20.80 days) was taken for germination by the seeds of 90 days maturity. Early germination was obtained from 130 days of maturity (9.8 days) onwards till ripening (8.4 days) which were significantly superior to others. Ripening of berries occurred at 180 days.

Table 31. Effect of maturity of seed and germination *in vitro*

Age of seed (days after spathe unfurling)	Lima x Eureka Red				Eureka Red x Red Dragon			
	Germination percentage		No. of days taken for germination		Germination percentage		No. of days taken for germi-nation	
	MS Media	Nitsch Media	Range	Mean	MS Media	Nitsch Media	Range	Mean
70	0	0	-		0	0	-	
80	0	0	-		7	7	25	
90	0	7	20		7	20	25	
100	13	13	20		7	26	20-24	
110	20	20	17-20	20.0	73	66	20-26	24.8
120	100	100	11-18	12.6	86	93	12-14	12.6
130	100	100	5-11	9.8	100	100	5-9	7.2
140	100	100	5-11	8.4	100	100	5-9	6.8
150	100	100	5-11	9.4	100	100	5-9	6.6
160	100	100	5-11	8.8	100	100	5-9	7.0
170	100	100	5-11	8.4	100	100	5-9	7.0
180	100	100	5-11	8.4	-	-	-	
CD (0.05)				2.65				1.40
SEm ±				0.91				0.48

In the case of cross 'Eureka Red' x 'Red Dragon', early germination (7.2 days) was seen in seeds of 130 days of maturity onwards. Ripening of the berries was observed in 170 days.

4.4.2 Standardisation of media

Seeds taken out of berries were sterilized and cultured in different media.

4.4.2.1 Standardisation of surface sterilization

The results of the experiment on different methods of surface sterilization of seeds are presented in Table 32.

Among the different treatments tried, none recorded total survival. Maximum survival percentage with less incidence of contamination was obtained with the combination in which the seeds were treated with bavistin 0.1 per cent for 30 minutes and then kept in mercuric chloride 0.1 per cent for 10 minutes and also with treatment with mercuric chloride 0.1 per cent for 10 minutes alone, without pretreatment with bavistin. Both the treatments recorded a survival percentage of 95.

Treatments of silver nitrate 0.1 per cent for 10 minutes and mercuric chloride 0.2 per cent for 10 minutes recorded the survival of 60 per cent. Silver nitrate 0.1 per cent treatment for longer period (20 minutes) resulted in blackening of the seeds reducing the survival percentage. Higher concentration of mercuric chloride also caused the same effect. Flaming also caused death of the seeds.

4.4.2.2 Effect of media on germination and development

The results of various media on germination and culture development are presented in Table 33.

4.4.2.2.1 Effect of different media on days taken for germination and germination percentage

No significant difference could be observed on the days taken for germination and germination percentage among the treatment combinations.

Table 32. Details of surface sterilization of seeds

Medium - MS + BA 1.0 mg/l

Treatment	Concentration (%)	Duration (minutes)	Percentage of		
			Blackened or bleached	Contaminated	Surviving
1. Silver nitrate	0.1	20	45	5	50
2. Silver nitrate	01	10	30	10	60
3. Bavistin + Mercuric chloride	0.1 0.1	30 10	-	5	95
4. Mercuric chloride	0.1	10	-	5	95
5. Mercuric chloride	0.2	10	35	5	60
6. Mercuric chloride + flaming	0.1	10	80	5	15

Values taken as the average of 20 observations of the cross Lima x Red Dragon

4.4.2.2.2 Effect of different media on number of days for first leaf formation

Significant difference among treatments was observed with regard to the days taken for first leaf formation.

Early development of leaf was noticed in medium MS + 0.5 mg l^{-1} BA (17 days) which was on par with MS + 1 mg l^{-1} BA, ½ MS, ½ MS + 1 mg l^{-1} BA, ½ MS + 0.5 mg l^{-1} BA and ¼ MS. Longest period for leaf formation (25.33 days) was taken by two media, MS and Nitsch.

4.4.2.2.3 Effect of media on number of days for callus development

After the germination of seeds, the media with growth substances produced bulging of the shoot portion and developed callus. The basal media alone did not produce callus. When different growth substances were added, callus development was noticed and significant difference was observed in the number of days taken for callus development.

Early development of callus was observed in ½ MS supplemented with 1 mg l^{-1} BA (19.33 days) and was on par with MS + 1 mg l^{-1} BA and ½ MS + 0.5 mg l^{-1} BA. Maximum number of days for callus development was recorded on MS + 0.5 mg l^{-1} BA (35 days). The basal media alone showed no response.

4.4.2.2.4 Effect of different media on number of days for multiple shoot formation

Multiple shoots were produced from the callus, developed from the shoot portion of the seedlings. Here also the basal media showed no response.

Among the other media tried, ½ MS + 1 mg l^{-1} BA produced multiple shoots earlier (38.33 days) than the other media, which was significantly superior to others. The other media took 50 days to 56.67 days for multiple shoot formation.

Table 33. Effect of different media on germination of hybrid seeds and further development

Explant - Seeds
Cross - Lima x Eureka Red

Treatments	Days taken for germination	Germination percentage	Days taken for first leaf formation	Days taken for callus development	Days taken for multiple shoot formation	Remarks
MS	6.33	93.33	25.33	*	*	Callus and multiple shoot formation not observed
½ MS	6.67	95.00	18.00	*	*	,,
¼ MS	6.33	93.33	21.00	*	*	,,
Nitsch	7.33	93.33	25.33	*	*	,,
MS + 0.5 mg/l BA	7.67	91.67	17.00	35.00	56.67	
MS + 1 mg/l BA	7.00	93.33	18.00	21.67	50.00	
½ MS + 0.5 mg/l BA	7.33	95.00	21.00	22.67	50.00	
½ MS + 1 mg/l BA	7.00	93.33	19.00	19.33	38.33	
CD (0.05)	NS	NS	4.09	6.03	10.17	
SEm ±	0.68	3.82	1.37	1.85	3.12	

4.4.2.3 Effect of growth substance combinations on callus initiation and growth

Studies on callusing were made using the seeds cross 'Lima' x Eureka Red, with the basal media of ½ MS. Cultures were kept in dark, for initiation of callus, and observations were taken 4 weeks after inoculation. The results are presented in Table 34.

IAA and IBA alone and also in combination with BA did not initiate callus in cultures. BA alone and in combination with 2,4-D and NAA showed initiation and growth of callus. Maximum callus development was recorded in combinations in which higher concentration of BA with lower concentration of auxins namely, 2,4-D and NAA were used.

Among the 25 combinations of growth substances tried, NAA (3 mg l^{-1}) + BA (6 mg l^{-1}) produced the maximum number of cultures initiating callus (40%), the highest growth score (3.0) and CI value (120) followed by the treatments 2,4-D (1 mg l^{-1}) + BA (2 mg l^{-1}) and NAA (0.5 mg l^{-1}) + BA (1 mg l^{-1}) which recorded 30 per cent of cultures initiating callus and callus index of 69.

4.4.2.4 Effect of growth substance combinations on shoot regeneration

Data on the effect of various treatments on shoot regeneration and development are presented in Table 35 and Fig.13. All the treatments showed shoot regeneration. However, the highest percentage of cultures initiating shoots (60%) was in treatment combination of BA 0.5 mg l^{-1} + IAA (1 mg l^{-1}) in ½ MS media, which was followed by the treatments NAA (2 mg l^{-1}) + BA (0.5 mg l^{-1}), NAA (3 mg l^{-1}) + BA (1 mg l^{-1}) and BA (1 mg l^{-1}) + IAA (2 mg l^{-1}). The treatment combination BA (0.5 mg l^{-1}) + IAA (1 mg l^{-1}) also showed multiple shoot formation.

4.4.2.5 Effect of combinations of growth substances on shoot multiplication and growth

Data pertaining to the results of the experiment on the effect of growth substances on the shoot multiplication and growth are presented in Table 36 and Fig.14.

Table 34. Effect of growth substances on callus initiation and growth

Cross - Lima x Eureka Red
Media - ½ MS

Treatment	Percentage of cultures initiating callus	Growth score	Callus index
BA 0.5 mg l^{-1}	10	1	10
BA 1.0 mg l^{-1}	20	1	20
IAA 1.0 mg l^{-1}	0	0	0
IBA 1.0 mg l^{-1}	0	0	0
IBA 2.0 mg l^{-1}	0	0	0
2,4-D 1.0 mg l^{-1}	0	0	0
2,4-D 1.0 mg l^{-1} + BA 1.0 mg l^{-1}	0	0	0
2,4-D 1.0 mg l^{-1} + BA 0.5 mg l^{-1}	0	0	0
2,4-D 1.0 mg l^{-1} + BA 2.0 mg l^{-1}	30	2.3	69
2,4-D 0.5 mg l^{-1} + BA 0.5 mg l^{-1}	0	0	0
2,4-D 0.5 mg l^{-1} + BA 1.0 mg l^{-1}	20	2	40
2,4-D 0.5 mg l^{-1} + BA 2.0 mg l^{-1}	0	0	0
2,4-D 1.5 mg l^{-1} + BA 1.5 mg l^{-1}	10	1	10
NAA 0.5 mg l^{-1} + BA 1.0 mg l^{-1}	30	2.3	69
NAA 1.0 mg l^{-1} + BA 1.0 mg l^{-1}	10	1	10
NAA 1.0 mg l^{-1} + BA 0.5 mg l^{-1}	0	0	0
NAA 1.0 mg l^{-1} + BA 2.0 mg l^{-1}	20	2	40
NAA 2.0 mg l^{-1} + BA 2.0 mg l^{-1}	0	0	0
NAA 3.0 mg l^{-1} + BA 6.0 mg l^{-1}	40	3	120
NAA 4.0 mg l^{-1} + BA 6.0 mg l^{-1}	20	2.5	50
IBA 0.5 mg l^{-1} + BA 1.0 mg l^{-1}	0	0	0
IBA 1.0 mg l^{-1} + BA 1.0 mg l^{-1}	0	0	0
IAA 1.0 mg l^{-1} + BA 0.5 mg l^{-1}	0	0	0
IAA 1.0 mg l^{-1} + BA 1.0 mg l^{-1}	0	0	0
IAA 0.5 mg l^{-1} + BA 1.0 mg l^{-1}	0	0	0

Observations taken from 20 cultures

4.4.2.5.1 Number of shoots (after 4 weeks)

Regarding the number of shoots, significant difference was obtained among the different media tried. Highest number of shoots (6.8 shoots) was obtained in the growth substances combination BA (6 mg l^{-1}) + NAA (2 mg l^{-1}) + 2,4-D (2 mg l^{-1}), which was on par with BA (6 mg l^{-1}) + NAA (3 mg l^{-1}). These two treatments were significantly superior to the other treatments.

4.4.2.5.2 Length of longest shoot (after 4 weeks)

A similar trend was noticed with respect to the size of the shoot also. Longest shoots of 0.68 cm was produced by BA (6 mg l^{-1}) + NAA (2 mg l^{-1}) + 2,4-D (2 mg l^{-1}) which was on par with BA (6 mg l^{-1}) + NAA (3 mg l^{-1}) which produced 0.56 cm long shoots. Shortest shoots of 0.14 cm was observed in the medium containing BA (6 mg l^{-1}) + NAA (2 mg l^{-1}).

4.4.2.5.3 Number of shoots (after 8 weeks)

From the Table 36, it is clear that maximum number of shoots (14.20) was observed in the treatment combination of BA (6 mg l^{-1}) + NAA (2 mg l^{-1}) + 2,4-D (2 mg l^{-1}) which was significantly superior to all other treatments, followed by the treatment combination BA (6 mg l^{-1}) + NAA (3 mg l^{-1}) which produced 10.4 shoots on an average. Least number of shoots (2.4) was produced by BA (4 mg l^{-1}) + NAA (2 mg l^{-1}).

4.4.2.5.4 Length of the longest shoot (after 8 weeks)

Again the treatment combination BA (6 mg l^{-1}) + NAA (2 mg l^{-1}) + 2,4-D (2 mg l^{-1}) showed the maximum length for the longest shoot (2.82 cm). This treatment was significantly superior to all other treatments. It was followed by the treatment combination BA (6 mg l^{-1}) + NAA (3 mg l^{-1}) which recorded 2.36 cm length.

Table 35. Effect of growth substances on shoot regeneration

Media - ½ MS

Treatment (mg l^{-1})			Percentage of cultures showing shoot regeneration	Remarks
NAA	BA	IAA		
1	0.5	-	30	
2	0.5	-	50	
3	1	-	50	
-	0.5	1	60	Also produced multiple shoots
-	1	2	50	
-	1	1	30	

Percentage of observations taken from 20 cultures

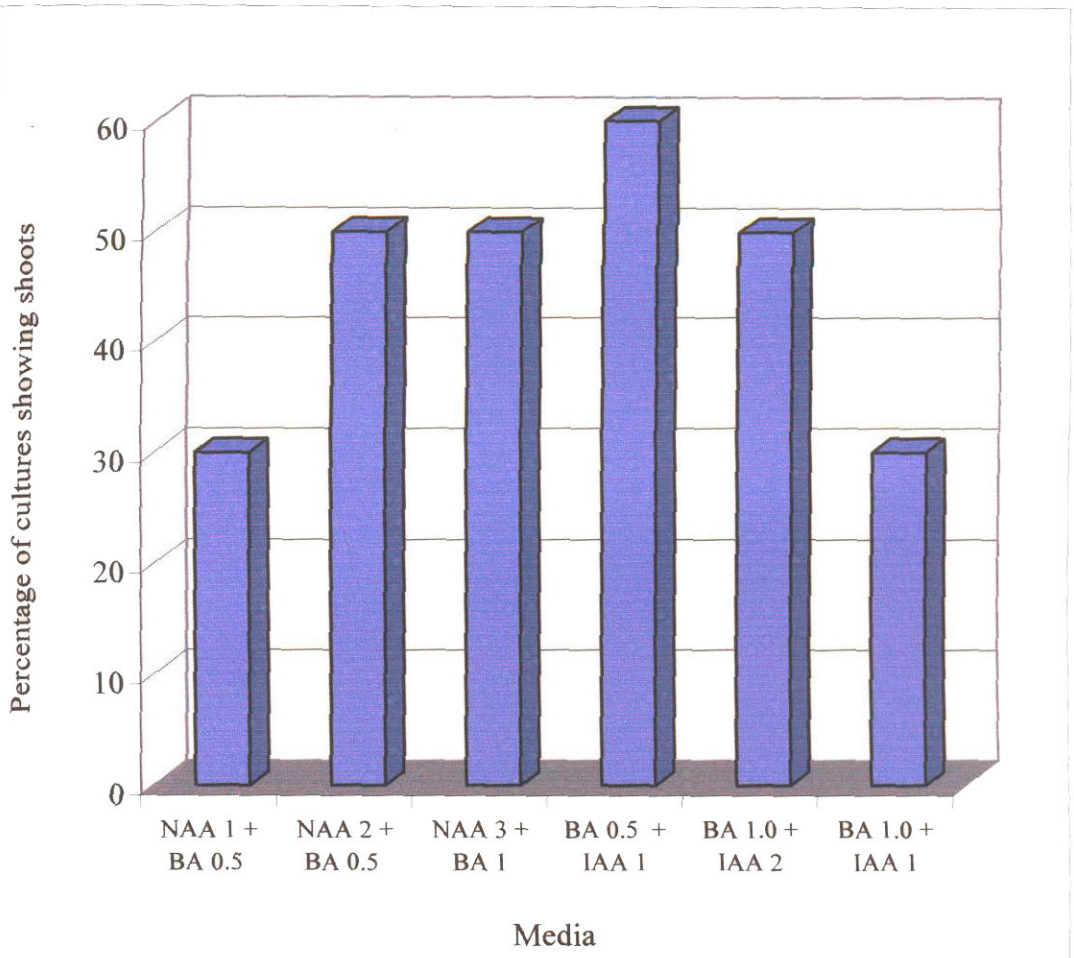


Fig. 13. Effect of growth substances on shoot regeneration

4.4.2.6 Effect of growth substances on root formation

The different media tried for growth and multiplication of shoots failed to produce roots. IBA and IAA alone and in combination with other growth substances were added to the media. The effects of different treatments are given in Table 37 and Fig.15.

4.4.2.6.1 Days taken for rooting

Early rooting was observed in the treatment ½ MS with BA (0.5 mg l^{-1}) + IAA (1 mg l^{-1}) which took 26.83 days for development of root on an average, this was on par with ¼ MS (27.67 days). Maximum time for rooting was taken by ½ MS media supplemented with BA 0.5 mg l^{-1} (48.67 days).

4.4.2.6.2 Number of shoots and roots

The data revealed that significant differences were shown by different media on the number of roots as well as the number of shoots after 8 weeks of inoculation. Maximum number of shoots was produced in the ½ MS + BA (0.5 mg l^{-1}) + IAA (1 mg l^{-1}) (14.17 shoots) which was significantly superior to all the other treatments, followed by ½ MS + BA (0.5 mg l^{-1}), which produced 9.17 shoots. Least number of shoots (1.17) was produced in the medium ½ MS + IBA (8 mg l^{-1}). The highest number of roots was produced in the treatment ¼ MS (7.5) followed by ½ MS + BA (0.5 mg l^{-1}) + IAA (1 mg l^{-1}) which produced 6.33 shoots on an average.

4.4.2.6.3 Percentage of cultures showing root formation

Highest percentage (60%) of cultures showing root formation was observed in two treatments, ¼ MS basal medium and ½ MS + BA (0.5 mg l^{-1}) + IAA (1 mg l^{-1}). The lowest percentage (10%) was in ½ MS + BA (0.5 mg l^{-1}).

4.5 IRRADIATION STUDIES

The hybrid seeds obtained were subjected to gamma rays at 2-20 Gy. The seeds were cultured and the cultures were irradiated using gamma rays at 0.5-10.0 Gy.

Table 36. Effect of growth substances on shoot multiplication and growth

Stage of inoculation - Shoot regeneration
Cross - Lima x Eureka Red
Media - ½ MS

Growth substances (mg l^{-1})			4 weeks		8 weeks	
BA	NAA	2,4-D	Number of shoots	Length of longest shoot (cm)	Number of shoots	Length of longest shoot (cm)
4	2	0	1.8	0.14	2.4	0.46
4	2	2	1.8	0.18	2.8	0.48
6	2	0	2.0	0.18	4.6	0.58
6	2	2	6.8	0.68	14.2	2.82
6	3	0	6.0	0.56	10.4	2.36
6	3	2	2.4	0.22	4.0	1.40
CD (0.05)			0.49	0.21	3.26	0.37
SEm ±			0.51	0.07	1.12	0.13

Table 37. Effect of media on root formation

Cross - 'Lima' x 'Eureka Red'

Media	Days taken for root formation	8 weeks after inoculation		Percentage of cultures showing root formation
		No. of shoots formed	No. of roots formed	
MS	30.17	2.17	1.67	20
½ MS	30.00	2.00	3.33	50
¼ MS	27.67	3.67	7.50	60
½ MS + IBA 1 mg/l	39.17	3.67	1.33	20
½ MS + IBA 2 mg/l	30.00	2.33	1.33	15
½ MS + IBA 4 mg/l	30.67	1.67	5.67	30
½ MS + IBA 6 mg/l	40.00	1.33	3.67	50
½ MS + IBA 8 mg/l	44.00	1.17	1.67	20
½ MS + IAA 1 mg/l	39.50	3.67	2.33	40
½ MS + BA 0.5 mg/l	48.67	9.17	2.00	10
½ MS + BA (0.5 mg/l) + IAA (1 mg/l)	26.83	14.17	6.33	60
½ MS + BA (0.5 mg/l) + IBA (1 mg/l)	34.33	7.50	3.17	50
CD (0.05)	2.554	1.104	0.8215	
SEm±	0.902	0.391	0.291	

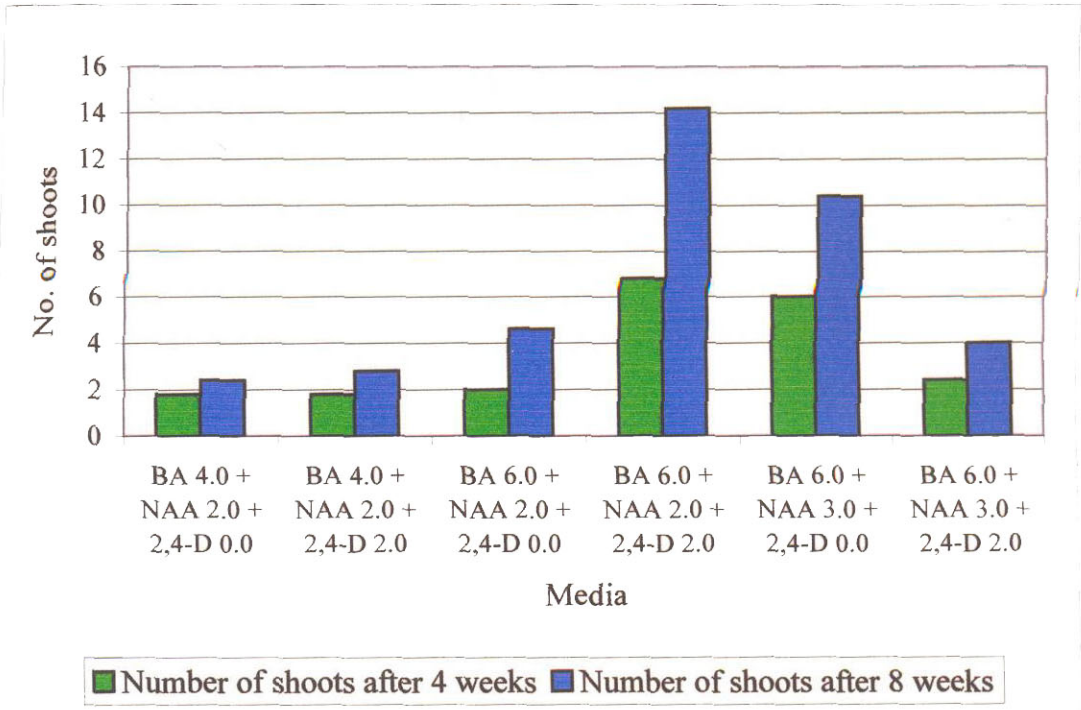


Fig. 14. Effect of growth substances on shoot multiplication

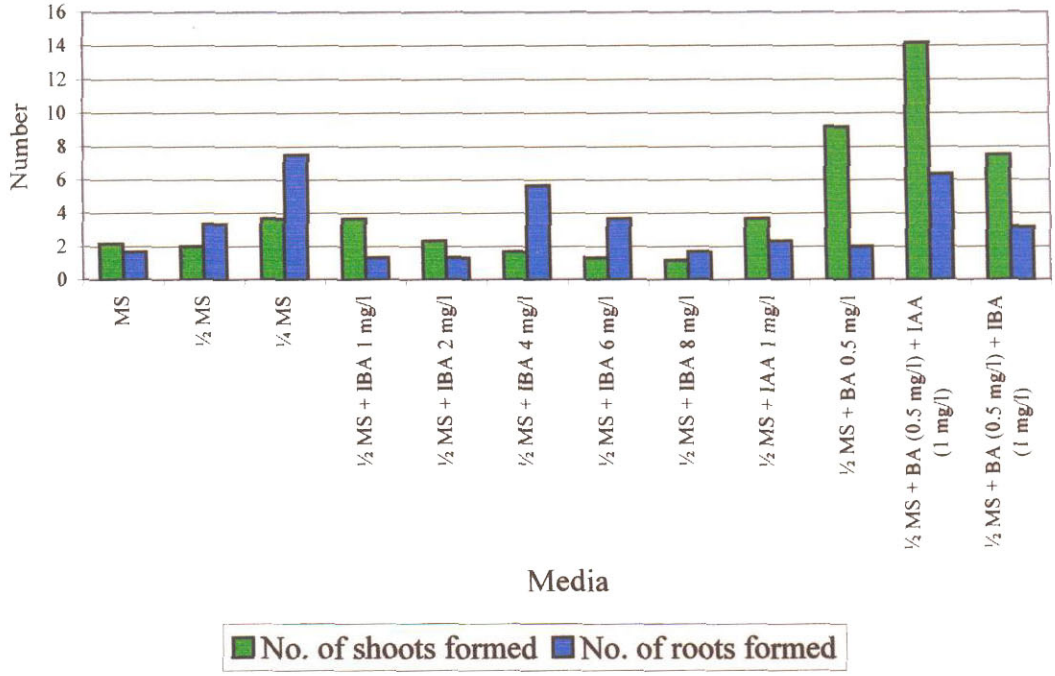


Fig. 15. Effect of growth substances on root formation

4.5.1 Irradiation of seeds

Data pertaining to the effect of irradiation on seeds are presented in Table 38.

4.5.1.1 Effect of irradiation on germination of seeds

Seeds of the cross 'Lima' x 'Red Dragon' were subjected to irradiation. Significant differences were observed among the different treatment doses. Seed irradiation reduced the germination percentage and further growth. 100 per cent germination was observed in case of 2 Gy and in control with no irradiation. Germination was 50 per cent in 14 Gy. The germination percentage gradually reduced to 40 per cent in 20 Gy.

4.5.1.2 Effect of irradiation on days taken for germination

Early germination (6.33 days) was observed in seeds with no irradiation. Seeds which were irradiated with 2 Gy dose germinated after 10.67 days which was found to be on par with 4 Gy, 8 Gy, 6 Gy and 10 Gy doses. Higher doses of irradiation increased the days taken for germination.

4.5.1.3 Effect of irradiation on number of days for leaf formation

The non irradiated treatment produced leaves early (25.33 days) which was significantly superior to the other treatments, followed by doses 2 Gy (44.67 days) and 4 Gy (49.00 days). Only three treatments produced leaves. The leaves formed in the dose 2 Gy were pale green in colour and showed a cluster type growth and no further development was observed. Dose 4 Gy showed differentiation of leaves but they later turned brown in colour. In the other higher doses leaves did not differentiate.

4.5.1.4 Effect of irradiation on size of leaf after 60 days

Significant variation was observed in the size of first leaf after 60 days among the different treatments. Maximum size was observed in the non irradiated

Table 38. Effect of irradiation on germination of hybrid seeds

Media - MS basal
Stage - Full maturity
Cross - Lima x Red Dragon

Doses gamma rays (Gy)	Percentage of cultures showing germination	Days for germination	Days for leaf formation	Size of first leaf after 60 days (mm)	Remarks
0	100	6.33	25.33	5.330	Normal growth
2	100	10.67	44.67	3.000	Leaves were pale green and formed as clusters
4	70	11.33	49.00	2.333	Leaves formed, later turned brown
6	70	11.67	-	-	No leaves formed. Cultures turned brown
8	60	11.33	-	-	,,
10	60	12.33	-	-	,,
12	60	15.67	-	-	,,
14	50	16.00	-	-	,,
16	50	15.00	-	-	,,
18	40	15.67	-	-	,,
20	40	16.33	-	-	,,
CD (0.05)		1.911	5.116	0.941	
SEm±		1.732	1.478	0.272	

treatment (5.33 mm) which was significantly superior to the others. This was followed by the treatment 2 Gy (3.00 mm) and 4 Gy (2.33 mm). Other treatments failed to produce leaves.

4.5.2 *In vitro* irradiation

4.5.2.1 Effect of irradiation at callus initiation stage

Irradiation was done with gamma rays at doses 0 to 10.0 Gy. The effects of irradiation on callus are presented in Table 39. After irradiation, the cultures were inoculated to the medium ½ MS + BA 0.5 mg l^{-1} + IAA1 mg l^{-1} for shoot regeneration.

4.5.2.1.1 Effect on percentage of cultures showing regeneration

Lower doses of irradiated callus (0.5 Gy and 1.5 Gy) as well as non irradiated callus showed 60 per cent of shoot regeneration. Fifty per cent cultures showed shoot regeneration at 2.0 Gy. At the dose 5.0 Gy, 10 per cent regeneration was observed. Higher doses reduced the percentage and no regeneration of shoots was observed from doses beyond 5.5 Gy.

4.5.2.1.2 Effect on number of shoots after 2 months

Significant differences were observed among the different treatments regarding the number of shoots after 2 months. Maximum number of shoots were formed (3.33) in case of non irradiated callus which was found to be on par with the treatments 0.5 Gy, 1.0 Gy, 2.0 Gy and 2.5 Gy. The treatment with 5.0 Gy produced 1.0 shoot.

4.5.2.1.3 Effect on number of leaves after 2 months

Highest number of leaves (6.00) was observed in non irradiated callus which was found to be on par with doses 0.5 Gy, 1.5 Gy and 1.0 Gy. The number of leaves produced was found to be reduced as the treatment dose increased.

Table 39. Effect of irradiation at callus formation stage

Cross - Lima x Eureka Red
Media - ½ MS + BA 0.5 mg/l + IAA 1 mg/l

Irradiation dose gamma rays (Gy)	Percentage of cultures showing regenerat-ion	Two months after irradiation			Remarks
		No. of shoots	No. of leaves	No. of roots	
0	60	3.333	6.000	2.667	Normal growth
0.5	60	3.000	5.667	2.333	Slow growth
1.0	50	2.667	5.333	2.000	Slow growth
1.5	60	1.667	5.667	2.333	Slow growth
2.0	50	2.333	4.333	1.333	Very slow growth
2.5	40	2.333	4.667	0.667	Light brown in colour
3.0	40	1.667	3.667	0.333	Root tip turned brown and splitting of roots also observed
3.5	30	2.000	2.333	0.667	Browning of cultures observed
4.0	20	1.333	2.333	-	,,
4.5	20	1.333	1.333	-	,,
5.0	10	1.000	1.667	-	,,
5.5	0	-			,,
6.0	0	-			,,
6.5	0	-			,,
7.0	0	-			,,
7.5	0	-			,,
8.0	0	-			,,
8.5	0	-			,,
9.0	0	-			,,
9.5	0	-			,,
10.0	0	-			,,
CD (0.05)		1.103	1.063	1.190	
SEm±		0.375	0.362	0.400	

4.5.2.1.4 Effect on number of roots after 2 months

Significant difference was observed among the different doses tried. Here also, as in the above cases, maximum number of roots was observed in non irradiated culture (2.67) which was found to be on par with 0.5 Gy, 1.5 Gy and 1.0 Gy. The root tips turned brown in higher doses above 3.0 Gy. Above 4.0 Gy, the treatments failed to produce roots.

4.5.2.2 Effect of irradiation at multiple shoot regeneration stage

Irradiation with gamma rays was done in cultures at the multiple shoot regeneration stage with doses of gamma rays at 0 to 10 Gy. After irradiation, the cultures were inoculated to the media ½ MS + NAA 2 mg l^{-1} + 2,4-D 2 mg l^{-1} + BA 6 mg l^{-1}, for producing multiple shoots. The results are presented in the Table 40.

4.5.2.2.1 Effect on percentage of cultures showing growth

Growth and differentiation of multiple shoots were observed in non irradiated cultures and irradiated cultures up to 3.5 Gy. Sixty per cent of cultures showed growth in non irradiated cultures as well as in cultures irradiated with gamma rays of 0.5 Gy dose, followed by irradiation with dose 1.0 Gy. As the dose of gamma rays increased, the percentage was found to be decreased. No growth was observed beyond a dose of 4.0 Gy.

4.5.2.2.2 Effect on number of shoots after 8 weeks

Significant differences among the treatments were observed regarding the number of shoots after 8 weeks. Non irradiated cultures produced 16.00 shoots, which was found to be on par with dose of 0.5 Gy (14.67 shoots). Gamma rays of dose 3.5 produced 3.33 shoots and beyond that dose, no shoots were produced.

4.6 PLANTING OUT AND HARDENING

The plantlets which reached plant out stage were taken out of the culture vessels. They were washed thoroughly in running water to remove all the traces of

Table 40. Effect of irradiation on multiple shoot regeneration

Media - ½ MS + NAA 2 mg/l + 2,4-D 2 mg/l + BA 6 mg/l
Stage of irradiation - Start of multiple shoot regeneration
Cross - Lima x Eureka Red

Irradiation dose gamma rays (Gy)	Percentage of cultures showing growth	After 8 weeks		Remarks
		No. of shoots	Length of longest shoot (mm)	
0	60	16.00	2.80	Normal growth
0.5	60	14.67	2.73	Slow growth
1.0	50	10.33	2.00	Slow growth
1.5	40	9.33	1.10	Very slow growth leaves pale and formed as clusters
2.0	20	9.00	1.17	Leaves whitish
2.5	30	7.33	0.63	,,
3.0	10	3.33	0.40	,,
3.5	10	3.33	0.17	,,
4.0	0	0	-	Tips of the cultures turned brown and no further growth observed later cultures turned brown fully
4.5	0	0	-	,,
5.0	0	0	-	,,
5.5	0	0	-	,,
6.0	0	0	-	,,
6.5	0	0	-	,,
7.0	0	0	-	,,
7.5	0	0	-	,,
8.0	0	0	-	,,
8.5	0	0	-	,,
9.0	0	0	-	,,
9.5	0	0	-	,,
10.0	0	0	-	,,
CD (0.05)		4.240	0.595	
SEm±		1.414	0.198	

agar and treated with a fungicide solution (Indofil 0.05%) for 10 minutes. These were planted in different media, after taking initial observations.

4.6.1 Influence of different potting media on the survival of plantlets

The plantlets were kept under 80 per cent shade net and watered regularly to maintain humidity. After one week, nutrient sprayings were also given regularly with N, P, K 30:10:10 at 0.1 per cent concentration. Survival percentage was recorded after 2 months from 20 observations. The results are presented in the Table 41 and Fig.16.

Table 41. Effect of different media on survival percentage

Potting media	Survival percentage (after 2 months)
Sand	40
Vermiculite	5
Cocopeat	2
Sand + vermiculite (1:1)	20
Sand + cocopeat + Husk pieces (1:1:1)	60
Sand + Trichoderma treated cowdung + husk (1:1:1)	60

Observations taken from mean of 20 observations

Survival percentage was found to be highest (60%) in two media, viz., sand + cocopeat + husk pieces (1:1:1) and sand + trichoderma treated cowdung + husk pieces (1:1:1). When cocopeat alone was used as the medium, the survival percentage was only two.

4.6.2 Influence of container on survival of plantlets

The plantlets were planted in different types of containers and survival percentage was recorded, after 2 months from 20 replications. Highest survival

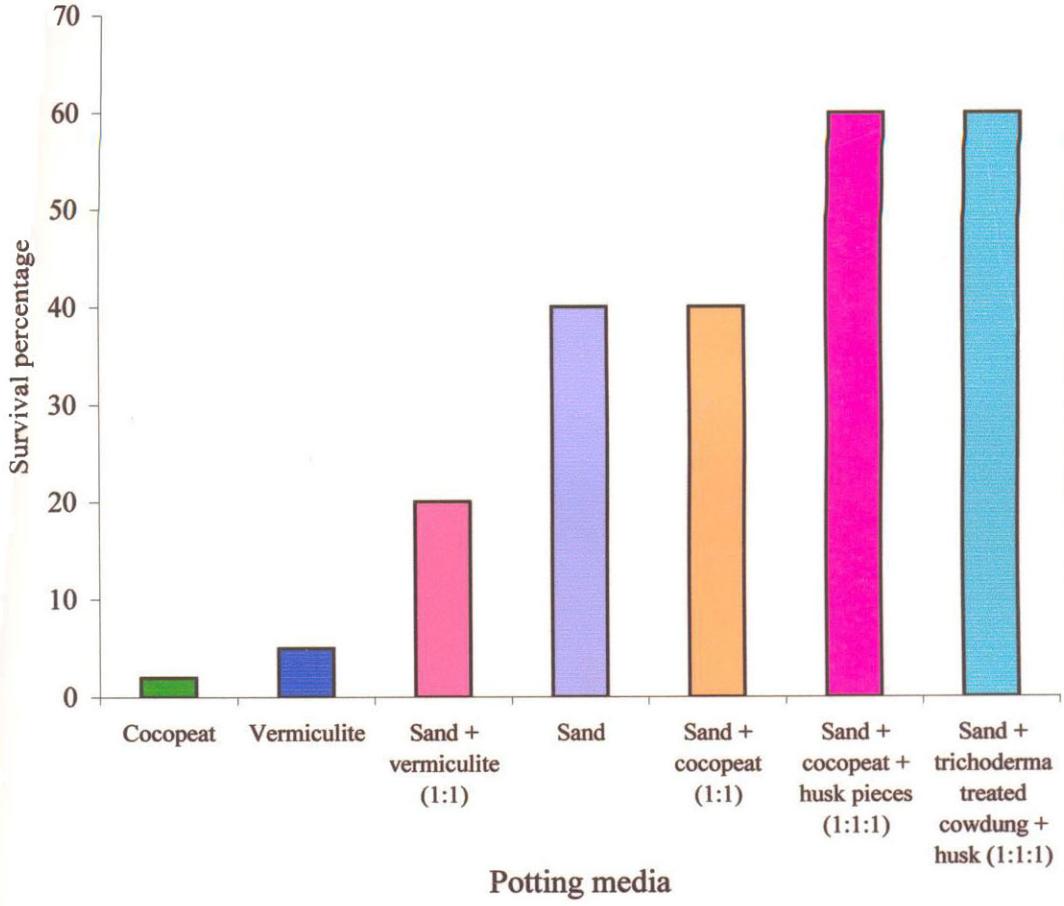


Fig. 16. Influence of potting media on survival of plantlets

percentage (60%) was observed in white plastic tea cup with holes (Table 42). This was followed by clay pot which was slightly smaller than the white plastic cup.

Table 42. Influence of container on survival percentage of hybrid seeds

Media - Sand alone

Type of container	Survival percentage
White plastic tea cup (holes made on sides and bottom)	60
Plastic thumb size pot	20
Clay pot (tea cup size)	50
Polythene cover with holes (tea cup size)	20

Percentage taken from 20 observations

The protocol developed for the production of hybrid seedlings through *in vitro* culture of immature seeds is given in the next page (Plates 6, 7, 8 and 9).

4.7 EVALUATION OF HYBRIDS

The hybrids were evaluated morphologically, cytologically and by isozyme analysis.

4.7.1 Morphological studies

Growth parameters like height of the plantlet, number of leaves, length of the largest leaf were observed at an interval of 4 months.

4.7.1.1 Vegetative characters

Growth performance of the hybrids is presented in Table 43 and Fig.17.

Highest plants were observed in Eureka Red x Lima (3.3 cm) which were on par with Candy Queen x Lima and Lima x Red Dragon (3.0 cm) at planting time.

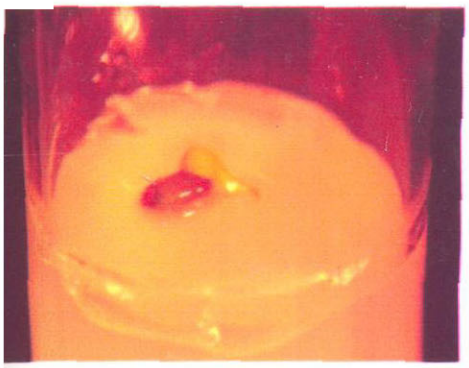
At that time, the hybrid Lima x Eureka Red possessed highest number of leaves (4.0) followed by the crosses Candy Queen x Red Dragon, Candy Queen x Agnihothri (3.3 each). The leaf size was maximum in Eureka Red x Red Dragon (1.6 cm).

The hybrid Eureka Red x Red Dragon produced highest plants (10.0 cm) after 4 months followed by the hybrid Eureka Red x Lima (9.0 cm). Lowest height was recorded in the hybrid Lima x Eureka Red. Maximum number of leaves was observed in the hybrids Lima x Eureka Red, Candy Queen x Red Dragon and Red Dragon x Nitta (6.0) after 4 months and the longest leaf was recorded in Eureka Red x Red Dragon (10.0 cm).

After 8 months, the maximum height was recorded by the hybrid Eureka Red x Red Dragon (22.0 cm) followed by the hybrid Lima x Eureka Red (18.5 cm). Plant height was minimum in Red Dragon x Lima (7.3 cm). After 8 months, the hybrid Lima x Eureka Red produced 7 leaves on an average followed by the hybrid Lima x Nitta and Red Dragon x Nitta (6.0 leaves). The hybrid Eureka Red x Red Dragon produced longest leaves (18.0 cm).

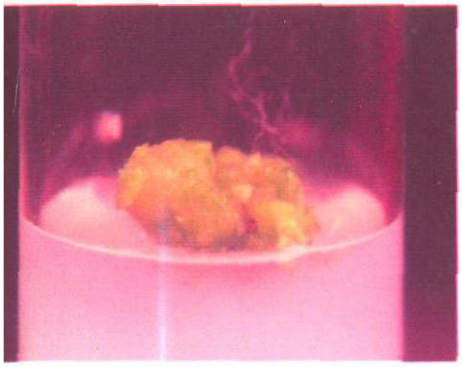
A maximum height of 32.6 cm was observed in 'Lima' x Eureka Red and least height of 9.8 cm for Red Dragon x 'Nitta' at the end of 12^{th} month. An average of 6 leaves were found in all the hybrids at about one year after planting. The 'Lima' x Eureka Red cross had a maximum of 8 leaves. Leaf size was also maximum in 'Lima' x Eureka Red (25.0 cm) at the end of 12^{th} month and it was minimum in 'Candy Queen' x 'Agnihothri' (5.0 cm).

Hybrids of 'Lima' x 'Eureka Red' produced flowers 11 months after planting and those of 'Candy Queen' x 'Lima' and 'Lima' x 'Red Dragon', 12 months after planting.



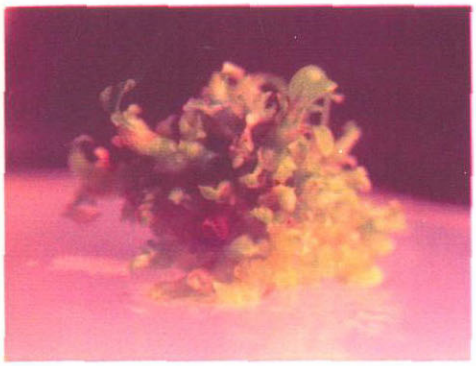
Seed germination

Callus initiation

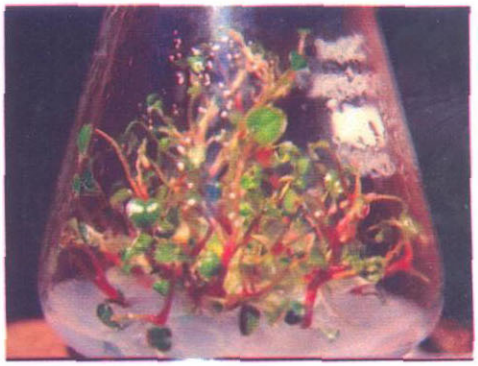


Callus multiplication

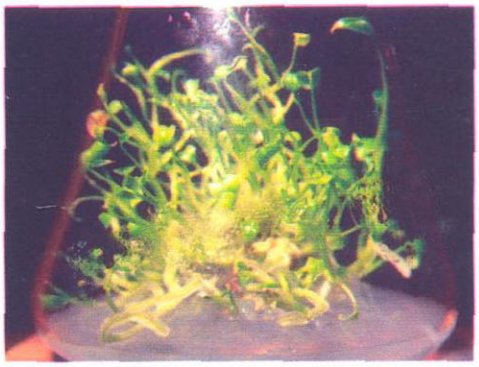
Callus multiplication



Shoot regeneration



Multiple shoot formation



Root formation

Plate 6. Seed inoculation and further development

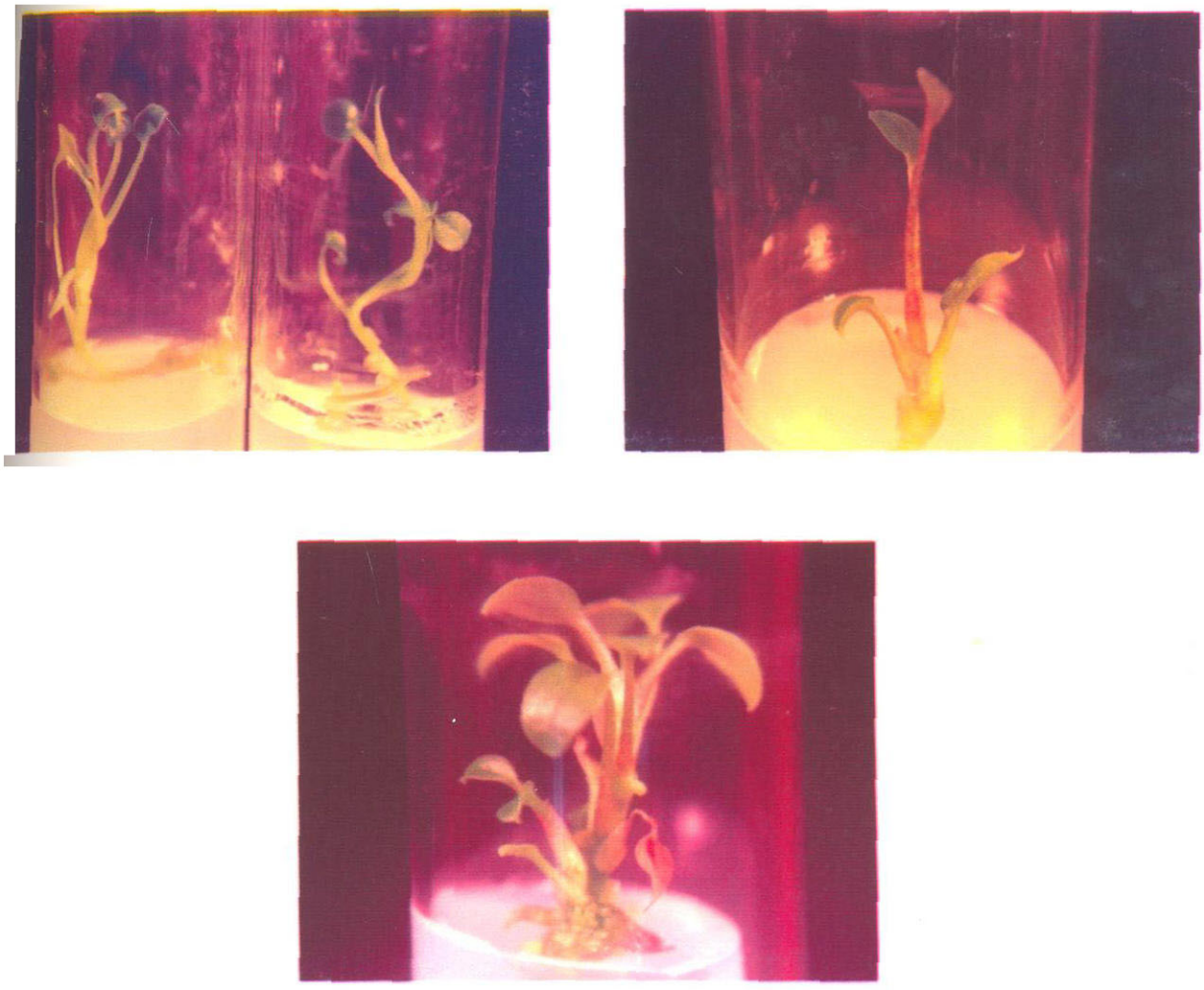


Plate 7. Plantlets ready for planting out

Plate 8. Hybrid seedlings in community pots

Plate 9. Planting out and hardening

Table 43. Growth performance of hybrids in the field

Hybrid	At planting			4 months after planting			8 months after planting			12 months after planting		
	Plant height (b) (cm)	Number of leaves	Length of largest leaf (cm)	Plant height (cm)	Number of leaves	Length of largest leaf (cm)	Plant height (cm)	Number of leaves	Length of largest leaf (cm)	Plant height (cm)	Number of leaves	Length of largest leaf (cm)
Nitta x Lima	2.0	2.6	0.8	6.2	5.0	2.8	13.0	5.0	5.8	15.5	5.3	5.8
Candy Queen x Lima	3.0	3.0	0.90	6.0	5.0	5.0	11.3	5.0	10.0	25.0	6.2	20.0
Candy Queen x Red Dragon	2.6	3.3	0.9	5.8	6.0	2.0	9.5	5.0	4.8	20.1	5.7	18.2
Candy Queen x Agnihothri	2.0	3.3	0.8	6.3	5.0	2.5	8.3	4.8	4.6	10.5	4.3	5.0
Lima x Nitta	2.0	3.0	0.9	6.0	5.0	2.5	13.0	6.0	8.0	16.0	5.7	12.0
Lima x Red Dragon	3.0	3.0	1.5	6.5	5.0	3.5	13.0	5.2	9.0	26.0	6.2	20.0
Lima x Eureka Red	2.5	4.0	0.70	4.2	6.0	1.2	18.5	7.0	12.0	32.6	8.0	25.0
Lima x Agnihothri	2.3	3.0	0.9	6.0	5.0	2.2	8.3	4.8	5.6	10.2	4.3	5.2
Red Dragon x Nitta	2.6	2.6	0.70	5.0	6.0	4.8	8.0	6.0	5.0	9.8	5.0	5.5
Red Dragon x Lima	2.0	3.0	0.8	5.2	5.0	4.6	7.3	4.6	5.6	10.2	4.3	5.8
Red Dragon x Eureka Red	2.3	3.0	0.9	6.5	4.3	3.8	12.5	4.6	6.0	14.0	5.7	6.8
Eureka Red x Lima	3.3	3.3	1.2	9.0	5.0	4.5	13.0	5.0	9.8	18.5	5.0	15.0
Eureka Red x Red Dragon	2.8	3.0	1.6	10.0	4.0	10.0	22.0	4.0	18.0	29.0	5.6	22.0
CD (0.05)	0.57	0.57	0.30	0.73	1.56	0.64	1.37	1.38	0.80	2.49	1.33	2.29
SEm ±	0.21	0.21	0.10	0.26	0.52	0.22	0.49	0.48	0.29	0.88	0.48	0.82

Average of observations taken on 6 plants

Hybrids of 'Lima' x 'Eureka Red' and 'Lima' x 'Red Dragon' produced suckers, one year after planting.

4.7.1.2 Floral characters

During the period of study, 3 plants from 3 hybrids namely Lima x Eureka Red, Lima x Red Dragon and Candy Queen x Lima had flowered. Data pertaining to the floral characters of the hybrids which flowered are presented in Table 44 and Plate 10. Variations could be observed in floral characters.

'Lima' x 'Eureka Red' hybrid took 24 days from spike emergence to spathe unfurling, whereas hybrid of 'Lima' x 'Red Dragon' and 'Candy Queen' x 'Lima', took 28 days.

The longest flower stalk was observed in 'Lima' x 'Eureka Red' hybrid and in 'Candy Queen' x 'Lima' hybrid (35 cm). The hybrid 'Lima' x 'Red Dragon' had 27 cm long stalk. The spathe length was maximum (8.3 cm) in the hybrid of 'Lima' x 'Red Dragon' and minimum (7.0 cm) in 'Candy Queen' x 'Lima'. Width of the spathe was found to be highest (7.0 cm) in 'Lima' x 'Eureka Red' and it was the lowest (5.5 cm) in 'Candy Queen' x 'Lima'.

Shortest spadix was seen in the hybrid 'Candy Queen' x 'Lima'. The angle of the spadix to the spathe was also found to be less in this cross. The angle was found to be wider in hybrid 'Lima' x 'Red Dragon'.

Description of hybrids

The hybrid from 'Lima' x 'Eureka Red' flowers had a stalk length of 35 cm and spathe size of 8 x 7 cm. It had a spadix of 4.5 cm at an angle of 45° to the spathe. The spathe is pinkish red and spadix, orange yellow in colour.

In the hybrid derived from 'Lima' x 'Red Dragon', flower stalk was 27 cm long, spathe size of 8.3 x 6 cm. The spadix was long (6 cm) inclining at an angle of 50° to the spathe. The hybrid had a light red coloured spathe with green colouration towards the basal lobes. Spadix is orange in colour.

The hybrid 'Candy Queen' x 'Lima' had 35 cm long stalk with 7 x 5.5 cm sized spathe. It had a short spadix of 4.0 cm length was inclined at 40° to the spathe. The spathe and spadix were red in colour.

4.7.2 Cytological studies

Mitotic chromosome counts were made on the root tip cells in all the six varieties and the hybrids of *Anthurium andreanum.* Somatic chromosome number of 30 + 2B was recorded. Very little abnormalities were recorded.

4.7.3 Isozyme studies

Leaves were selected as source of enzyme extraction. Six varieties and four hybrids were screened for variation. The results revealed that there was remarkable variation for peroxidase and SOD isoenzymes among the varieties selected as parents and the hybrid plants. No results were obtained with catalase and esterase isoenzymes.

4.7.3.1 Peroxidase activity

Bands were present in all the parent varieties and hybrids (Fig.18 and Plate 11). All samples showed a similar band with almost equal Rf value ranging from 0.544 to 0.563. The varieties 'Nitta' and Eureka Red exhibited only one band. The variety 'Lima' had one band Prx 6 with Rf value 0.607 in addition to the bands with Rf values 0.408 and 0.544.

Table 44. Floral characters of hybrids in the field

Hybrids	Time taken from planting out to flowering (months)	Time taken from spike emergence to unfurling (days)	Stalk length (cm)	Size of spathe		Size of spadix (cm)	Angle of spadix to spathe	Colour of spikes
				Length (cm)	Width (cm)			
Lima x Eureka Red	11	24	35	8.0	7.0	4.5	45°	Pinkish red spathe. Orange yellow spadix
Lima x Red Dragon	12	28	27	8.3	6.0	6.0	50°	Light red coloured spathe with green colour towards the base. Orange spadix
Candy Queen x Lima	12	28	35	7.0	5.5	4.0	40°	Red spathe with red spadix

Lima x Red Dragon

Lima x Eureka Red

Candy Queen x Lima

Plate 9. Hybrid flowers

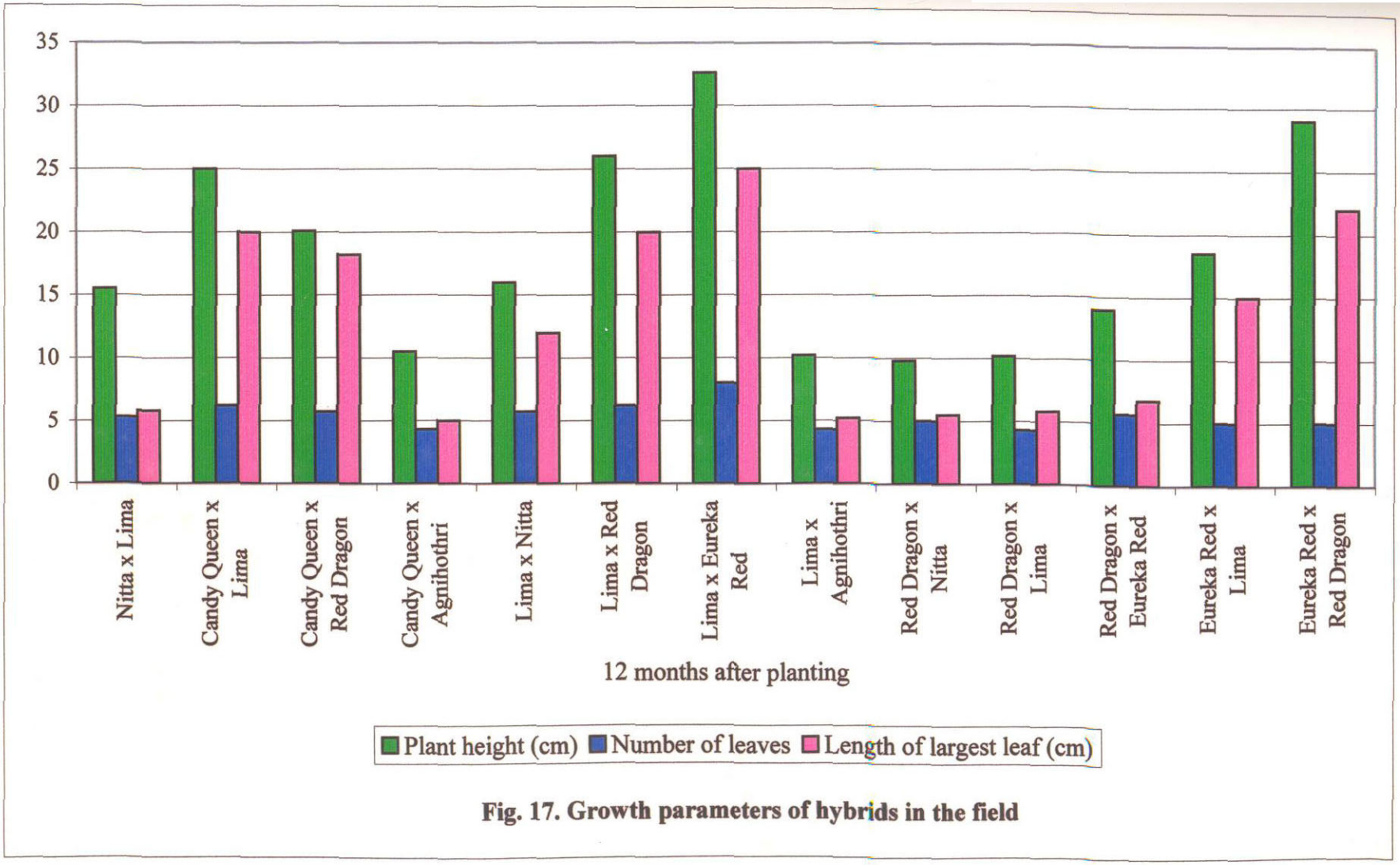


Fig. 17. Growth parameters of hybrids in the field

Protocol for immature hybrid seed culture in *Anthurium andreanum*

Explant
(Immature seeds of 130 days maturity)

↓

Mercuric chloride 0.1% 10 minutes

Surface sterilization

↓

Inoculation

½ MS + BA 1 mg l^{-1} — 1 week

↓

Germination

½ MS + BA 1 mg l^{-1} — 3 weeks

↓

Callus initiation

½ MS + BA 1 mg l^{-1} — 6 weeks

↓

Callus multiplication

½ MS + BA 0.5 mg l^{-1} + IAA 1 mg l^{-1} — 4 weeks

↓

Shoot regeneration

½ MS + BA 6 mg l^{-1} + NAA 2 mg l^{-1} + 2,4-D 2 mg l^{-1} — 8 weeks

↓

Shoot multiplication

½ MS + BA 0.5 mg l^{-1} + IAA 1 mg l^{-1} — 4 weeks

↓

Rooting

Indofil 0.05% 10 minutes

↓

Planting out in plastic tea cup with holes

Sand + Trichoderma treated cowdung + husk (1:1:1) — 8 weeks

↓

Repotting in earthern pots (15 cm diameter)

Sand + Trichoderma treated cowdung + husk (1:1:1) — 12 weeks

↓

Repotting in earthern pots (25 cm diameter)

Sand + Cowdung clumps + Coconut husk + Earthern crocks (2:1:1:0.5) — 30 weeks

↓

Flowering

The hybrids 'Lima' x 'Eureka Red' produced 2 bands. One band was common in both the parents (Prx-12 with Rf value 0.549). The other band Prx-13 was present in the parent 'Lima' with almost similar Rf values. The band with Rf value of 0.408 which was present in 'Lima' was absent in the hybrid.

The hybrid from the cross 'Eureka Red' x 'Red Dragon' exhibited one band, Prx-14 with Rf value 0.553 which was common in 'Red Dragon' and 'Eureka Red'. The band Prx-8 with Rf value 0.417 present in 'Red Dragon' was absent in the hybrid.

The common band present in both parents 'Candy Queen' and 'Lima' was also present as Prx-15 with Rf value 0.534 in the hybrid from 'Candy Queen' x 'Lima'. The other bands of the parents were absent in the hybrid. But the hybrid had one additional band Prx-16 with Rf value 0.568.

The hybrid 'Lima' x 'Nitta' produced one band Prx-17 with Rf value 0.549 which was similar to their parents. The hybrid was lacking the other 2 bands of 'Lima', but possessed an additional band Prx-18 with Rf value 0.573.

4.7.3.2 SOD activity

The bands were present in three varieties and two hybrids (Fig.19 and Plate 12). The variety 'Nitta' produced only a single band SOD 1 with Rf value of 0.383. The variety 'Red Dragon' produced 4 bands and 'Agnihothri' produced 3 bands. The band SOD 6 with Rf value of 0.359 was almost similar to the band SOD 2 of the variety 'Red Dragon'. The other bands were positioned in different places and had different Rf values.

The hybrid from 'Candy Queen' x 'Lima' had two bands SOD 9 and SOD 10 with Rf values 0.762 and 0.791 respectively, even though their parents produced no bands.

The hybrid from 'Lima' x 'Nitta' produced three bands which were different from those of their parents. The band SOD 13 with RF value of 0.077 was almost similar to that of the hybrid with parents 'Candy Queen' x 'Lima'.

Fig. 18. Zymogram of peroxidase enzyme activity in anthurium comparing varieties and hybrids

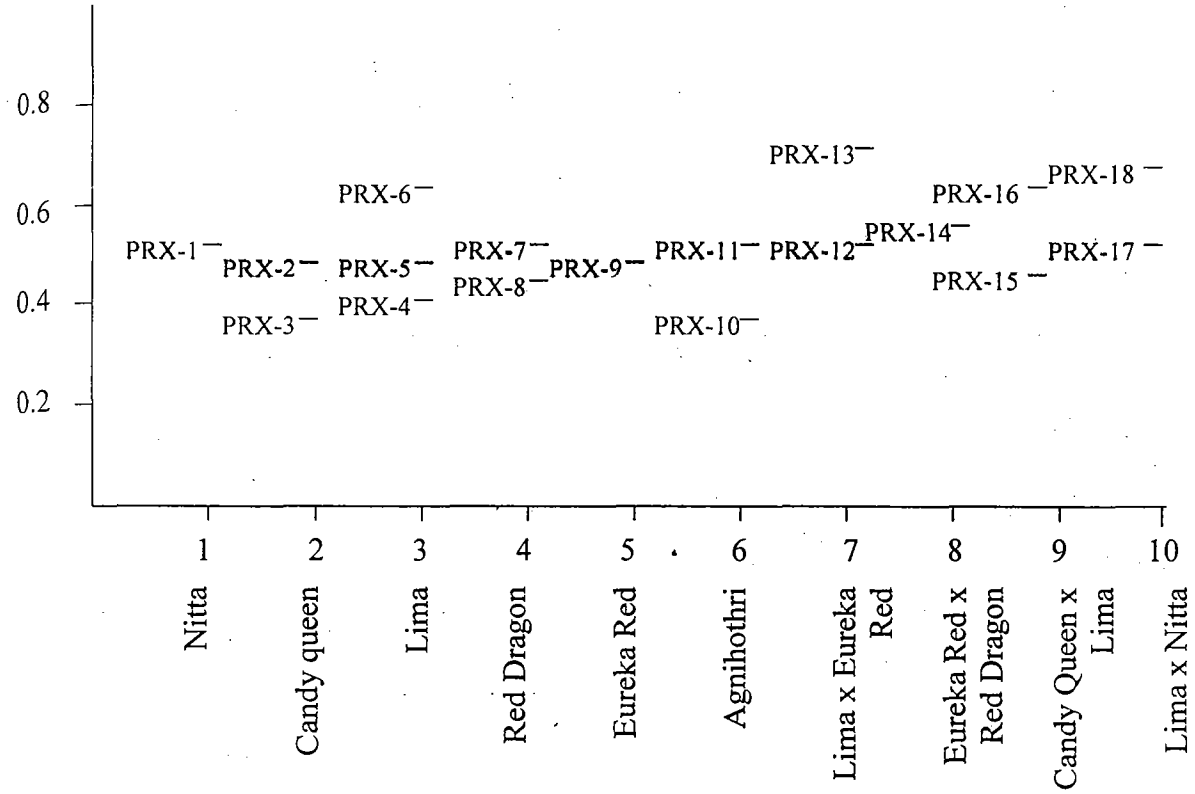
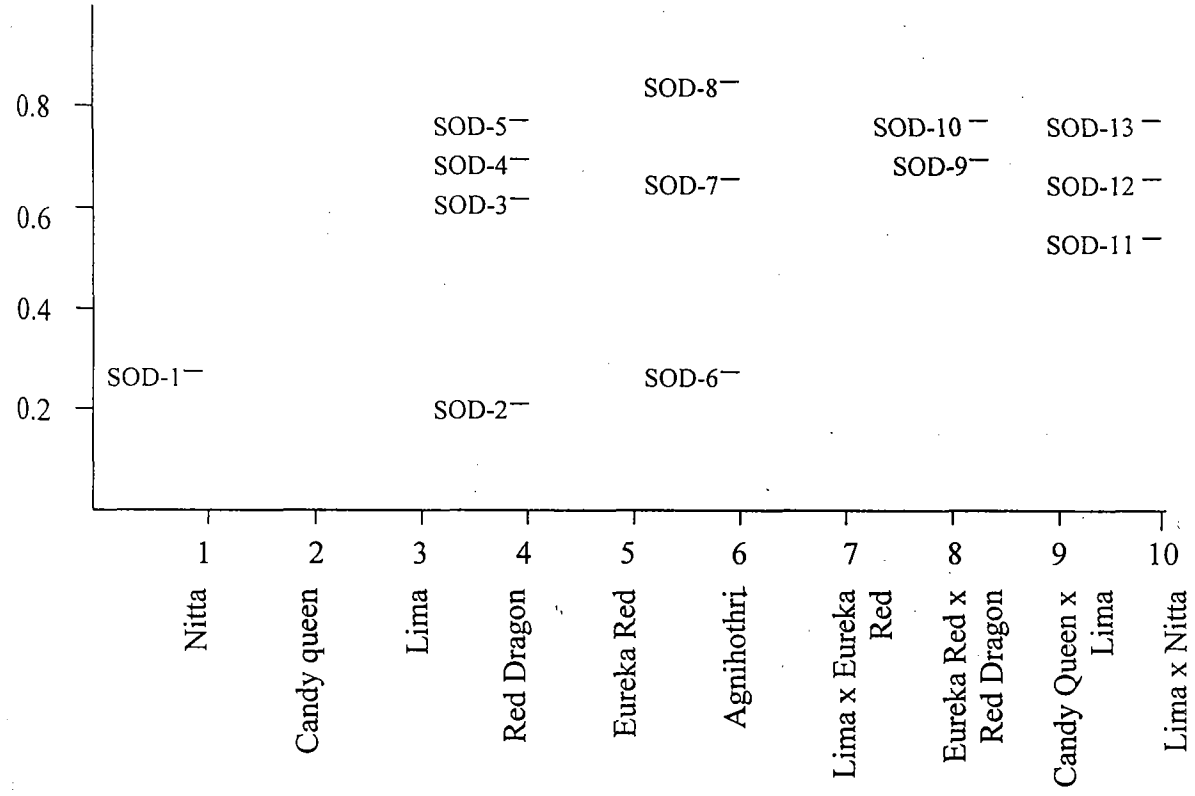


Fig. 19. Zymogram of SOD activity in anthurium comparing varieties and hybrids



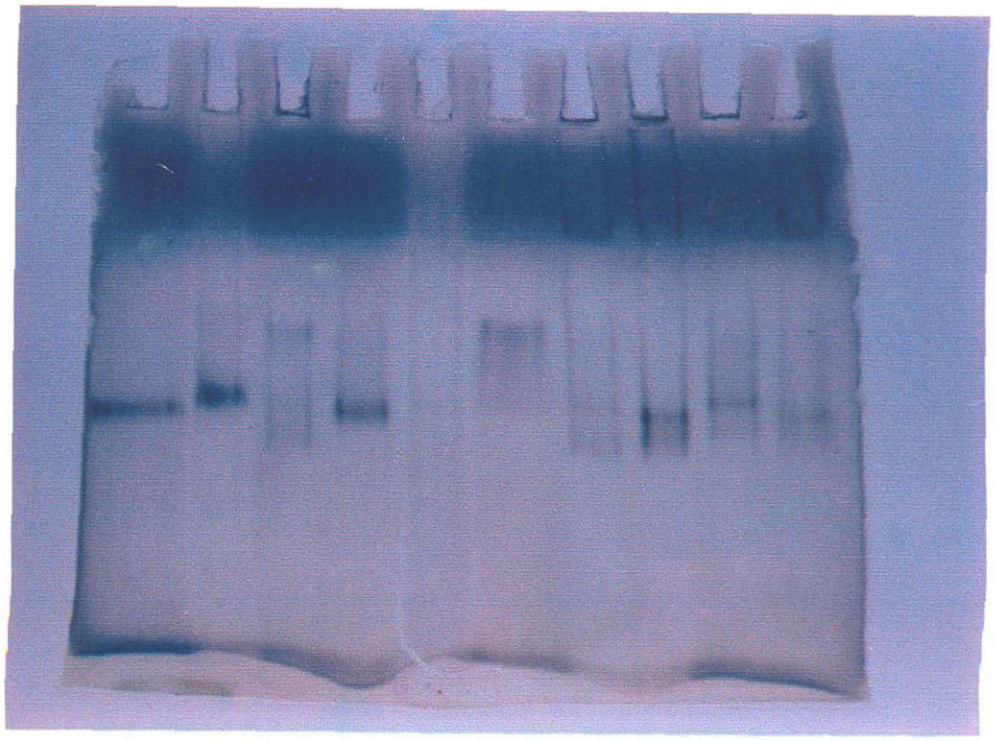


Plate 11. Peroxidase activity of *A. andreanum* varieties and hybrids

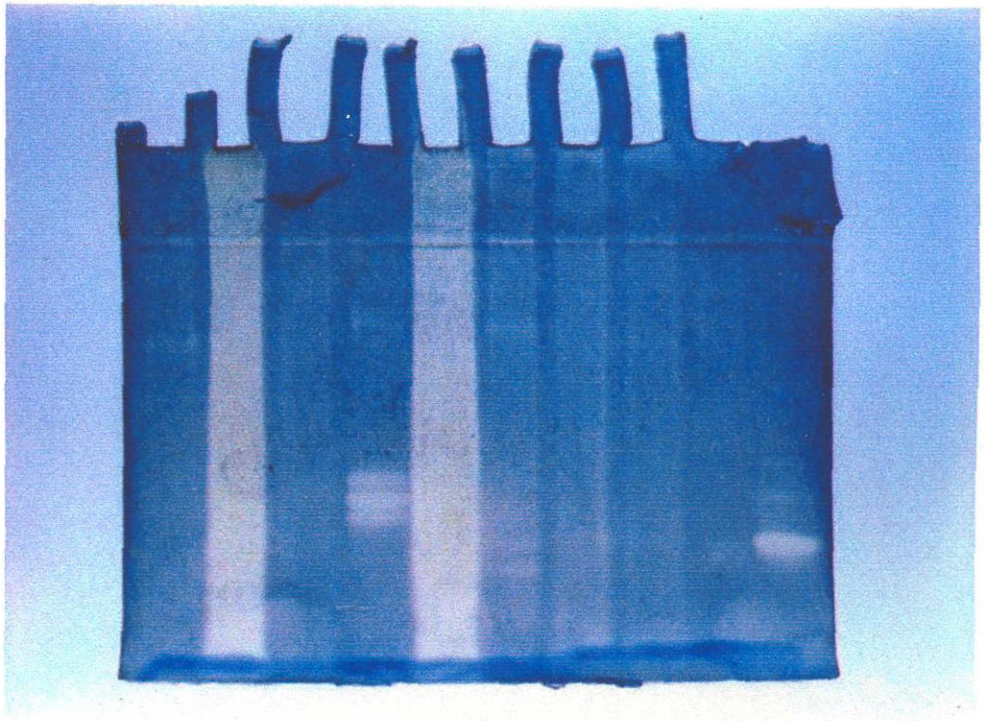
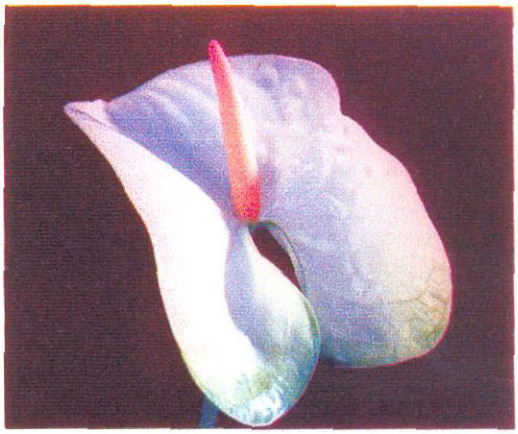


Plate 12. SOD activity of *A. andreanum* varieties and hybrids

Discussion



5. DISCUSSION

A. andreanum is an economically important cut flower crop which is known for its bewitchingly beautiful flowers. The present study was undertaken with the objective of improving *Anthurium andreanum* varieties. Information were collected on varietal characters, correlations among characters, pollen characters and compatibility among species and varieties. Standardization of the maturity of seed for *in vitro* seed culture, refinement of medium for immature seed culture and *in vitro* mutagenesis were also undertaken. Hybrids were evaluated on the basis of their growth performance and isozyme analysis. This chapter deals with the brief discussion on the findings in the light of the work already done.

5.1 CHARACTERIZATION

5.1.1 Vegetative characters

The morphological characters of parent varieties help in understanding the variability that exists among them. The varieties under the present study showed significant variation with respect to vegetative characters.

The variety 'Lima' was tallest (19.22 cm) followed by 'Nitta' (13.56 cm) and the varieties 'Red Dragon' and 'Agnihothri' possessed the least height (4.22 cm). Tisdale *et al.* (1985) reported that plant height can be used as an index of plant growth. Significant variation in plant height of different varieties of anthurium was reported by Bindu and Mercy (1994), Sindhu (1995), Renu (1999) and Rajeevan *et al.* (2002).

Internodal length also gives an indication of plant height. Internodal length was more in varieties 'Lima' (3.67 cm) and 'Nitta' (3.11 cm). These varieties were tall and tend to topple down which requires staking. Varieties with short internodes are desirable. The variety 'Red Dragon' had the shortest internode (1.73 cm).

The varieties differ with regard to the ability to produce suckers. Higaki and Rasmussen (1979) reported that some varieties produce suckers readily while others had to be stimulated to produce suckers. According to Mercy and Dale (1994)

most of the good commercial and hybrid varieties are shy suckering or did not sucker at all. In the present study, suckering was recorded in all varieties, but the varieties differed in the ability to produce suckers. Highest number of suckers was recorded for 'Red Dragon' (3.22) and 'Agnihothri' had the least number (2.33). In commercial cultivation, suckers are not retained in the plants. Since they affect the growth and flower production. Suckers are to be removed at the earliest stage possible.

5.1.2 Floral characters

The present investigation revealed that one spike was produced from the axil of each leaf and the sequence of leaf, flower and new leaf was maintained throughout the life of the plant. The close correlation between the number of leaves and the number of flowers was reported by Gajek and Schwarz (1980). But the varieties differed in the annual production of leaves and flowers (spikes).

The annual production of spikes was more in the variety 'Eureka Red' which produced 8.9 spikes followed by the variety 'Red Dragon' (8.0) and it was least in the variety 'Nitta' which produced 6.5 spikes.

Higaki and Rasmussen (1979) reported six to eight new leaves and buds per year on a stem axis in *Anthurium*. Mercy and Dale (1994) observed five to eight leaves and spikes per year whereas it was four to eight according to Sindhu (1995). The number of spikes produced by the varieties studied under this investigation ranged from 6.5 to 8.9. Year round flowering was obtained in all the varieties. Flowering also depends upon the intensity of light, temperature, nutrition, watering and other practices like use of growth regulators pruning of leaves and removal of suckers.

Spathe size is an important character which determines the value of the cut flower. The largest spathes were produced by the variety 'Candy Queen' (17.67 cm length and 13.33 cm width each) followed by the variety 'Lima'. 'Red Dragon' and 'Eureka Red' had the smallest spathes (12.33 and 11.89 respectively). According to the classification proposed by Mercy and Dale (1994) the varieties 'Candy Queen' and 'Lima' comes under super large. The varieties 'Nitta' and 'Agnihothri' had large

spathes while the varieties 'Red Dragon' and 'Eureka Red' had medium sized spathes. None of the varieties produced small, mini and micro sized flowers. Renu (1999) had reported medium sized spathes for 'Nitta Orange' (23.5 cm length + width of spathe). In the present study, 'Nitta' had a spathe size of 25 cm which comes under the class large (25-29 cm). The difference in flower size may be due to the difference in age. Higake and Poole (1978) had reported increase in flower size with aging.

The differences in spathe size of different varieties were also reported by Sindhu (1995), Bindu and Mercy (1994) and Henny (1999).

Short spadix reclining to the spathe is a desirable feature for anthurium flowers. Here the shortest spadix was observed in 'Red Dragon' (6.22 cm) which was on par with 'Eureka Red' and 'Nitta'. The longest spadix was observed in 'Lima' (9.11 cm). The spadix length ranged from 6.22 cm to 9.11 cm. Renu (1999) had also reported short spadix for the variety 'Nitta'. The five varieties studied by Bindu and Mercy (1994) showed a spadix length ranging from 4.0 cm to 9.5 cm.

High spathe spadix ratio is a desirable character which indicates bigger spathes with smaller spadices. The spathe spadix ratio was highest in the variety 'Nitta' (2.15) followed by 'Candy Queen' (2.06). A high spathe spadix ratio for 'Nitta' was also reported by Renu (1999). The varieties studied in this experiment showed a range from 1.75 to 2.15. The six varieties studied by Sindhu (1995) showed a range in spathe spadix ratio from 2.00 to 2.86.

Ideal anthurium varieties should have a short spadix curving towards the tip of the spathe and held at an angle less than 45° for convenience in packing. Such ideal position of spadix with an angle of 40° was observed in only one variety, 'Candy Queen'. The widest angle was recorded in 'Nitta' (65°) followed by 'Eureka Red' (60°) and 'Agnihothri' (60°).

The peduncle of spike was found to be straight in all the varieties except in 'Candy Queen', where it was bending and leaning towards the ground, which may affect the quality of the flower.

Varying degrees of smoothness and blistering were reported in *A. andreanum* by Birdsey (1956), Mercy and Dale (1994) and Renu (1999). Among the varieties studied, spathes of Red Dragon, 'Lima' and 'Eureka Red' were found to be with less blisters, whereas 'Nitta', 'Candy Queen' and 'Agnihothri' had more blisters. Chlorophyllous pigmentation at the base of the lobes of spathe was observed in the varieties 'Lima' and 'Red Dragon'. This variety was reported as an obake variety by Rajeevan *et al.* (2002).

The spadix colour was light pink in 'Red Dragon' and light yellow in all other varieties. According to Mercy and Dale (1994), the spadix had a single colour (red, pink or cream) in ordinary anthurium varieties whereas hybrids had yellow, white, pink or red colours in two or more bands. Sindhu (1995) reported that the spadices of six varieties studied by her had either a single colour or two or more bands of colours. Henny (1999) observed that the *Anthurium* hybrid 'Red Hot' had a spadix which was orange red apically blending to red basally. Spadix and spathe with contrasting colours are valued in the international market.

If the size of the plant increased, the flower size also was found to increase. Beyond a particular height of the plant, the spathe length remained more or less same with no significant variation. In varieties, 'Red Dragon' and 'Eureka Red', the flower quality (colour and thickness of spathe) was adversely affected when the size increased. No difference was noticed for the other varieties. Higaki and Poole (1978) had also reported increase in flower size with aging.

Seasonal variation was noticed in the flowering behaviour of *A. andreanum* varieties. Most varieties ('Nitta', 'Lima', 'Eureka Red' and 'Agnihothri') produced highest number of flowers during the hot months from February to April. The variety 'Candy Queen' produced more number of flowers (2.3) during August to October and 'Red Dragon' produced 2.3 flowers during both, May to July and August to October. The flower production was found to be very low during the months from November to January in all the varieties. The relative humidity during that period was low (November 81%, December 72% and January 76% in the morning). Long flower production interval was observed during that period in all the varieties studied.

5.1.3 Flowering pattern

Flowers of *Anthurium* are bisexual and are closely congested and arranged in a series of spirals on the spadix (Croat and Bunting, 1978). An individual flower has 4 colourless perianth segments arranged in a four sided configuration which envelop 4 stamens with 4 loculed anther. Pistil is cylindrical and bicarpellary. As the pistil develops a stout style exerts to expose a receptive stigma. The structure of anthurium true flowers was explained by Croat (1980). Paull (1982) reported that in *Anthurium* species flower maturation started from the basal portion and proceeded regularly towards the apex. The maturity of the flowers initiated from the basal portion. All the varieties studied were protogynous and attained female fertility within 7 days of unfurling of spathe. An interphase of 3 to 20 days was found in between female phase and male phase, which prevents self pollination.

Studies conducted by Bindu and Mercy (1994) and Mercy and Dale (1994) revealed the protogynous nature of *A. andreanum* varieties. Observation in the present study also confirms this.

The duration from spike emergence to unfurling of spathe was maximum in the variety 'Agnihothri' (25.33 days) whereas the varieties 'Lima' and 'Candy Queen' took only 19.00 days. The number of days from unfurling of spathe to initiation of female phase was observed to vary from 0 to 7 days. Dichogamy exists in anthurium where female flowers opened first and initiation of female phase was identified by the slight projection by stigmas and presence of viscous exudate on the spadix. In the variety 'Nitta', female phase was initiated on the same day of unfurling of spathe, whereas the variety 'Lima' took 4 to 7 days for the initiation of female phase. According to Renu (1999) the number of days from the day the spadix became visible to initiation of female phase was observed to vary from 3.6 to 6.8 days. A period of 4-7 days after the opening of the spathe to the initiation of female phase was also reported by Mercy and Dale (1994).

The female receptivity ranged from 12.00 days in the variety Red Dragon to 20.33 days in Eureka Red. According to Croat (1980) the duration of female phase

may range from half a day to as long as 28 days. The duration was reported to be three to twelve days by Bindu and Mercy (1994) and as three to seven days by Mercy and Dale (1994).

A brief interphase between female phase and male phase was observed in all the varieties, which ranged from 3.67 days to 20 days. The variety Red Dragon had the shortest interphase period while 'Lima' had the longest.

Croat (1980) observed that the duration of interphase was several days in most *Anthurium* species, whereas in a few of them the time lag was very short. Interphase of four to seven days was noticed by Bindu and Mercy (1994). Sindhu (1995) stated that interphase lasted for four to ten days. Interphase of 4.8 to 10.2 days was recorded by Renu (1999).

Like female phase, the male phase also started from the base of the spadix and proceeded upwards. The male phase was identified by the appearance of stamens on the spadix. The average male phase ranged from 9.33 days in the variety 'Lima' to 20.67 days in Red Dragon. Croat (1980) reported that in some *Anthurium* species, the male phase lasted for several weeks. In a study by Bindu and Mercy (1994), the male phase ranged from 3 to 7 days. They also opined that the anthesis and anther dehiscence are favoured by moderately low temperature and high relative humidity. Sindhu (1995) reported that male phase lasted for 3 to 8 days depending on the variety. The present study showed a longer male phase than those reported by others. Renu (1999) reported a slightly longer male phase (5.4 days to 10.4 days). The difference obtained in the study might be due to varietal variation as well as due to the change in climate and location. Chen-JungBin *et al.* (1999) also reported the variation in pollen shedding habits of different varieties.

The present study revealed that the longevity of unfertilized spikes on the plant ranged from 43 days in Agnihothri to 90 days in the variety 'Lima'. According to Paull (1982) the non reversible visible changes accompanying the senescence of *Anthurium* flowers were loss of spathe gloss, necrosis of spadix and greening of spathe

and spadix. Mercy and Dale (1994) noted that the senescence was marked by yellowing of peduncle and withering of spathe and spadix which took nearly 4 to 7 months from the emergence of young spadix. The longevity, varied from 2.5 months in 'Nitta Orange' to 3.7 months in 'Ceylon Red', in the case of unfertilized spadices according to Renu (1999).

In case of fertilized spadices the days taken to attain seed maturity differed among the varieties. Shortest (145 days) period was recorded in the variety 'Red Dragon' and longest (208.3 days) in 'Agnihothri'. A range of 3.8 months to 7.5 months for seed maturity was reported by Renu (1999) and 4.0 to 7.0 months by Mercy and Dale (1994).

5.1.4 Pollen studies

Pollen morphology is a useful tool for classifying plants (Erdtman, 1952 and Nair, 1970). Tarasevich (1989) studied pollen grains of 34 *Anthurium* species and reported that different sections of *Anthurium* were heterogeneous for pollen grain morphology.

Pollen grains of all the varieties and species studied were more or less round in shape. Largest (25.4 µ) pollen grain was observed in the variety 'Agnihothri' and smallest in 'Red Dragon' and *Anthurium amnicola* (16.6 µ). Bindu (1992) reported pollen grain size of 81.8 x 68.0 µ in 'Pink' and 87.2 x 86.4 µ in 'Lady Jane'.

The most effective and most suitable test for pollen viability is staining with acetocarmine. In the present study, acetocarmine staining method was used to find the pollen fertility of the selected varieties. The highest pollen fertility was observed for *Anthurium crystallinum* (69.8%) followed by 'Agnihothri' (64.6%). Lowest pollen fertility was recorded in 'Lima' (16.6%). Mitu and Acatrinei (1974) reported that the germination of pollen grain was proportional to pollen grain stainability as acetocarmine preferentially stains the chromosome or nucleus. Lalithambika (1978) observed that the pollen sterility of *A. andreanum* varied from 70-75 per cent.

Bindu and Mercy (1994) reported that the pollen fertility in *A. andreanum* varied from 20.4 per cent to 28.8 per cent. Renu (1999) reported that pollen fertility ranged from 14 per cent in 'Lady Jane' to 42 per cent in 'Liver Red'.

In the present study also all varieties, except 'Agnihothri' (64.6%) and 'Red Dragon' (50.2%) showed low pollen fertility. The high sterility of the *A. andreanum* varieties may be due to their hybrid nature as the sterility is a condition frequently associated with hybridity.

The pollen emergence pattern of the selected varieties was studied for a period of 2 years, from May 1999 to April 2001. The study revealed significant difference among the varieties. During 1999, pollen emergence was best during the months of May to August.

During 2000, pollen emergence started late (from July), may be due to the low humidity and high temperature recorded during the year comparing with that of the year 1999. During the month of May, 1999 the temperature was 30.7°C (maximum) and relative humidity was 92 per cent (morning). But during May 2000 the temperature was 33.7°C (maximum) and relative humidity was 88 per cent (morning). In most of the varieties, the pollen emergence started in July and extended to October. No pollen emergence was observed during the months October to April, in general. In 'Nitta', the pollen emergence was low in both the years.

Suppression of pollen production during the hot months of March to June was reported by Renu (1999) which is in close confirmity with the results obtained in this experiment. Generally when the temperature is higher, the anther emergence has been found to be suppressed. The optimum temperature for *A. andreanum* has been reported to be between 18°C and 28°C (Rajeevan *et al.*, 2003). Sindhu (1995) also observed suppression of male phase from March to August.

No pollen production was observed in the variety 'Candy Queen' and *A. ornatum* and very low pollen production in 'Nitta'; may be due to the sensitivity of the varieties to the temperature and humidity prevailing in this area.

Singh (1990) also observed that the details of anthesis vary with crop species and are affected by environmental factors such as humidity and temperature.

5.1.5 Post harvest studies

Inflorescences were harvested when 1/3 flowers opened on the spadix. Inflorescence harvested at this stage lasted for a longer period (Salvi, 1997).

Senescence is generally associated with the plugging of stem vascular tissues accompanied by the loss in weight, visible changes including spathe gloss loss, necrosis of spadix blueing of spathe, stem collapse and abscission of spathe and spadix from the stem (Akamine, 1976).

Considering the overall performance of the varieties, 'Eureka Red' was found to be the best in terms of vase life showing delayed fading (17.67 days), spathe necrosis (18.00 days) and spadix necrosis (16.33 days), which was followed by the varieties 'Red Dragon' and 'Lima'. In term of vase qualities, the inferior varieties were 'Candy Queen', 'Agnihothri' and 'Nitta'. A similar trend was noticed in the case of longevity in packing also. In general, spikes with thick or medium thick spathes exhibited long life in vase as well as in packing.

5.2 VARIATION AND CORRELATION STUDIES

The total variability was partitioned into heritable and non heritable components with the help of genetic parameters like genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance, which help in selection. GCV provides a valid basis for comparing and assessing the range of genetic diversity for quantitative characters and PCV measures the extent of total variation.

In general, PCV was slightly higher than GCV in most of the characters indicating the influence of environment. The apparent variation is not only due to genotypes but also due to the influence of environment. In all the characters, the

differences between GCV and PCV were very low. These small differences point out that the environmental influence on these characters is less.

Plant height recorded the highest PCV (70.57%) and GCV (69.96%), followed by male phase (PCV 63.82% and GCV 58.39%) indicating a greater extend of variability for these characters, thereby suggesting scope for improvement of these characters through selection. Reduced plant height is a desirable character in anthurium (Prasad *et al.*, 1998), which can be exploited efficiently and effectively for bringing considerable improvement through selection. Extension of male phase will be highly desirable since availability of pollen grains is a prerequisite in hybridization. Lower values of PCV and GCV were estimated for the characters length of spathe (15.15% for both PCV and GCV)), interval of flower production (16.82% and 11.68%, respectively) and length of spadix (17.39% and 13.69%, respectively) indicating low magnitude of variability.

Assessing heritability is valuable in any plant breeding programme, since it provides basis for selection on phenotypic performance. Heritability estimates the transmissibility of characters from one generation to other and it provides a measure of the value of selection for different attributes. But high heritability does not necessarily mean a high genetic advance for a particular character (Allard, 1960). Heritability along with genetic advance is more useful than heritability alone in predicting the resultant effect of selecting the best individuals (Johnson *et al.*, 1955).

In the present study, heritability was of moderate to high magnitude for most of the characters. Highest heritability was exhibited by the character flower stalk length (99.21%) followed by plant height (97.43%), longevity of spike on plant (89.74%), length of spathe (81.86%), width of spathe (85.61%) and duration of male phase (84.00%). These characters also showed relatively higher genetic advance values, which are controlled by additive gene action and hence selection may be effective.

Length and width of spathe and length of flower stalk together constitute the size of flower which enhance the cut flower value of anthurium in the market as these are important traits desired by the end user. These characters can be improved through selection. High heritability for the characters like spathe size, plant height and days to initiation of female phase was reported by (Sindhu, 1995).

In a cut flower like anthurium, the economic characters are length of spathe, width of spathe, stalk length and longevity. These are generally complex in nature and influenced by many factors. Improvement of the above economic characters is possible, only by knowing the association of various characters. Characters genetically related to each other tend to move in the same direction under selection. Such a correlated response to selection is the basic property of quantitative traits under the control of polygenic system. The genotypic correlation between characters provides a reliable measure of genetic association between the characters and helps to differentiate the vital association useful in breeding from the non-vital ones (Falconer, 1981).

In the present study, the plant height was positively correlated with the stalk length, length of spathe, width of spathe, length of spadix and longevity of spike on the plant. Selection for good stalk length spathe size and longevity of spike on the plant may also be associated with undesirable traits like increased height and long spadix.

A significant and positive association between length of spathe and width of spathe was observed, both at phenotypic (0.818) and genotypic (0.897) level. Both these characters contribute to flower size which is an important criterion in commercial varieties. The flower size (length and width of spathe) exhibited high positive correlations with the plant height and length of flower stalk. The study also revealed that the length of spadix was positively correlated with length and width of spathes.

The number of suckers was negatively correlated with most of the characters like plant height (-0.547 phenotypically and -0.551 genotypically) and

spathe size (-0.098 both phenotypically and genotypically). This indicate that the taller plants with large flowers will have less number of suckers. The Association of large spathe with less suckering explains the reason for low sucker production in commercial varieties.

Duration of male phase and female phase are negatively correlated (-0.193 phenotypically and -0.226 genotypically) with each other and also negatively correlated with most of the other characters like plant height and size of spathe and spadix. Female phase is positively correlated with the longevity of spike, which is an important character. Bigger plants with longer flowers will have shorter male and female phases.

5.3 HYBRIDIZATION AND COMPATIBILITY STUDIES

Hybridisation was done in all possible combinations. A total of 387 crossings were done which included 42 of the 54 possible combinations. The responses of varieties to crossing differed. Pollination involving the variety 'Candy Queen' and *A. ornatum* as pollen parents could not be attempted as no pollen emergence was observed in them during the period of study.

Kamemoto and Nakasone (1955) suggested hybridisation and selection as the common methods for improving anthurium. Both interspecific and intraspecific hybridisations were used by early anthurium breeders. Birdsey (1956) attributed much of the variations in blistering pattern of spathes of *A. andreanum* to interspecific hybridisation. Sheffer and Kamemoto (1976a) evaluated the interspecific cross compatibilities among 56 species of *Anthurium* and they concluded that interspecific hybrids with *A. andreanum* and *A. scherzerianum* were not readily obtainable. But they got hybrids of *A. andreanum* with six other closely related species. Sheffer and Kamemoto (1977) recorded good cross compatibility between *A. andreanum, A. concinnatum, A. haffmani, A. linderianum, A. micromysterium, A. nymphaefolium* and *A. pinchinchae*. Kamemoto and Sheffer (1978) reported a new species hybrid from the cross *A. scherzerianum* x *A. wendlingerii*. An interspecific hybrid 'Southern

Blush' was produced by Henny *et al.* (1988) by crossing a large pink flowered *A. andreanum* cultivar with *A. amnicola*. Attempts to transfer systemic resistance from *A. antioquiense* to cultivated *A. andreanum* were done by Kuehnle *et al.* (1995) and they produced resistant F_1 hybrids. An interspecific hybrid 'Red Hot' which originated from hybridisation of *A. amnicola* Dressler with an unnamed selection of *A. andreanum* (accession code G.79) was described by Henny (1999). One of the resulting F_1 hybrids was again crossed with 'Lady Jane' to produce the progeny from which 'Red Hot', a miniature type was selected. Henny and Norman (2001) also described a new interspecific hybrid 'Show Biz' which produced numerous attractive light red spathes.

Compatibility analysis was done on the basis of three important parameters such as percentage of spadices bearing fruits, percentage of fruit set per spadix and percentage of germination of seed.

In this experiment, interspecific pollinations were done with *A. andreanum* as the female parent. All the selected *A. andreanum* varieties were crossed with the pollen from the *A. crystallinum* and *A. amnicola.* Among all the crosses, the only interspecific cross which was found to set seeds was *A. andreanum* var. Red Dragon x *A. amnicola.* Even though this cross produced seeds, they failed to germinate. In general, the interspecific crossings done (12 combinations) in this experiment were incompatible and failed to set seeds except the combination Red Dragon x *A. amnicola* where only one spadix set seeds among the 7 crossings done and one per cent seed set per spadix was obtained which failed to germinate later.

Among the cross combinations attempted, it was evident that the variety 'Lima' had the maximum cross compatibility, followed by 'Candy Queen' and 'Red Dragon'. The variety 'Agnihothri' was compatible only with the variety 'Lima'. The seed set percentage was very low for 'Agnihothri' but the seedlings did not survive later which showed that the variety could not be considered as a good female parent. 'Nitta' was found to be compatible with 'Lima' only, but the seed set percentage was high and 40 per cent germination also obtained.

Among all the combinations, the variety 'Lima' had the largest number of compatible crosses, high seed set percentage and high germination percentage and hence it can be regarded as a good female parent. The varieties 'Candy Queen', 'Eureka Red' and 'Red Dragon' can also perform well as good female parents.

Hundred percentage seed set was recorded for the crosses 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon'. Cent per cent germination of seeds was obtained for the cross 'Candy Queen' x 'Lima' and this cross again recorded maximum per cent of (21%) double seeded berries. Occurrence of double seeded berries in anthurium was earlier reported by Zimmer (1986). Highest percentage of seed set was recorded (44%) in 'Lima' and lowest (5%) in 'Agnihothri'. Hybridisation work by Sheffer and Kamemoto (1976a) revealed 81.0 per cent fruiting spadices through self pollination, 65.4 per cent through intraspecific crosses and 28.0 per cent through interspecific crosses. Renu (1999) studied 10 varieties and obtained 22.50 per cent fruit set in selfings and 31.06 per cent in crossings.

Studies conducted by Sindhu (1995) revealed that a large number of combinations were incompatible. In the present study also, most of the crosses were unsuccessful including all the selfings, which were found incompatible. Here only 14 crosses were found to be unsuccessful which set seeds and produced seedlings. The combination 'Candy Queen' x 'Lima', 'Lima' x 'Red Dragon', 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon' with highest compatibility score (8) are expected to produce commercially valuable hybrids as all these varieties have desirable commercial characters. The male parents involved in these crosses namely 'Lima', 'Red Dragon' and 'Eureka Red' were the best compatible male parents among the varieties and species studied.

The percentage of fruit set per spadix was maximum in the cross 'Candy Queen' x 'Lima' and it was minimum for 'Red Dragon' x 'Lima' among the successful crosses. Absence of full fruit set in spadix was identified as a major problem in the development of *Anthurium* cultivars by Zimmer (1986). Renu (1999) also reported less than 50 per cent fruit set per candle in most of the varieties.

Among the crosses, the duration from the day of opening of spathe to the day of berry ripening ranged from 135 days in 'Candy Queen' to 210 days in 'Agnihothri'. Similar duration for fruit ripening was observed by Sindhu (1995) and Renu (1999). The duration for fruit maturity in anthurium was recorded as 6-8 months by Singh (1987), 6-7 months by Geier (1987), 5-12 months by Zimmer (1986) and as 4-7½ months by Mercy and Dale (1994). Pierik *et al.* (1974) opined that breeding of *A. andreanum* was handicapped by the long period from fertilization to ripening of seeds (6-7 months). Kuehnle *et al.* (1995) concluded that production of horticulturally desirable varieties took many years since it is a perennial crop, with a long juvenile phase and slow seed germination.

Compatibility among the varieties based on the performance as pollen parents was also analysed. Of all the male parents tried, the variety 'Eureka Red' produced highest percentage of berries per spadix as pollen parent. The percentage of germination of seeds was highest when 'Red Dragon' was used as pollen parent, followed by 'Lima'. Based on the overall performance as pollen parents, the most successful varieties were 'Eureka Red', 'Red Dragon' and 'Lima'. *A. crystallinum* had high pollen fertility but the crosses were incompatible with *A. andreanum* varieties.

Among the compatible crosses, 'Agnihothri' had a pollen fertility percentage of 64.6 but had a poor seed set.

Out of the 17 successful combinations, the crosses with highest compatibility score of 8 were 'Candy Queen' x 'Lima', 'Lima' x 'Red Dragon', 'Lima x 'Eureka Red' and 'Eureka Red' x 'Red Dragon'. In general considering all the cross combinations, involving the six female parents, 'Lima' appeared to be the most compatible, showing the best performance with a total compatibility score of 28 with 4 successful crosses. It could be considered as a good female parent. The variety 'Candy Queen' appear to be the next best female parent with a total score of 22 with 4 successful crosses. The varieties 'Red Dragon' (score 20) and 'Eureka Red' (score 15) also performed well as female parents. The varieties 'Nitta' and 'Agnihothri' showed lowest compatibility score of 7 from a single surviving cross with the variety 'Lima'.

Comparing the performance of male parents, the most successful variety was 'Lima' with a total compatibility score of 31 from 5 successful cross combinations, followed by the variety, 'Red Dragon' with a score of 23 from 3 successful combinations. The variety 'Eureka Red' also performed well with a compatibility score of 18. The variety 'Lima' performed well both as female parent and male parent.

Hybridisation undertaken between varieties of *A. andreanum* and also between species of *Anthurium* resulted in a few successful hybrid combinations. The incompatibility might have occurred because of the failure of fertilization or failure of zygotic survival. Fertilization might have failed because of the inability of pollen tube to reach the embryosac and consequent nonavailability of sperms for fertilization. Failure of zygotic survival might be due to the presence of lethal genes, genotypic disharmony between genomes, chromosome elimination, cytoplasmic incompatibility or endosperm abortion.

The varieties used in this study were found self incompatible. The self incompatibility might be due to the failure of pollen grains to germinate, pollen grains failed to enter the stigma or due to the embryo degeneration at a very early stage after fertilization.

5.4 *IN VITRO* STUDIES AND IMMATURE SEED CULTURE

Depending upon the variety, anthurium took 5-7 months for maturity of seeds on the plant. This time lag could be substantially reduced if seedlings could be produced by the *in vitro* culture of seeds isolated from the immature berries. In this experiment, seeds from 70 days maturity onwards were taken from the two crosses, 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon' and cultured in MS and Nitsch media. The results revealed that the seeds obtained from 'Lima' x 'Eureka Red' germinated from 100 days of maturity in MS media and from 90 days in Nitsch media. The germination percentage was low (7 to 20%) upto 110 days. Hundred percentage germination was obtained from seeds of 120 days of maturity. Seeds of 120 days maturity took 12-18 days for germination and of 130 days, just 5-11 days. For full ripening of berries in this cross, it took 180 days after flower opening.

In the case of seeds from 'Eureka Red' x 'Red Dragon', germination was observed from 80 days of maturity in both the media. Hundred per cent germination

was obtained in 130 days of maturity, which took 5-8 days for germination. For full ripening of berries it took 170 days after flower opening.

For seed germination in sterile sand in petri dishes, Bachthaler (1978) suggested reddish (half ripe) and red (ripe) stage of maturity of seeds as the most suitable. Szendel *et al.* (1982) obtained best germination in high peat substrate at early maturity stage (yellow green to light orange). Post pollination investigation carried out by Matsumoto *et al.* (1998) in *A. andreanum* cv. 'Kalapana' revealed that the development from a single celled zygote to fully mature seed took 24 weeks. Differentiation of the shoot apex, cotyledon and protoderm occurred at 14 weeks. By 20 weeks, shoot apex had visible leaf primordia and the root apex was clearly defined. According to them the cotyleon was well developed and 100 per cent germination occurred at 20 weeks and thereafter. In general the present investigation revealed that the seeds can be germinated *in vitro* 40-50 days before field maturity with almost 100 per cent germination.

Different chemicals were used for surface sterilization of anthurium seeds. Results revealed that maximum survival percentage was obtained in the treatments Bavistin 0.1 per cent for 30 minutes + mercuric chloride 0.1 per cent for 10 minutes and also in the treatment mercuric chloride 0.1 per cent. These treatments showed no blackening. Use of mercuric chloride 0.1 per cent for surface sterilization in anthurium explants has also been reported by Anu (1998) with initial treatment with Bavistin 0.1 per cent.

All the basal media tried in this experiment as well as the combination of basal media with growth substances responded well. Early leaf formation was observed in the combination MS + BA 0.5 mg l^{-1}. After the germination of seeds, fast growth and multiplication was recorded when BA was added to the basal media. Early development of callus was observed in ½ MS supplemented with 1 mg l^{-1} BA. The basal media alone could not produce further development of callus or multiple shoot. The same media combination (½ MS + 1 mg l^{-1} BA) produced multiple shoots earlier than the other media. According to Murashige (1974) BA has been the most effective cytokinin for meristem, shoot tip and bud cultures, followed by kinetin. Sreelatha (1992) also reported that kinetin 2.0 mg l^{-1} and BA 1.0 mg l^{-1} were equally effective in inducing multiple shoots.

Benzyl adenine alone and in combination with 2,4-D and NAA showed callus initiation and growth of the callus. Maximum callus development was recorded in the combination in which higher concentrations of BA with lower concentrations of auxins like 2,4-D and NAA were used. The effect of 2,4-D and BAP on callus initiation has been reported by Kuenhle and Sugii (1991). According to Sreelatha (1992), treatments with BA and 2ip, produced callus growth.

Devinder Prakash *et al.* (2001) and Atta-Alla *et al.* (1998) reported the use of seeds of *Anthurium* germinated *in vitro* as a source of explants. According to Devinder Prakash *et al.* (2001), MS medium supplemented with 1.0 or 0.5 mg l^{-1} 2,4-D induced callus. Highest callus production from seeds with 2.0 mg l^{-1} 2,4-D was reported by Somaya *et al.* (1998). Malhotra *et al.* (1998) observed callus induction in *A. andreanum* on Nitsch medium supplemented with BA 1 mg l^{-1} 2,4-D 0.1 mg l^{-1} and ammonium nitrate 200 mg l^{-1}.

The treatment combination, ½ MS + BA (0.5 mg l^{-1}) + IAA (1 mg l^{-1}) recorded maximum shoot regeneration. This combination also showed multiple shoot formation. The effect of BA and IAA on multiple shoot formation has also been reported by Sreelatha (1992). Increase in number of shoots in a medium containing 2 mg l^{-1} BA and 0.2 mg l^{-1} NAA was reported by Atta-Alla *et al.* (1998).

In the present study, regarding the number of shoots and size of shoots, the combination ½ MS with BA (6 mg l^{-1} + NAA (2 mg l^{-1}) + 2,4-D (2 mg l^{-1}) was found to be the best.

Different growth substance combinations were tried for rooting. Early rooting was observed in ½ MS with BA (0.5 mg l^{-1}) + IAA (1 mg l^{-1}). The same combination produced more number of shoots also in addition to roots. The media ¼ MS alone produced maximum number of roots. Both these media recorded highest percentage of cultures showing roots. Auxin is essential for root initiation and widely used auxins for rooting were NAA and IBA. But in this experiment, IAA was found to be the best for root initiation. Mahanta and Paswan (2001) also observed 80 per cent of rooting in MS medium supplemented with IAA 1.0 mg l^{-1}.

Though auxin is considered essential for rooting, better rooting in a medium free of plant growth substances was reported by Nair *et al.* (1984) and Maria and Segura (1989).

5.5 IRRADIATION STUDIES

Ionising radiations can interact with cells to produce genetic effect. Genetic diversity can be introduced with the hope of obtaining spectacular types in ornamental crops like anthurium. It has been reported that tissue culture greatly facilitates the application of mutagenesis to tissues or cells (Nickel, 1973).

Ionising radiation has been found to be a good mutagen. It can interact with the cells to produce a genetic effect in the immediate vicinity of its ionisation track (Muller, 1954).

In the present study, gamma rays upto 20 Gy was tried on mature seeds. The germination percentage decreased from 100 to 50 in doses 0 to 14 Gy. But leaf formation was noted up to 4 Gy. Leaves appeared to be pale green in colour. Irradiation reduced the germination percentage and further growth. It also reduced the leaf size. The lower doses of gamma rays 0.5 Gy and 1.5 Gy showed 60 per cent shoot regeneration when callus was irradiated as in non irradiated callus. Shoot production, leaf production and root production were observed in lower doses of gamma rays. The doses beyond 2.0 Gy showed slow growth and browning and beyond the dose 5.0 Gy, no growth was observed.

In the case of irradiation on multiple shoot regeneration stage, upto the dose 3.5 Gy, shoots were produced. Beyond that no further growth was observed and the cultures turned brown. Lower doses produced more number of shoots and pale green and clustered leaves.

5.6 PLANTING OUT AND HARDENING

The plantlets were washed, treated with a fungicide solution and planted in different media and kept under the shade net. Two media recorded highest survival percentage. They were sand + coco peat + husk pieces (1:1:1) and sand + trichoderma treated cowdung + husk (1:1:1). Sand alone produced 40 per cent survival. According to Sreelatha (1992) sand was the best potting medium for planting out. Geier (1989)

also observed that anthurium plantlets could be established without losses in a part or sand media. Cocopeat along with FYM or leaf mould was recommended by Jawaharlal *et al.*, 2001.

Among the different containers tried, white plastic tea cup with holes made on sides and bottom was found to be the best, which showed a survival percentage of 60 per cent, followed by clay tea cup. A survival rate of 60 per cent was reported by Mahanta and Paswan (2001) when the *in vitro* plantlets were transferred to plastic pots with soilrite-perlite (10:1) medium.

5.7 EVALUATION OF HYBRIDS

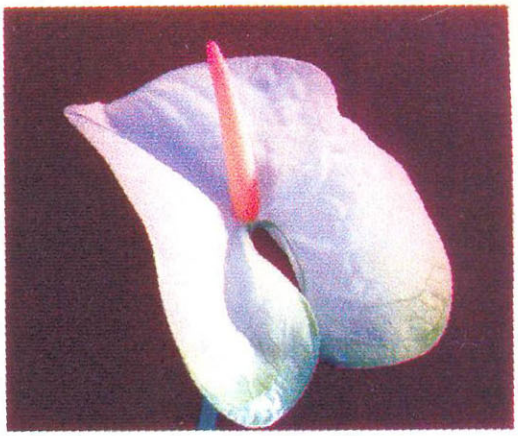
Regarding the growth of the hybrids, an average of 6 leaves was found in all the hybrids at about one year after planting. Better growth was observed in the plantlet from the cross 'Lima' x 'Eureka Red' which flowered at 11th month after planting. This also produced suckers. Flowering was observed in 'Candy Queen' x 'Lima' at 12th month of planting. 2½ to 3 years was reported to be required for the majority of seedlings to flower. When immature seeds were cultured in vitro and produced plantlets, these plantlets could be transferred to the field by 6 to 7 months. These plantlets flowered by 12 months. So in total from hybridization to flowering, it took only 22 to 24 months. But in conventional method, it takes 30 to 35 months.

Even though the floral characters on the first spike were not an indication of the actual performance of the plant, colour variations were noticed in the hybrid flowers.

Chromosome counts of the root tip cells in all the varieties of *A. andreanum* and their hybrids, showed a somatic chromosome number of 2n = 30+2B. This is in conformity with the earlier observations of Bindu (1992) and Balachandran (1998) in different varieties of *A. andreanum*. Sheffer and Croat (1983) reported that the most common somatic number of the genus anthurium is 30 and B chromosomes are basic features of the genus *Anthurium*.

In this experiment four enzymes were studied but only two were found to be effective in studying the variation. Earlier studies on electrophoresis on anthurium revealed that out of the seven enzymes studied peroxidase was the most useful isoenzyme system for distinguishing cultivars (Kobayashi *et al.*, 1987). In the present study also peroxidase was found to be the most stable, and was expressed in all parent varieties and hybrids. Data on peroxidase activity suggested that there was significant difference among the various varieties and hybrids regarding the banding pattern. Variation in banding pattern was also observed with the enzyme SOD.

Summary



6. SUMMARY

Investigations on improvement of *Anthurium andreanum* by *in vivo* and *in vitro* methods were carried out at the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during the period from 1998 to 2001. The main objectives were to evolve new varieties of *Anthurium andreanum* and to standardise the age of seed and media for successful *in vitro* seed culture. The results and salient findings are summarized here.

For the study, six commercially important varieties of *A. andreanum*, viz., 'Nitta', 'Candy Queen', 'Lima', 'Red Dragon', 'Eureka Red', 'Agnihothri' and three other species of *Anthurium*, viz., *A. crystallinum, A. ornatum* and *A. amnicola* were used.

Characterisation of the varieties was done and the results showed significant variation with respect to the morphological characters. The variety 'Lima' was the tallest (19.22 cm), with long internodes (3.67 cm). The shortest varieties were 'Red Dragon' and 'Agnihothri' (4.22 cm each). Bigger leaves were produced by the varieties 'Candy Queen' (681.3 cm^2), 'Agnihothri' (579.4 cm^2) and 'Lima' (556.8 cm^2). The variety 'Red Dragon' produced highest number of suckers (3.22) and least by the varieties 'Lima' and 'Eureka Red' (1.22).

One inflorescence each was produced from the axil of each leaf. Year round flowering was obtained in all the varieties. The varieties 'Eureka Red' (8.9) and 'Red Dragon' (8.0) produced more number of spikes per year. The production of flowers was least in the variety 'Nitta' (6.5). The productivity of flower spikes ranged from 6 to 9 in the varieties studied. Seasonal variation was noticed in the flowering behaviour of *A. andreanum*. Most varieties ('Nitta', 'Lima', 'Eureka Red' and 'Agnihothri') produced the highest number of flowers during the hot months from February to April. The flower production was very low during the months from November to January in all the varieties.

The largest spathe was produced by the variety 'Candy Queen' (17.67 cm x 13.33 cm) and the varieties 'Red Dragon' (12.33 cm x 8.00 cm) and 'Eureka Red' (11.89 cm x 8.44 cm) had the smallest spathes. Short spadix observed in 'Red Dragon', 'Eureka Red' and 'Nitta' (6.22, 6.78 and 6.89 cm, respectively) is ideal for a good anthurium variety. Peduncle of spike was straight in all the varieties, except 'Candy Queen', where it was bending towards the ground. Spathes were blistered and glossy in 'Nitta', 'Candy Queen' and 'Agnihothri' and smooth and thick in 'Lima', 'Red Dragon' and 'Eureka Red'. Green colouration at the basal lobes of the spathe was noticed in 'Red Dragon' and 'Lima'.

All the varieties studied were protogynous and attained female fertility within 7 days of unfurling of spathe. The female receptivity phase ranged from 12.00 days in the variety 'Red Dragon' to 20.33 days in 'Eureka Red'. An interphase of 3 to 20 days between female phase and male phase was observed in all the varieties, which prevented self pollination.

Like female phase, male phase also started from the base of the spadix and proceeded upwards. The male phase ranged from 9.33 days in the variety 'Lima' to 20.67 days in 'Red Dragon'. Longevity of spike on the plant ranged from 43 days in 'Agnihothri' to 90 days in 'Lima', in case of unfertilized spikes. In case of fertilized spikes, time taken to attain seed maturity was shortest in 'Red Dragon' (145 days) and longest in 'Agnihothri' (208 days).

Pollen grains of all the varieties were more or less round in shape. Largest pollen grains were observed in the variety 'Agnihothri' (25.4 μ) and highest pollen fertility in *Anthurium crystallinum* (69.8%). Smallest pollen grains were produced by the variety 'Red Dragon' and *A. amnicola* (16.6 μ) and pollen fertility was lowest in 'Lima' (16.6%). Pollen production was low in variety 'Nitta' and no pollen production was observed in the variety 'Candy Queen' and in *Anthurium ornatum*. Pollen emergence was absent during the months October to April.

In terms of post harvest qualities, the varieties 'Eureka Red', 'Red Dragon' and 'Lima' were found to be superior, both in vase and in packing. In vase, spathes

retained colour for longer period in varieties 'Eureka Red' (17.67 days) and 'Red Dragon' (15.67 days) and no colour change was observed in the variety 'Lima'. Longer periods for necrosis of spathe and necrosis of spadix were also exhibited by the varieties, 'Eureka Red', 'Red Dragon' and 'Lima' (18.00, 17.67 and 17.33 days respectively for spathe necrosis and 16.33, 14.67 and 14.67 days respectively for spadix necrosis).

The magnitude of variation and the heritability estimates were made. PCV was slightly higher than GCV in most of the characters studied, indicating the influence of environment also. The small differences point out that the variations observed are mainly due to genetic reasons and environmental influence is less. Among the 10 characters studied, plant height recorded the highest PCV (70.57) and GCV (69.96), suggesting scope for improvement of this character through selection, as reduced plant height is a desirable character in anthurium.

Heritability was of moderate to high magnitude for most of the characters. Highest heritability was exhibited by the character flower stalk length (99.21%) followed by plant height (97.43%), longevity of spike on plant (89.74%), length of spike (81.86%), width of spike (85.61%) and male phase (84.00%). These characters also showed relatively higher genetic advance values, and can be improved through selection.

A significant and positive association between length of spathe and width of spathe was observed both at phenotypic and genotypic level (r_p = 0.818. r_g = 0.897). These characters also exhibited high positive correlation with the plant height and length of flower stalk. Thus selection for taller plants will help in obtaining flowers with longer stalks and increased flower size.

Hybridisation was done in all possible combinations. Out of these 42 combinations tried, 17 were found compatible and produced seeds. The interspecific crossings done in this experiment were incompatible and failed to set seeds except the combination 'Red Dragon' x *A. amnicola*, where seed set was obtained but seeds

failed to germinate. Out of the 17 successful combinations, the crosses with highest compatibility score of 8 were 'Candy Queen' x 'Lima', 'Lima' x 'Red Dragon', 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon'. In general, considering all the cross combinations, involving the six female parents, 'Lima' appeared to be the most compatible, showing the best performance with a total compatibility score of 28 from 4 successful crosses. The varieties 'Candy Queen' (score 22), 'Red Dragon' (score 20) and 'Eureka Red' (score 50) also performed well as good female parents. Comparing the performance of male parents, the most successful variety was 'Lima' with a total compatibility score of 31 from 5 successful cross combinations. The varieties 'Red Dragon' (score 23) and 'Eureka Red' (score 18) also performed well as male parents. The variety 'Lima' was the best performer both as female and male parent.

Hundred per cent seed set was recorded for the crosses 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon'. All the seeds germinated from the cross 'Candy Queen' x 'Lima' and this cross again recorded highest percentage of double seeded berries (21%) and highest percentage of fruit set per spadix (52.5%).

The time taken for seed maturity varied in *Anthurium* varieties from 145 to 208 days. This time lag could be substantially reduced by the *in vitro* culture of seeds isolated from immature berries. In this experiment, seeds from 70 days maturity onwards were used for *in vitro* culture. Hundred percentage germination was obtained from seeds of 120 days of maturity in 'Lima' x 'Eureka Red' and 130 days in 'Eureka Red' x 'Red Dragon'. Thus the seeds could be successfully germinated *in vitro* 40-50 days prior to the time taken by conventional means.

For *in vitro* seed culture, sterilization with mercuric chloride 0.1 per cent for 10 minutes was found good. All the basal media tried (MS, ½ MS, ¼ MS and Nitsch) as well as combinations of basal media with growth substances responded well regarding germination of seeds. After germination of seeds, fast growth and multiplication was observed in ½ MS with 1 mg l^{-1} BA.

BA alone and in combination with 2,4-D and NAA showed callus initiation and growth of the callus. Maximum callus development was recorded in combination

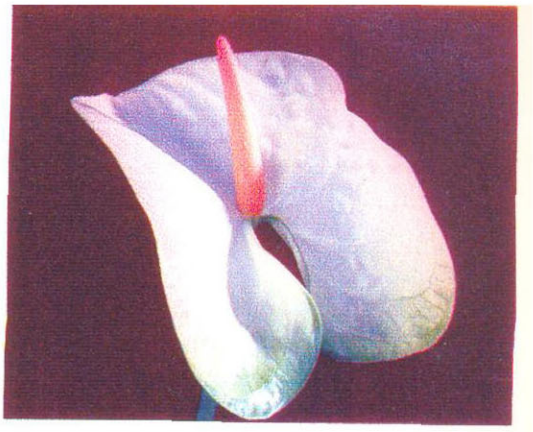
½ MS with BA 6 mg l^{-1} + NAA 3 mg l^{-1}. This combination recorded the maximum number of cultures initiating callus (40%) the highest growth score (3.0) and CI value (120). The treatment combination ½ MS + BA 0.5 mg l^{-1} + IAA 1 mg l^{-1} recorded maximum shoot regeneration (60%). The shoot multiplication and growth were maximum in the medium ½ MS with BA 6 mg l^{-1}, NAA 2 mg l^{-1} and 2,4-D 2 mg l^{-1}. This combination produced 6.8 shoots, 4 weeks after inoculation and 14.2 shoots, 8 weeks after inoculation. The medium ½ MS with BA (0.5 mg l^{-1}) and IAA (1 mg l^{-1}) was found good for rooting and growth enhancement.

Irradiation of seeds reduced the germination percentage and further growth. The lower doses of gamma rays produced pale green leaves with reduced leaf size. The lower doses of gamma rays 0.5 Gy and 1.5 Gy showed 60 per cent shoot regeneration when callus was irradiated. The doses beyond 2.0 Gy showed slow growth and browning of the tissues and beyond the dose 5.0 Gy, no growth was observed. Fifty per cent germination was obtained at the dose 2.0 Gy. When irradiation was done on multiple shoot regeneration stage, shoots were produced up to 3.5 Gy, beyond which no growth was observed.

The survival of plantlets was better in the media sand + cocopeat + husk pieces (1:1:1) and sand + trichoderma treated cowdung + husk pieces (1:1:1). White plastic tea cup with holes at the bottom and sides was found good for planting out of the plantlets. An average of 6 leaves was found in all the hybrids one year after planting. When immature seeds were cultured *in vitro* the plantlets could be transferred to the field by 6-7 months. These plantlets flowered by 12th month. So in total from hybridization to flowering, it took only 22-24 months, whereas in conventional method it took 30-35 months.

Colour variations were noticed in the hybrid flowers. All the varieties of *A. andreanum* and their hybrids recorded a somatic chromosome number of 2n = 30+2B. Comparison made between parents and hybrids based on isozyme analysis revealed differences in banding pattern. Variation in banding pattern was observed with two enzymes, viz., peroxidase and superoxide dismutase. The enzyme peroxidase was found to be most stable and was expressed in all varieties and hybrids.

References



REFERENCES

Abdussamed, K.P. 1999. Regulation of flower and post harvest behaviour of *Anthurium andreanum* Lind. M.Sc.(Ag.) thesis, Kerala Agricultural University, Thrissur, p.135

Ajithkumar, P.V. and Nair, S.R. 1996. Establishment and hardening of *in vitro* derived plantlets of *Anthurium andreanum. J. of Orn. Hort.* 4: 9-12

Akamine, E.K. 1976. Post harvest handling of tropical ornamental cut crops in Hawaii. *Florida Agricultural Experiment Stations. Journal Series No.6096*

Allard, R.W. 1960. *Principles of Plant Breeding*. John Wiley and Sons, Inc., New York, pp.89-98

Anais, G., Darrasse, A., Prior, P. and Cadic, A. 2000. Breeding anthuriums (*Anthurium andreanum* L.) for resistance to bacterial blight caused by *Xanthomonas campestris* pv. *diffenbachiae*. *Acta. Horticulturae* No.508. 135-140

Anthura (Corporate Author). 1997. Variety: 'Champion'. *Plant Varieties J.* 10(1): 12

Anthura (Corporate Author). 2001. 'Antinkeles' syn 'Pink Champion' Application No.2001/013. *Plant Varieties J.* 14:1, 24

Anu, G.K. 1998. Improvement of propagation efficiency in *Anthurium andreanum* Andre. M.Sc. thesis, Kerala Agricultural University, Thrissur. p.146

Arndt, G. 1991. Anthurium (*A. scherzerianum*) var. 'Arabella' (Commercial synonym Aradt's Flamenco Arabella). *Plant Varieties J.* 4(1): 14

Atta-Alla, H., McAlister, B.G. and Van-Staden, J. 1998. *in vitro* culture and establishment of *Anthurium parvispathum. South African J. Bot.* 64:5, 296-298

*Bachthaler, E. 1977. Germination of *Anthurium scherzerianum* hybrids. *Gartenbauwissen schaft.* 42(3): 136-138

*Bachthaler, E. 1978. *Anthurium scherzerianum* hybrids: seed germination at different stages of berry ripeness. *Gartnerborse-und-Gartenwelt* 78(50): 1249-1250

*Bachthaler, E. 1979. Studies on the storage of seeds of *Anthurium scherzerianum* hybrids. *Gartenbauwissen schaft* 44(6): 251-255

Balachandran, M. 1998. Improvement of *Anthurium andreanum* Lind. *in vitro*. Ph.D. thesis, Kerala Agricultural University, Thrissur, p.208

Bhatt, N.R. and Desai, B.B. 1989. Anthurium. *Commercial Flowers* (eds. Bose, T.K. and Yadav, L.P.). Naya Prakash Publishers, Calcutta, India, pp.623-641

Bhattacharjee, S.K. 1977. Packing of fresh cut flowers for export. *Hort. Bulletin* 8(2 and 3): 1-6

Bhojwani, S.S. and Bhatnagar, S.P. 1974. *The Embriology of Angiosperms*. Vikas Publishing House, New Delhi, pp.43-46

Bindu, M.R. 1992. Chromosome behaviour and pollen analysis in *Anthurium* sp. M.Sc. (Agri.) thesis, Kerala Agricultural University, Thrissur, p.106

Bindu, M.R. and Mercy, S.T. 1994. Cytological studies in *Anthurium andreanum* L. Ist National Seminar on Anthurium 6-9 May, 1994, Trivandrum. *Abstract*: 14

Bindu, M.R. and Mercy, S.T. 1996. Pollen studies in *Anthurium andreanum* Lind. *J. Trop. Agric.* 34(2): 96-98

Bindu, M.R. and Mercy, S.T. 1997. Chromosome behaviour and Karyotype analysis in *Anthurium andreanum* Lind. *South Indian Hort.* 45(3-4): 134-138

Birdsey, M.R. 1956. *The cultivated Aroids.* Gillick Press, Berkeley, p.71

Chen-JungBin, Chen-WenHuei, Wu-GwoDean, Chen-J.B., Chen-W.H. and Wu-G.D. 1999. *Investigation of flowering characteristics and establishment of crossing techniques for A. andreanum* Hort. Report No.164, Taiwan Sugar Research Institute, pp.41-57

*Christensen, O.V. 1971. Morphological studies on the growth and flower formation of *Anthurium scherzerianum* Schott. and *A. andreanum* Lind. *Tistsskrift for plant eavl.* 75(6): 793-798

Criley, R.A. 1989. Culture and cultivar selection for anthurium in Hawaii. *Acta Hort.* 246: 227-236

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

Croat, T.B. and Bunting, G.S. 1978. Standardisation of *Anthurium* descriptions. *Aroideana* 2: 5-25

Devinder-Prakash, Choudhary, M.L., Prasad, K.V., Nagesh, N. and Prakash, D. 2001. Regeneration of plantlets from petiole explants of *Anthurium andreanum* Lind. cv. 'Mauritius Orange'. *Phytomorphology* 51(1): 83-85

Erdtman, G. 1952. *An Introduction to Pollen Analysis*. The Chronica Botanica Co., Waltham, p.239

Ettinger, T.L. and Preece, J.E. 1985. Aseptic micropropagation of Rhododendron. P.J.M. hybrids. *J. Hort. Sci.* 60(2): 269-274

Falconer, D.B. 1981. *Introduction to Quantitative Genetics,* Longman, New York, p.340

Finnie, J.F. and Van Staden, J. 1986. *In vitro* culture of *Anthurium andreanum. South African J. Bot.* 52(4): 343-346

Forsyth, W.G.C. and Simmonds, N.W. 1954. A survey of the anthocyanin of some tropical plants. *Proc. Roy. Soc. Bot.* 142: 549-564

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

Gajanana, T.M., Subrahmanyam, K.V. 1999. Economics of production and marketing of anthurium in Karnataka. *Agricultural Economics Res. Rev.* 12(1): 48-55

*Gajek, W. and Schwarz, K.H. 1980. *Anthurium andreanum* hybrids, valuable all the year round cut flowers which use a limited energy input. *Gartenbau.* 27(11): 343

Gamborg, O.C. and Shyluck, J.P. 1981. Nutrition, media and characteristics of plant, cell and tissue culture. *Plant Tissue Culture Methods and Applications in Agriculture* (ed. Thorpe, P.A.). Academic Press, New York, pp.21-24

Geier, T. 1987. Micropropagation of *Anthurium scherzerianum*: Propagation schemes and plant conformity. *Acta Hort.* 212: 439-443

Geier, T. 1989. Anthurium. *Handbook of Plant Cell Cultures, Vol.5 Ornamental species* (eds. Ammirato, P.V., Evans, D.A., Sharp, W.R. and Bajaj, Y.P.S.). Mc Graw Hill Publishing Company, New York, pp.228-252

Haris, H. and Hopkinson. 1976. *Handbook of enzyme electrophoresis in human genetics.* North Holland Publishing Co., New York/Oxford. p.536

Henny, R.J. 1989. Development, testing and release of new ornamental cultivars (Aroid). *Acta. Hort.* 252: 71-76

Henny, R.J. 1999. 'Red Hot' *Anthurium. Hort. Sci.* 34(1): 153-154

Henny, R.J. and Norman, D.J. 2001. *Anthurium* 'Show Biz'. *HortSci.* 36(6): 1140-1141

Henny, R.J., Poole, R.T. and Conover, C.A. 1988. 'Southern Blush' a hybrid anthurium for foliage producers. *HortSci.* 23(5): 922-923

Higaki, T. and Poole, R.T. 1978. A media and fertilizer study in anthurium. *J. Amer. Soc. Hort. Sci.* 103: 98-100

Higaki, T. and Rasmussen, H.P. 1979. Chemical induction of adventitious shoots in *Anthurium. Hort. Sci.* 14: 64-65

Iida, T., Yabe, K., Wasida, S. and Sakurai, Y. 1986. *Mass propagation of Begonia tuberhydrida* Voss. *plantlets using tissue culture.* Research Bulletin No.18, Aichi-kem Agricultural Research Center, Japan, pp.186-190

*Ito, T. 1942. Chromosomen and sexualitat von der Araceae I Somatische Chromoscmenzahlen einigen arten. *Cytologia.* 20: 313-325

Iwata, R.Y., Tang, C.S. and Kamemoto, H. 1979. Anthocyanins of *Anthurium andreanum* Lind. *J. Amer. Soc. Hort. Sci.* 104(4): 464-466

Iwata, R.Y., Tang, C.S. and Kamemoto, H. 1985. Concentration of anthocyanin affecting spathe colour in anthuriums. *J. Amer. Soc. Hort. Sci.* 110(3): 383-385

Jawaharlal, M., Joshna, J.P., Arumugam, T., Subramanian, S. and Vijayakumar, M. 2001. Standardisation of growing media for Anthurium (*A. andreanum* var. Temptation) under shade net house. *South Indian Hort.* 49: 323-325

Jeong, J.H., Kwon, S.T., Lee-Jong, S. and Roh, M.S. 1996. Variations of morphological characteristics of *Lilium hansonii* related with protein and isozyme bands, *Acta Hort.* 414: 145-150

Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimates of genetic and environmental variability in soyabeans. *Agron. J.* 47: 314-318

*Kalkaman, E.C. 1983. Anthurium. *Vakblad Voor de Bloemisterij*, 38: 69-71

Kamemoto, H. 1962. Some factors affecting the keeping quality of anthurium flowers. *Hawaii Farm Sci.* 11(4): 2-4

Kamemoto, H. and Kuehnle, A.R. 1996. *Breeding Anthuriums in Hawaii.* University of Hawaii Press, Honolulu, p.132

Kamemoto, H. and Nakasone, H.Y. 1955. Improving anthurium through breeding. *Hawaii Farm. Sci.* 3: 4-5

Kamemoto, H. and Nakasone, H.Y. 1963. *Evaluation and improvement of anthurium clones*. Technical Bulletin. Hawaii Agricultural Experimental Station, Hawaii, p.58

Kamemoto, H. and Sheffer, R.D. 1978. A new species hybrid, *Anthurium scherzerianum* x *Anthurium wendlingerii. HortSci.* 13(2): 177-179

Kamemoto, H., Nakasone, H.Y. and Aragaki, M. 1969. Improvement of anthurium through breeding. *Proc. Trop. Raj. Amer. Soc. Hort. Sci.* 12: 267-273

Kamemoto, H., Iwata, R.Y. and Marutani, M. 1988. Genetics of major spathe colours in anthurium. *Research series, College of Trop. Agric. Hum. Reso.* Hawaii 56: 11

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

Klapwijk, D. and Spek, H.J.J. Van der. 1984. Development rate, flower growth and production of anthurium. *Netherlands J. Agric. Sci.* 36: 219-224

Kobayashi, R.S., Brewbaker, J.L. and Kamemoto, H. 1987. Identification of *Anthurium andreanum* cultivars by gel electrophoresis. *J. Am. Soc. Hort. Sci.* 112(1): 164-167

Kuanprasert, N., Kuehnle, A.R. and Tang, C.S. 1998. Floral fragrance compounds of some Anthurium (Araceae) species and hybrids. *Phytochemistry* 49(2): 521-528

Kuehnle, A.R. and Sugii, N. 1991. Callus induction and plantlet regeneration in tissue cultures of Hawaiian anthuriums. *HortSci.* 26(7): 919-921

Kuehnle, A.R., Chen, F.C. and Sugii, N. 1992. Somatic embryogenesis and plant regeneration in *Anthurium andreanum* hybrids. *Plant Cell Reports* 11: 438-442

Kuehnle, A.R., Chen, F.C. and Sugii, N. 1995. Novel approaches for genetic resistance to bacterial pathogens in flower crops. *HortSci.* 30(3): 456-461

Kuo, C.G. and Tssay, J.S. 1977. Propagation of chineese cabbage by axillary bud culture. *HortSci.* 12: 459-460

Lalithambika, R. 1978. Cytological studies on twelve species of anthurium with special reference of B chromosomes. M.Sc. thesis, University of Kerala, Trivandrum, p.188

Lightbourn, G.J. and Prasad, P.V.D. 1990. *In vitro* techniques for rapid multiplication of four varieties of *A. andreanum* in Jamaica. *Proc. Am. Soc. Trop. Hort.* 34: 3-4

Lo, O.F., Chen, C.J. and Ross, J.G. 1980. Vegetative propagation of temperate foliage grasses through callus culture. *Crop Sci.* 20: 363-367

Lowry, J.B. 1972. Anthocyanins in tropical phyto chemistry. *Malaysian J. Sci.* 1(4): 133-140

Mahanta, S. and Paswan, L. 2001. *In vitro* propagation of anthurium from axillary buds. *J. Ornamental Hort. New Series* 4(1): 17-21

*Malhotra, S., Puchooa, D. and Goofoolye, K. 1998. Callus induction and plantlet regeneration in three varieties of *Anthurium andreanum. Revue. Agricole-et-Sucriere-de-Ile-Maurice* 77(1): 25-32

Mangolin, C.A., Pacoli, A.J. and Machado, M.F.P.S. 1994. Isozyme patterns in callus cultures and in plants regenerated from calli of *Careus peruvianus. Biochemical Genetics*, 32: 237-247

Maria, C.C. and Segura, J. 1989. *In vitro* propagation of Lavender. *HortSci.* 24(2): 375-376

Marutani, M., Sheffer, R.D. and Kamemoto, H. 1993. Cytological analysis of *Anthurium andreanum* (Araceae), its related taxa and their hybrids. *Am. J. Bot.* 80: 93-103

Matsumoto, T.K., Kuehnle, A.R. and Webb, D.T. 1998. Zygotic embryogenesis in *Anthurium* (Araceae). *Am. J. of Bot.* 85(11): 1560-1568

*Maurer, M. 1979. Raising *Anthurium scherzerianum* F_1 hybrids. *Gb-+-Gw.* 79(35): 832-834

McCown, B. and Amos, R. 1979. Initial trials with commercial micropropagation of birch selections. *Proc. Int. Plant Prop. Soc.* 29: 387-393

Mercy, S.T. and Dale, B. 1994. *Anthurium.* St. Joseph's press, Thiruvananthapuram, p.64

*Mitu, M. and Acatrinei, G. 1974. Pollen germinating ability in some foreign pear cultivars. Facultatea germinativa a pollenulai la citeva soiuri straine de par Lucrari Stintifice, *Institutional Agronomic Jon. Jonesiu de la Brad.* 1: 275-279

*Montes, S., Aldaz, J.P., Cevallos, M., Cabrera, J.C. and Lopez, M. 2000. Application of the growth regulator Pectimorf in rapid propagation of *Anthurium cubense. Cultivos-Tropicales* 21(3): 29-31

Muller, H.J. 1954. The nature of the genetic effects produced by radiation. *Radiation Biology* Vol.II (ed. Hellaender, A.) Mc Graw Hill, New York, p.351

Murashige, T. 1974. Plant propagation through tissue culture. *Ann. Rev. Plant Physiol.* 25: 135-166

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-497

Nair, P.K.K. 1970. *Pollen Morphology of Angiosperms - A Historical and Phytogenetic study*. Scholar publishing House, Lucknow, pp.67-73

Nair, S., Gupta, P.K., Shrigurkar, M.V. and Mascarenhas, A.F. 1984. *In vitro* organogenesis from leaf explants of *Annona squamosa* Linn. *Plant Cell Tissue Organ Culture* 3: 29-40

Nickel, I.G. 1973. Test tube approaches to by pass sex. *Hawaiian Plantlets Record* 58: 293-314

Nirmala, Singh, K.S.F. and Chandravadana, H.V. 1999. Anthocyanins of *Anthurium andreanum* Lind - presence of an unknown pigment. *J. Applied Hort.* 1(1): 29-31

Nitsch, J.P. and Nitsch, C. 1969. Haploid plants from pollen grains. *Science* 163: 85-87

Obara-Okeyo, P. and Kako, S. 1997. *In vitro* and *in vivo* characterization of cymbidium cultivars by isozyme analysis. *J. Hort. Sci.* 72(2): 263-270

Oglesby Plant Laboratory Inc. (Corporate author). 1996. 'Ruth Morat' Syn Lady Ruth. *Plant Varieties J.* 9(3): 17

Omura, M., Matsuta, N., Moriguchi, T. and Kozaki, I. 1987. Adventitious shoot and plantlet formation from cultured pomegranate leaf explants. *HortSci.* 22(1): 133-134

Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers*. I.C.A.R., New Delhi, 4: 97-123

Paull, R.E. 1982. *Anthurium (Anthurium andreanum* Andre) Vase life evaluation criteria. *HortSci.* 17(4): 606-607

Pierik, R.L.M., Leeuwon, P. Van, and Rigter, G.C.C.M. 1979. Regeneration of leaf explants of *Anthurium andreanum* Lind. *in vitro*. *Netherlands J. Agric. Sci.* 27(3): 221-226

*Pierik, R.L.M., Meys, J.A.J. Van Der., and Steegmans, H.H.M. 1974a. Vegetative propagation of *Anthurium andreanum* in propagating tubes. *Vakblad voor de Bloemisterij* 29(6): 12-15

Pierik, R.L.M. Steegmans, H.H.M. and Meys, J.A.J. Van. Der. 1974b. Plantlet formation in callus tissue of *Anthurium andreanum* Lind. *Sci. Hort.* 2(2): 193-198

*Pierik, R.L.M., Steegmans, H.H.M., Schaik, W. Van., and Eyk-bos, G. Van. 1975. With the aid of shaking machines. Callus propagation of *Anthurium andreanum. Vakblad voor de Bloemisterij* 30: 26-27

Prasad, K.V., Prakash, D., Aswath, C. and Chaudhary, M.L., 1998. *Know about anthurium.* Division of ornamental crops, Indian Institute of Horticultural Research, Bangalore, pp.8-15

Pyati, A.N. and Murthy, H.N. 1995. *In vitro* seed germination and seedling development of *Dendrobium ovatum* (Willd). *Krunzl. J. Orchid Soc. India* 9(1-2): 69-74

Rajasekharan, P. and Kumar, M.P. 1994. Somatic embryogenesis and *in vitro* plant development of *Anthurium andreanum* Lind. First national seminar on Anthurium. 8-9 May, 1994. Tropical Botanical Garden and Research Institute; Thiruvananthapuram. *Abstract*: 17

Rajasekharan, P., Mohan Kumar, P. and Haridas, P. 1994. *In vitro* propagation of *Anthurium andreanum* L. First national seminar on Anthurium. 8-9 May, 1994. Tropical Botanical Garden and Research Institute, Thiruvananthapuram. *Abstract*: 14

Rajeevan, P.K. and Valsalakumari, P.K. 2000. *Anthurium* - a potential crop for product diversification in Indian cut flower industry - *Floriculture Today* 4(2): 21-28

Rajeevan, P.K. and Valsalakumari, P.K. 2001. Anthurium. *Hand book of Horticulture* (ed. Chadha, K.L.). ICAR, New Delhi, pp.573-578

Rajeevan, P.K., Valsalakumari, P.K., Geetha, C.K., Leena Ravidas, Vinod Kumar and Bhattacharjee, S.K. 2002. *Anthurium.* Technical Bulletin IARI, New Delhi p.42

Randhawa, G.S. 1990. Role of carbon and nitrogen source in *in vitro* germination of *Anthurium andreanum* seeds. M.Sc. thesis, UAS, Bangalore, p.181

Rao, A.N. and Avadhani, P.N. 1963. Effects of chlorax on the germination of *Vanda* seeds. *Curr. Sci.* 32: 467-468

Renu, R.S. 1999. Inter varietal hybridization in *Anthurium andreanum* Lind. M.Sc. thesis, Kerala Agricultural University, Thrissur, p.131

Sadasivam, S. and Manickam, A. 1992. *Biochemical Methods for Agricultural Sciences*.Wiley Eastern Limited. pp.58-64

Salvi, B.R. 1997. Optimisation of shade, nutrients and growth regulators for cut flower production in *Anthurium andreanum* Lind. Ph.D. thesis, Kerala Agricultural University, Trichur, p.278

Salvi, B.R., Prabhakaran, P.V., Valsalakumari, P.K. and Geetha, C.K. 1995. (Unpublished) Estimation of leaf area in anthurium (*Anthurium andreanum*). Kerala Agricultural University, Thrissur, Kerala

Salvi, B.R., Valsalakumari, P.K. and Rajeevan, P.K. 2001. Nursery techniques for anthurium. *Floriculture Today* 5(12): 44-45

Salvi, B.R., Valsalakumari, P.K., Rajeevan, P.K. and Geetha, C.K. 1997. Effect of holding solutions on cut anthurium flowers. *Hort. J.* 10(2): 14-17

Salvi, B.R., Valsalakumari, P.K., Rajeevan, P.K. and Geetha, C.K. 1998. Agrotechniques for growing anthruiums in Kerala. National Seminar on anthurium production, *Souvenir and abstracts*, IIHR, Bangalore, pp.16-17

Satyadas, J. 1985. Karyomorphological studies on eight species and varieties of *Anthurium* with special reference to B chromosomes. M.Sc. thesis, University of Kerala, Trivandrum, p.212

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

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

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

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

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

Sheffer, R.D. and Kamemoto, H. 1977. Interspecific hybridization involving *Anthurium andreanum* Lind. and related species. *Proc. Trop. Reg. Amer. Soc. Hort. Sci.* 19: 275-283

Sindhu, K. 1995. Cross compatibility in *Anthurium andreanum* Lind. M.Sc.(Ag.) thesis, Kerala Agricultural University, Thrissur, p.111

Singh, F. 1987. Anthurium Vyeing for a place among commercial flower crops. *Ind. Hort.* pp.14-16

Singh, F. 1992. Enthrolling anthurium. *Vatika*, 3: 17-20

Singh, F. 1998. Anthurium production - the global scenario. National Seminar on anthurium production, 2-3 June, 1998, Coorg at Chethali. *Abstract*: 8

Singh, R.K. and Choudhary, B.D. 1977. *Biometrical methods in Quantitative Genetic Analysis*. Kalyani Publishers, New Delhi, p.250

Sobhana, A. 2000. Improvement of *Dendrobium* through hybridization and *in vitro* mutagenesis. Ph.D. thesis, Kerala Agricultural University, Thrissur, p.230

Somaya, K.U., Narayanaswamy, P. and Jayaprasad, K.V. 1998. Micropropagation studies in *Anthurium andreanum* Lind. *Karnataka J. of Agric. Sci.* 11(2): 466-470

Sreelatha, U. 1992. Improvement of propagation efficiency of *Anthurium* species in *in vitro*. Ph.D. thesis, Kerala Agricultural University, Thrissur, p.120

Srivastava, D. 1982. Studies on the pollen biology of certain cultivated Malvaceae. *Advances in Pollen Spore Research, Vol.9* (ed. Nair, P.K.K.). Today and Tomorrows Printers and Publishers, New Delhi, pp.10-12

Stanley, R.G. and Linskens, H.F. 1974. *Pollen Biology, Biochemistry and Management.* Springer-Verlag, Berlin Heidelberg, New York, pp.39-85

Steen, J.V.D. and Vijverberg, A.J. 1973. Yield difference in the culture of *Anthurium andreanum*. *Vakblad voor de Bloemisterij* 28(7): 10-11

Swaminathan, V. 1986. *In vitro* seed germination in Anthurium. M.Sc. thesis, UAS, Bangalore, p.176

Swanson, C.P. 1968. *Cytology and Cytogenetics*. Macmillan Co. Ltd. p.596

*Szendel, A.J., Hetman, J. and Laskowska, H. 1982. Evaluation of conditions for germinating *Anthurium andreanum* and *A. scherzerianum* seeds. *Prace. Instytutu sadawniltwa W skernie wicach.* 6: 54-57

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

Tisdale, S.L., Nelson, W.L. and Beaton, J.D. 1985. *Soil Fertility and Fertilizers*. Macmillan Pub. Co. Inc. New York p.733

Vallejos, C.E. 1983. Enzyme staining. *Isozymes in Plant Genetics and Breeding, Part A* (ed. S.D.Tanskley and T.J.Orton). Elsevier, Amsterdam, pp.469-516

Valsalakumari, P.K., Abdussamed, K.P., Rajeevan, P.K. and Geetha, C.K. 2001. Shade and nutrient management in *Anthurium andreanum*. *South Indian Hort.* 49: 326-331

Voyatzi, C. and Voyatziz, D.G. 1989. *In vitro* shoot proliferation rate of *Dieffenbachia exotica* cv. Marianna as affected by cytokinins, the number of recultures and the temperature. *Scientia Horticulturae* 40(2): 163-169

Wannakrairoj, S. and Kamemoto, M. 1990. Inheritance of purple spathe in *Anthurium*. *J. Amer. Soc. Hort. Sci.* 115(1): 169-171

Yam, T.W. and Weatherhead, M.A. 1988. Germination and seedling development of some Hong Kong orchid. *Lindleyana* 3(3): 1150-1160

Yonada, K., Lida, T., Asano, H. and Suzuki, M. 1993. *Identification of rose species and hybrids by leaf peroxidase isozyme phenotypes.* Technical Bulletin No.50. College of Agriculture and Veterinary Medicine, Nihon University, pp.22-25

*Zimmer, K. 1986. Problems in the development of *Anthurium* and *Spathiphyllum* cultivars. *Deutscher-Gartenbau* 40(12): 574-577

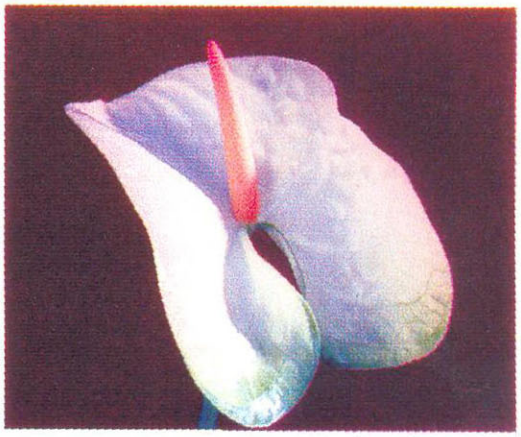
*Zimmer, K. 1990. New approaches to variety development in *Anthurium scherzerianum*. Selection for flowering behaviour. *Gb-+-Gw-Gartnerbarse-und-Gartonwelt*. 90(18): 870-873

*Zimmer, K. and Bahnemann, A. 1982. Cloning of temperature tolerant *A. scherzerianum* seeds. *Gartenbauwissen schaft* 47(2): 72-74

*Zirkle, C. 1937. Acetocarmine mounting media, *Science*. 85: 528

* Originals not seen

Appendices



APPENDIX - I

Meteorological data for the period under study

Month/ Year	Max. Temp. (°C)	Min. Temp. (°C)	Total Rainfall (mm)	No. of rainy days	Relative humidity		Sunshine (average light days)	Wind speed (Km/hr)
					Morning	Afternoon		
April 1999	33.4	25.6	39.0	4	88	58	10.3	3.3
May 1999	30.7	24.7	430.5	18	92	72	4.9	3.0
June 1999	29.4	23.0	500.2	23	94	75	5.0	2.5
July 1999	28.4	23.0	823.3	28	96	82	2.4	2.5
Aug 1999	29.8	22.9	260.1	12	94	73	5.5	2.3
Sept. 1999	31.6	23.4	28.4	3	89	63	7.1	2.1
Oct. 1999	30.5	23.2	506.2	15	94	75	4.8	1.6
Nov. 1999	31.4	22.7	9.1	1	81	57	8.2	3.6
Dec. 1999	30.7	22.7	0.0	0	72	48	8.8	6.6
Jan. 2000	32.9	23.2	0.0	0	76	43	9.2	7.1
Feb. 2000	33.3	22.8	4.6	1	85	52	8.6	3.7
Mar. 2000	35.6	23.9	0.0	0	87	46	9.7	9.7
April 2000	34.0	24.6	67.9	3	89	59	7.2	2.6
May 2000	33.7	24.4	117.2	8	88	56	8.5	2.9
June 2000	29.6	22.8	602.0	21	94	77	3.3	3.1
July 2000	28.8	21.9	354.3	15	93	70	4.8	3.8
Aug. 2000	29.1	22.6	518.8	19	94	79	3.1	3.4
Sept. 2000	30.7	23.0	198.1	10	91	70	5.9	3.2
Oct. 2000	30.7	22.7	262.2	10	91	68	5.6	2.7
Nov. 2000	33.3	23.1	413.0	5	77	54	6.7	5.7
Dec. 2000	30.4	22.0	11.2	2	70	48	7.9	7.8
Jan. 2001	32.6	23.2	0.0	0	71	41	8.0	8.5
Feb. 2001	34.5	22.9	12.2	1	86	48	8.0	4.2
Mar. 2001	34.9	24.0	4.4	0	84	54	8.2	4.1

Source: Department of Agricultural Meteorology, College of Horticulture

APPENDIX-II

Chemical composition of MS medium

Chemical	mg l^{-1}
Macronutrients	
KNO_3	1900.00
NH_4NO_3	1650.00
KH_2PO_4	170.00
$MgSO_4.7H_2O$	370.00
$CaCl_2.2H_2O$	440.00
Micronutrients	
H_3BO_3	6.200
$MnSO_4.4H_2O$	22.300
$ZnSO_4.7H_2O$	8.600
$Na_2M_0O_4.2H_2O$	0.250
$CaCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
KI	0.830
$FeSO_4.7H_2O$	27.800
Na_2EDTA	37.300
Vitamins	
Thiamine HCl	0.100
Pyridoxine HCl	0.500
Nicotinic acid	0.500
Others	
Glycine	2.00
Myo-inositol	100.00
Sucrose	30.00
pH	5.80

MS - Murashige and Skoog, 1962

APPENDIX-III

Chemical composition of Nitsch medium

Constituents	Quantity	Volume made up
Solution A		
Ammonium nitrate	36 g	1000 ml
Potassium nitrate	47.5 g	
Magnesium sulphate	9.25	
Potassium dihydrogen orthophosphate	3.4 g	
Solution B		
Calcium chloride	8.3 g	1000 ml
Selection C		
Sodium EDTA	3.73	1000 ml
Ferrous sulphate	2.78 g	
Solution D		
Boric acid	1.0 g	1000 ml
Manganese sulphate	2.5 g	
Sodium molybdate	0.025 g	
Copper sulphate	0.025 g	
Solution E		
Nicotinic acid	0.5	1000 ml
Pyridoxine HCl	0.05	
Thiamine HCl	0.05	
Glycine	0.2	
Folic acid	0.05	
Myoinositol	100 mg	
Sucrose	20 g	
Agar	6-9 g	
pH	5.8	

IMPROVEMENT OF *Anthurium andreanum* Lind BY *IN VIVO* AND *IN VITRO* METHODS

By
LEENA RAVIDAS

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Horticulture

Faculty of Agriculture
Kerala Agricultural University

Department of Pomology and Floriculture
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR-680656
KERALA, INDIA
2003

ABSTRACT

Investigations on 'Improvement of *Anthurium andreanum* by *in vivo* and *in vitro* methods' were carried out at the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during the period from 1998 to 2001. The main objectives were to evolve new varieties of *A. andreanum* and to standardise the age of seed and media for *in vitro* seed culture.

Six commercially important varieties of *A. andreanum*, viz., 'Nitta', 'Candy Queen', 'Lima', 'Red Dragon', 'Eureka Red', 'Agnihothri' and three species of *Anthurium* viz., *A. crystallinum, A. ornatum* and *A. amnicola* were selected for the study.

Characterisation of the varieties was done morphologically which showed significant variations with respect to vegetative and floral characters. Year round flowering was obtained in all the varieties. The varieties 'Eureka Red' and 'Red Dragon' produced more number of spikes per year (8.9 and 8.0, respectively). Among the varieties, 'Candy Queen' produced the largest spathe (17.67 cm x 13.33 cm). Shortest spadix was observed in Red Dragon. Peduncle was straight in all the varieties except in 'Candy Queen'.

The true flower of *Anthurium* is protogynous. The duration from spike appearance to unfurling ranged from 19.00 days in 'Lima' and 'Candy Queen' to 25.33 days in 'Agnihothri'. Female receptivity ranged from 12 days in 'Red Dragon' to 20.33 days in 'Eureka Red'. An interphase of 3 to 20 days was found in between female phase and male phase. Duration of male phase ranged from 9.33 days in 'Lima' to 20.67 days in 'Red Dragon'. Early ripening of seeds was obtained in 'Red Dragon' (145 days) while it took 208 days in 'Agnihothri'.

Pollen grains of all the varieties were more or less round in shape. 'Agnihothri' produced largest pollen grains (25.4 μ). Pollen fertility was highest in *A. crystallinum* (69.8%). Pollen production was profuse during the period from June to October.

The varieties 'Eureka Red', 'Red Dragon' and 'Lima' were superior regarding post harvest qualities. In vase, spikes retained colour for longer period in varieties 'Eureka Red' (17.67 days) and 'Red Dragon' (15.67 days) and no colour change observed in variety 'Lima'. Longer periods for necrosis of spathe and necrosis of spadix were also exhibited by the varieties 'Eureka Red', 'Red Dragon' and 'Lima'. The same trend was exhibited in packing also.

Heritability was of moderate to high magnitude for most of the characters. Values for PCV were slightly higher than those of GCV. Plant height recorded highest PCV (70.57) and GCV (69.96).

Out of the 42 combinations of hybridization tried, 17 were found compatible. All the self crosses and interspecific crosses were found incompatible. Among all the combinations, 'Lima' produced the largest number of compatible crosses as well as high seed set and germination percentage (compatibility score 28). The varieties 'Candy Queen', 'Red Dragon' and 'Eureka Red' also performed well as good female parents. Comparing the performance of male parents, the most successful variety was 'Lima' with a total compatibility score of 31 from 5 successful cross combinations, followed by the varieties 'Red Dragon' and 'Eureka Red'. Out of the 17 successful combinations, the highly compatible crosses were 'Candy Queen' x 'Lima', 'Lima' x 'Red Dragon', 'Lima' x 'Eureka Red' and 'Eureka Red' x 'Red Dragon'.

The protocol for immature hybrid seed culture (*in vitro*) in anthurium was developed. Seeds, 40-45 days before field maturity could be used for *in vitro* culture, thus reducing the time lag for the production of hybrid seedlings. Germination and further development were good in ½ MS + 1 mg l^{-1} BA. For callus initiation, ½ MS with BA 6 mg l^{-1}, NAA 3 mg l^{-1} was effective. For rooting and growth enhancement, ½ MS with BA 0.5 mg l^{-1} and IAA 1 mg l^{-1} proved good.

Irradiation of seeds reduced germination percentage and further growth. Callus irradiation at 0.5 to 10 Gy was not effective. Lower dosages induced pale green leaves and clustered appearance while higher doses induced browning of cultures.

Survival of plantlets was the best in the media sand + cocopeat + husk pieces (1:1:1) and sand + trichoderma treated cowdung + husk pieces (1:1:1). White plastic tea cups with holes at the bottom and sides were found good for planting out of plantlets. When immature seeds were cultured *in vitro*, plantlets could be transferred to the field by 6-7 months. These plantlets flowered by 12th month. So in total from hybridization to flowering, it took only 22-24 months whereas in conventional method it took 30-35 months.

Three hybrids produced flowers during the period of evaluation. The hybrid 'Lima' x 'Eureka Red' flowered 11th month of planting out and 'Lima' x 'Red Dragon' and 'Candy Queen' x 'Lima' flowered 12 months after planting out. Colour variations were noticed in the hybrid flowers. Variation in banding pattern was observed in isozyme analysis with the enzymes peroxidase and superoxide dismutase, while comparing parents and hybrids.