GENOTYPIC EVALUATION AND *IN VITRO* MULTIPLICATION OF ANTHURIUM (*Anthurium andreanum* Linden) HYBRIDS

by

ANAND S

(2017-11-039)

THESIS

Submitted in partial fulfilment of the

requirement for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM - 695522

KERALA, INDIA

2019

DECLARATION

I, hereby declare that this thesis entitled **"Genotypic evaluation and *in vitro* multiplication of anthurium (*Anthurium andreanum* Linden) hybrids"** is a bonafide record of research work done by me during the course of research and the 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.

Vellayani
Date: 12-07-2019

Anand S
(2017 - 11- 039)

CERTIFICATE

Certified that this thesis, entitled **"Genotypic evaluation and *in vitro* multiplication of anthurium (*Anthurium andreanum* Linden) hybrids"** is a record of research work done independently by **Mr. Anand S (2017-11-039)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellayani
Date: 12/7/2019

Dr. Beena Thomas
(Chairman, Advisory Committee)
Assistant Professor,
Department of Plant Breeding and Genetics,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Anand S (2017-11-039)**, a candidate for the degree of **Master of Science in Agriculture** with major in Plant Breeding and Genetics, agree that the thesis **"Genotypic evaluation and *in vitro* multiplication of anthurium (*Anthurium andreanum* Linden) hybrids"** may be submitted by **Mr. Anand S (2017-11-039)**, in partial fulfilment of the requirement for the degree.

Beena Thomas 12/7/19

Dr. Beena Thomas
Assistant Professor
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani
Thiruvananthapuram

Dr. C. Lekha Rani
Professor
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani
Thiruvananthapuram

Arya K 12/7/19

Dr. Arya K.
Professor and Head
Department of Plant Breeding and Genetics
College of Agriculture, Vellayani
Thiruvananthapuram

Swapna S

Dr. Swapna Alex
Professor and Head
Department of Plant Biotechnology
College of Agriculture, Vellayani
Thiruvananthapuram

C V Annadurai 12.7.19

EXTERNAL EXAMINER
Prof & Head (PBG)
ACTRI, Madurai.

Acknowledgement

First and foremost, praises and thanks to the ***Almighty,*** *for everything that happens to me...*

With great respect and devotion, I would like to record my deep sense of gratitude and indebtedness to my major advisor ***Dr. Beena Thomas****, Assistant Professor, Department of Plant Breeding and Genetics, for her valuable guidance, constant inspiration, abundant encouragement, critical scrutiny of the manuscript and valuable suggestions which render me to accomplish the research work successfully. I extend my sincere gratitude for the care and affection bestowed on me throughout the study period.*

It gives me immense pleasure to express my deep sense of gratitude to ***Dr. Arya K.****, Professor and Head, Department of Plant Breeding and Genetics, for her motherly approach, valuable advices and whole hearted approach right from the beginning, which helped me in the successful completion of the thesis.*

I awe my heartfelt thanks to my advisory committee, ***Dr. C. Lekha Rani,*** *Professor, Department of Plant Breeding and Genetics and* ***Dr. Swapna Alex,*** *Professor and Head, Department of Plant Biotechnology for their generous and timely help, unstinting support, suggestions and passionate approach rendered during the period of research work.*

I consider it as my privilege to express my deep felt gratitude to ***Dr. Jayalekshmi V. G.****, Professor (RC) and Head, Department of Seed Science and Technology, for her valuable suggestions, technical advices and incessant motivation throughout the research work.*

I am deeply grateful to my beloved teachers of Plant Breeding and Genetics, ***Dr. Mareen Abraham*** *and* ***Dr. Gayathri G*** *for generous support, timely advice, constructive criticisms and constant encouragement rendered to me during the course of my study and research work.*

I would like express my sincere gratitude to ***Dr. M. M. Viji, Dr. Roy Stephen, Dr. Sajitha Rani, Dr. Joy M*** *and* ***Dr. Kiran A. G.*** *for the valuable suggestions and whole hearted cooperation and support rendered throughout the course work and research programme.*

My heartfelt thanks to ***Dr. Bejoy Mathew, Dr. Radhika B. J., Mr. Sreekumar M. K,*** *and* ***Remya*** *of Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Palode for sharing their practical experience in tissue culture, which proved to be of immense help in successful completion of my research programme.*

No choice of words will suffice to express thanks to my loving friends ***Govind, Anki, Christy, Nibin eattan, Kavya, Nadirsha, Saranya and Swathy*** *for being with me from the beginning of course study till now and played a big part in completion of the research programme. Without their persistent help, this would not have been possible.*

Words are inadequate to express my thanks to ***Shahiba*** *and* ***Chako maash*** *for being the best seniors ever and for their unbound love, guidance and big hearted support.*

My loving gratitude to my dear friends ***Saayu, Jithumon, Sooraj, Ajmal, Dhanu, Suresh, Manu, Mappu, Amal, Alan, Melvin, Abuji, Gopan, Vipin, Subham, Ramesha, Chanda, Chedan, Reghu, Murukesh, Vinod, Pavan, Panju, Aswathy, Rehana, Navya, Thasni, Athulya, Athira, Arathy, Monisha,***

Nasreena, Susan, Ammu, Arya, Swathy, Aishu, Anju, Greeshma, Ponni, Reshma, Liz, Nivya, Safana, Deepti, Keerthana, Reni, Krishnaveni, Namitha and Lekshmi *for being my best batchmates and for making my college days memorable.*

I wish to express my heartfelt thanks to my beloved seniors ***Reshma chechi, Ajil, Gokul, Akhil, Fallukka, Naveen, Abhinav, Ajmal, Fazeeka, Chippy chechi, Asoontha chechi, Arya chechi, Amrutha chechi, Thouseem itha, Anju chechi, Niji chechi, Hari eattan, Thejasree chechi, Rehana chechi, Namitha chechi, Manu eattan, Nishanth bro and Nideesh eattan*** *for their love, care, support and timely guidence. I express my special thanks to my dearest sister* ***Smera*** *and to my dear juniors* ***Reshma, Mythri, Bhoomika, Darsana, Roshin*** *and all the other* ***junior batch students****. It is my pleasure to express my special thanks to* ***office assistants and lab assistants of my department*** *for their sincere cooperation and kindly approach offered during the study period.*

I am thankful to ***Kerala Agricultural University*** *for the technical and financial assistance for carrying out my study and research work. I would like to express my thanks to* ***Librarian, library staffs, farm labours*** *and* ***all other staffs*** *of COA, Vellayani for their support and assistance during the study period.*

It would be impossible to list out all those who have helped me in one way or another in successful completion of this work. I once again express my heartful thanks to all those who helped me in completing this venture in time.

Words are not enough to express my whole-hearted and affectionate gratitude to my ***Achan, Amma and Anjukutty*** *for their unconditional love, sacrifices and support bestowed on me throughout my career.*

Anand S.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS AND SYMBOLS USED

°	Degree
°C	Degree Celsius
<	Less than
>	Greater than
%	Per cent
µL	Micro litre
½ MS	Half strength Murashige and Skoog medium
BA	Benzyl Adenine
BAP	Benzylaminopurine
CD	Critical Difference
cm	Centimetre
CR	Ceylon Red
CRD	Completely Randomised Design
cv	Cultivar
d.f	Degrees of freedom
Day^{-1}	Per day
DT	Dragon's Tongue
EDTA	Ethylenediaminetetraacetic acid
et al.	And others
Fig.	Figure
g	Gram
$g\ L^{-1}$	Gram per litre

g^{-1}	Per gram
GCV	Genotypic coefficient of variation
HoR	Hounduras Red
HR	Honeymoon Red
i.e.	That is
IAA	Indole acetic acid
IBA	Indole butyric acid
KAU	Kerala Agricultural University
Kg	Kilo gram
KR	Kalympong Red
L^{-1}	Per litre
LJ	Lady Jane
LR	Liver Red
mg	Milligram
mg g^{-1}	Milligram per gram
mg L^{-1}	Milligram per litre
ml L^{-1}	Millilitre per litre
MR	Miniature Red
NAA	Naphthalene acetic acid
NO	Nitta Orange
No.	Number
OG	Orange Glory
P	Pink
PCV	Phenotypic coefficient of variation

Plant^{-1}	Per plant
PR	Pompon Red
PVP	Poly- Vinyl Pyrrolidone
S. E	Standard Error
Sl.	Serial
sp. or spp.	Species (Singular and Plural)
spadices^{-1}	Per spadices
spadix^{-1}	Per spadix
v/v	Volume / volume
Via	Through
viz.	Namely
w/v	Weight / volume
Year^{-1}	Per year

Introduction

1. INTRODUCTION

Anthurium is the largest genus in the family Araceae, constituting more than 800 species. The neotropical genus *Anthurium* is the largest and most complex genus in Aroids, which includes more than 100 genera. The generic name *Anthurium* is derived from two Greek words i.e., 'Anthos' and 'Oura', meaning 'flowering tail'. *Anthurium* species has a diploid chromosome number of 30. Anthuriums are commercially accepted as ornamentals for their showy and colorful spadix and are widely used both as cut flowers as well as potted garden plants. Anthuriums are commonly called as 'Painter's palette', 'Tailflower', 'Cresta de Gallo', 'Cockscomb', 'Flamingo Flower', 'Hawaiian Love Plant', and 'Tongue of Fire'. They are semi terrestrial, perennial and epiphytic plants, native of South-West Columbia which was brought to Europe in 1876 (Singh, 1987). Towards the middle of the nineteenth centuary, some varieties from Hawaii reached Kalympong of West Bengal derivatives of which are now known as Kalympong varieties.

Anthuriums are extremely diverse and are available in a wide range of sizes and colours. Two species of the genus with commercial importance are *Anthurium andreanum* Linden and *Anthurium scherzerianum* which have magnificent flowers and attractive foliage. Anthurium plants also have the capability to remove toxic substances such as benzene, formaldehyde and ammonia from air. They exude substances that kill moulds and viruses, thus keeping the atmosphere healthier.

Anthurium inflorescence consists of a colourful, shiny, heart shaped modified leaf called spathe surrounding a straight or slightly curved inflorescence called spadix or candle. The spadix is composed of a multitude of perfect flowers, with two-carpelled ovary and four anthers.

In the present scenario of ever increasing pressure of population in the country and shrinkage in available area under cultivation of food crops, substantial increase in

the production of high unit value export oriented floricultural items like anthurium proves to be a fruitful choice. Another advantage of anthurium is that it produces flowers all the year round, ensuring year round income to farmers. They can also be easily fitted into the agro climatic and socio economic situation of Kerala.

The growing floriculture and tourism industries have a high demand for cut flowers. This creates a greater demand for anthurium flowers in the domestic as well as international markets making it a remunerative agri-business sector in the country. Anthurium has very high genetic potential due to its heterozygous nature. There is ample scope in hybridization programmes by harnessing its variability, incorporated with effective selection and multiplication of superior genotypes.

The major constraint in the large scale cultivation of anthurium is the insufficiency of quality planting material. Anthuriums are conventionally propagated by seeds. They are protogynous and hence they are naturally cross pollinated in nature. Hence seed propagated progeny are not true to type and not uniform in their performance. Approximately one third of total seedlings have to be discarded before flowering (Geier, 1990) as the performance and quality of seedling plants cannot be predicted. Seed propagation is also associated with long period of seed maturity of six to eight months, short viability of seeds and long juvenile period. Vegetative propagation methods in anthurium includes division of suckers, top cuttings and nodal stem cuttings. The number of propagules by these methods are as few as two suckers $year^{-1}$ and the rate of multiplication is also found to be low. Hence these traditional propagation methods fail to achieve the required multiplication rates to keep up with the market demand.

Hybrid superiority is an important criterion in the global cut flower market. For entering into the global flower trade, our hybrids should be on par with the international standards. In the highly competitive market scenario there is a high demand for new hybrids and new varieties. However, they remain in the market only

for a short period only. Market requirements during this peak demand period can be met only by the development of efficient *in vitro* system.

In this context, an *in vitro* propagation system with higher rate of multiplication becomes relevant. Various methods of *in vitro* propagation through diverse means such as axillary bud proliferation (Kunisaki, 1980), somatic organogenesis (Pierik *et al.*, 1974a; Pierik *et al.*, 1974b; 1979; Kuehnle and Sugii, 1991) and somatic embryogenesis (Geier and Reuther, 1981; Kuehnle *et al.*, 1992; Rajasekaran and Mohankumar, 1994) have been successfully developed in anthurium. Of these methods, somatic organogenesis has been found to be the best route towards *in vitro* propagation of anthurium.

Various anthurium genotypes show pronounced difference in the *in vitro* response. These differences in morphogenetic potential among genotypes of anthurium was evident from studies conducted by Pierik (1975) and Leffring *et al.* (1976). Protocols have been evolved only for a few genotypes of *A. andreanum* and protocol development should go hand in hand with the development of new hybrids in the market.

The improved propagation methods and hybridization programmes involving indigenous and exotic varieties of anthurium will no doubt increase the development of superior genotypes. Their mass multiplication through *in vitro* techniques can boost up the availability of disease free planting materials at a low price when compared to the imported planting materials. High multiplication rate in *in vitro* propagation is necessary to reduce the per plant cost and to increase the market potential. Tissue culture studies need to be taken up to develop refined *in vitro* mass multiplication protocols with maximum proliferation rate in anthurium. This can give a longer leap towards biotechnological advancement in the country and will be a mile stone in attaining self-reliable stand in the global floriculture industry.

In light of all the above, present study was attempted to assess the variability among twenty genotypes of *Anthurium andreanum* Linden hybrids so as to identify suitable hybrids with commercial qualities and to standardize a protocol for *in vitro* mass multiplication of these selected hybrids.

Review of Literature

2. REVIEW OF LITERATURE

In the present world scenario of ever increasing population and reduced cultivation area, high unit value crops such as anthurium is gaining popularity among the farmers. Today, floriculture is considered as a lucrative profession with higher potential return compared to the other fields and other horticultural crops. The demand of anthurium in the global floriculture market will go on increasing at a faster rate due to the liberalization of economy and globalization of trade.

Anthuriums are blessed with beautiful long lasting flowers having wide range of genetic variability. Therefore, anthuriums are cultivated in many parts of the world to produce cut flowers for both domestic and international markets. This commanding position of anthurium in the floriculture market can be exploited by harnessing its variability and by development of superior hybrids. This will in turn benefit the economy of our country.

In addition the climatic conditions of Kerala are ideal for anthurium cultivation. So formulating effective breeding programme to develop novel attractive anthurium hybrids and to improve the planting material availability to the growers through *in vitro* mass multiplication techniques has its own significance. In light of this, present investigation is formulated to assess the variability among anthurium hybrids and to mass multiply the superior genotypes through *in vitro* techniques. A brief review of works relevant to the study is presented below.

2.1 CULTIVATION PRACTICES

Anthuriums, like other aroids can be grown as indoor plants or in outdoors with mild climate in shades. For large scale cultivation a warm green house with 75 per cent shade is preferable as the morphological characters, flower production and quality of flowers are affected by the intensity of light (Mercy and Dale, 1994).

A field trial was conducted by Srinivasa (2006b) to study the influence of different shade levels on the growth and flowering of anthurium cultivar Honduras. The results showed that all the vegetative and reproductive characters recorded the maximum values at 80 per cent shade level. Prasad *et al.* (2001) reported that a low to medium range light intensity of 2,000 to 6,000 lux and shade level of 60-80 percent are required for proper growth and flowering of anthurium. Foliar application of GA_3 (500 ppm) enhanced growth and flowering in various anthurium cultivars (Dhaduk *et al.*, 2007).

Anthuriums are basically halophytes. The ambient conditions to be maintained for the growth and development of the crop includes atmospheric humidity of 70-80 per cent, relative humidity of 75 per cent and atmospheric temperature range between 25 and 28°C during the day and 18 and 22°C during the night with optimum temperature of 22° to 25°C. Prasad *et al.* (2001) and Prakash *et al.* (2006) reported that proper growth and development of anthurium occurs at a temperature range from 21- 24°C during day and 18.3°C during night with atmospheric humidity of 50- 80 percent.

According to KAU (2007), for anthuriums the tolerable level of light in tropics during summer is 20-30 per cent. Excessive light causes yellowing and scorching of leaves. Very low light intensity causes excessive vegetative growth and low flowering. It is preferable to grow anthurium in the open, under artificial shade structures for better growth and yield. Plant prefer to grow under a relative humidity of not less than 60 per cent and a temperature of not more than 30°C.

Growth medium of anthurium mainly includes components such as sand, cow dung, brick pieces, charcoal and coconut husk which ensures 100 percent drainage (Mercy and Dale, 1994). Based on a study conducted in anthurium by Keshav and Prashant (2008), the total number of leaves produced ranged from 5.00 (FYM + Brick piece) to 7.66 (FYM + cocofibre). Maximum value of leaf lamina diameter (20.40 cm),

leaf sheath length (24.40 cm), plant height (48.73 cm), early flower initiation (206.33 days), maximum number of spadices (4.66), largest flower (33.60 cm^2) and longest flower stalk (31.20 cm) were recorded in FYM + cocofibre growth medium. Cuquel *et al.* (2012) observed better flower quality in anthurium when grown in a growth medium containing wood shaving and organic compost in 1:1 ratio. Islam *et al.* (2013) proposed the use of well decomposed coconut husk with cow dung, sand and small stones at proportion of 2: 1: 1: 1 as growing media for anthurium. Irrigation needs to be done on daily basis.

Fertilization is a major factor that determines the growth, development and flower yield of anthurium. Nitrogen deficiency can lead to reduction in yield and flower quality (Dufour and Guerin, 2005) in anthurium. According to Srinivasa (2006a) split application of 30:20:40 g NPK resulted in the maximum values for characters such as number of sucker (1.88), number of spadices (2.25), leaf length (17.63 cm), leaf width (8.52 cm), spadix length (5.00cm) and spadix girth (6.04 cm) in all the anthurium genotypes studied. Split application of 20:15:30 g NPK also showed improvement in spathe length (9.88 cm) and spathe width (7.81 cm). Other physiological traits such as anthocyanin content, carotenoid, wax and chlorophyll content did not show any significant difference with various fertilizer levels.

In an experiment conducted to study the effect of integrated nutrient management (INM) practices in improvement of flower yield of *Anthurium andreanum cv.* Meringue, 4 per cent panchagavya + 5 per cent RDF was shown to improve the floral characters such as days to first flowering (206.50), flower yield per plant (5.90). A pronounced increase in vegetative traits such as plant height (32.40 cm), number of leaves per plant (6.20), number of suckers per plant (4.20) and number of aerial roots per plant (2.60), number of primary roots per plant (12.50) and root length (15.30 cm) were also observed (Waheeduzzama *et al*, 2007).

In case of anthurium seasonal variations have commendable influence on flower yield and flower quality. A study conducted by Agasimani *et al.* (2011 b) on ten varieties of anthurium in two seasons i.e. summer and winter showed significant difference. The variety Esmeralda interestingly performed well in both the seasons compared to other varieties.

Elibox and Umaharan (2012) recommended regular phytosanitation measures in anthurium on weekly basis and the infected plant materials and weeds needs to be removed and burnt to control further spread of pest and diseases. Timely flower harvest at three-fourths ripe stage needs to be practiced daily.

2.2 ANTHURIUM DIVERSITY

Genetic diversity in anthurium helps in effective improvement, management and conservation of anthurium cultivars by breeders. Consequently, evaluation of the variation is a prerequisite tool to breed a population. It also allows the exploitation of genetic variability for the production of new superior hybrids (Cabral *et al.*, 2010).

Uniformity among the flowers along with proper performance and high quality are the most important traits that ensure the success of a variety in the market (Stancato and Tucci, 2010). Therefore, in response to market demand breeding programmes need to be formulated for production of superior and new anthurium hybrids.

2.2.1 Vegetative Characters

2.2.1.1 Plant Height

Renu (2000) studied the vegetative charters of ten anthurium varieties and reported a significant variation in plant height. The highest plant height was recorded in the variety Midori Green (29.7 cm) and the lowest in Pompon Red (70.9). In a study conducted by Mayadevi (2001) on twenty genotypes of anthurium, plant height varied

from 45.5 cm in Midori Green variety to 96.67 cm in White variety. Asish (2002) reported significant variation in plant height among 50 genotypes of anthurium. Plant height ranged widely between 22.17 cm to 64.80 cm. The highest plant height of 44.00 cm was observed in the hybrid PR x DT and the lowest for variety Carrie (21.25cm) in the variability studies attempted by Premna (2003).

Six new anthurium cultivars were compared by Talia *et al.* (2003). Queen variety recorded the maximum plant height and the variety Santé recorded the lowest. Pravin (2004) stated that plant height varied between 30.80 cm to 76.17 cm among the 14 anthurium varieties. In a study conducted by Srinivasa and Reddy (2005) the cultivar Honduras showed the highest plant height of 40.94 cm.

Cristiano *et al,* (2007) observed differences in stem height among anthurium varieties in which the variety Cheers recorded the longest (72 cm) and Choco the shortest (56 cm). Shiva and Nair (2008) stated significant variation in plant height ranging from 4.27 cm in Taurus to 9.22 cm in Mirage variety.

Madhukumar (2010) stated that significant variation was present in plant height among 40 genotypes of anthurium ranging from 17.33 cm in Nitta Orange to 43.25 cm in Liver Red. Jadhav *et al.* (2012) studied anthurium varieties under fertigation and observed that the variety Esmeralda had maximum plant height of 57.58 cm. Anand *et al.* (2013) reported that all the anthurium cultivars differed significantly with respect to the plant height. The cultivar Ria Bamboo Red showed the highest plant height (73.90 cm) followed by Tropical Red (71.50 cm), Peach (67.90 cm) and Honduras (64.10 cm). The least plant height was seen for Margreta (33.70 cm) and Mearague Red (45.40 cm). Sheena (2015) reported that the cultivar Marjike had the maximum plant height (59.24 cm) and Red Amour showed the lowest plant height (29.29 cm).

Gopi (2016) stated that genotype Cascade White recorded the highest plant height (50.6 cm) followed by Chikoos (46 cm) and Liver Red (44 cm). The lowest plant

height was observed in Nitta Orange (22 cm) variety. An investigation by Anand *et al.* (2017) reported that the cultivar Claisto recorded the maximum plant height of 66.00 cm followed by Fantasia (59.50 cm), Acropolis (54.50 cm) and Rosa (52.50 cm). The least plant height was noticed in the variety Marysia (24.00 cm).

2.2.1.2 Leaf Area

Mercy and Dale (1994) opined that for commercial floral anthurium, leaves should be small to medium sized, narrow and elongated so as to get market preference. Leaf area showed significant variation in anthurium ranging from 66.26 cm^2 to 88.89 cm^2 in a study conducted by Mayadevi (2001).

Asish (2002) studied 50 genotypes of anthurium and observed that the leaf area ranged beetween 41.32 cm^2 to 323.77 cm^2. Premna (2003) identified the maximum leaf area of 301.10 cm^2 in the variety Acropolis White and the minimum leaf area of 113.62 cm^2 in the variety Carrie. Pravin (2004) analyzed the variability in leaf area in 14 varieties of anthurium. Leaf area varied between 91.97 cm^2 and 287.76 cm^2. A similar study by Shiva and Nair (2008) briefed that anthurium leaf size ranged from 2.95 to 5.36 cm and the leaf area ranged from 5.33 cm^2 to 16.43 cm^2. Femina *et al.* (2006) reported an average leaf area of 80.22 cm^2 in the cultivar Pistache.

Agasimani *et al.* (2011a) found that the variety Esmeralda had the maximum leaf area of 237.24 cm^2 and the variety Chias showed the minimum leaf area of 100.10 cm^2. Sheena (2015) reported that leaf size in various anthurium cultivars ranged between 92.18 cm^2 (Red Armour) to 239.65 cm^2 (Marjike).

Anand *et al.* (2013) recorded the variability in leaf length and leaf diameter among anthurium varieties and found that Liver Red recorded the highest leaf length of 40.84 cm compared to the variety Lambada (16.41 cm) that recorded the lowest. Throughout the experimental period Liver red recorded the maximum breadth (21.60 cm) and Verdun Red (7.5 cm) had the minimum. These significant difference in varietal

traits are governed by the genetic constitution and also due to variation in environmental conditions.

Variability analysis by Gopi (2016) revealed that the maximum value for leaf area was exhibited by Cascade White (450.276 cm^2) cultivar followed by Boroque (417.29 cm^2) and Chikoos (391.49 cm^2). The minimum leaf area was observed in the variety Nitta Orange. Anand *et al.* (2017) noticed the highest leaf length Calisto (33.54 cm) and the lowest in Midori (21.73 cm). The maximum leaf breadth of 25.50 cm was found in Fantasia cultivar and the minimum was recorded in Marysia (13.40 cm).

2.2.1.3 Internode Length

An ideal anthurium must produce short internodes so that the height of the plant is limited (Singh, 1987). On comparing the internode length of five parents and ten anthurium hybrids, intermodal length ranged from 1 cm to 1.5 cm among the parents and 1.02 cm to 1.34 cm among hybrids (Mayadevi, 2001).

Ravidas (2003) pointed out that the varieties Red Dragon (1.72 cm) and Candy Queen (1.78) has lowest internode length. A significant difference in internodal length varying from 1.48 cm in Acropolis White and PR x DT to 1.20 cm in Carrie variety was reported by Premna (2003).

Madhukumar (2010) on evaluating 40 anthurium genotypes concluded that the cultivar Agnihotri had the minimum internodal length (0.97 cm) and Esmeralda (2.02 cm) had the maximum. In a similar study Sheena (2015) reported highest internodal length in the variety Marjike (0.99 cm) and shortest internode length in Red Armour (0.45 cm) variety.

Gopi (2016) reported high internode length in the varieties Tropical Peach (1.30 cm), Hawaiian Orange (1.24 cm), Chocos (1.20 cm), Emperor (1.16 cm), Lady Jane (1.16 cm), Merengue White (1.12 cm) and Tropical Red (1.06 cm).

2.2.1.4 Days from Emergence to Maturity of Leaves

According to Mayadevi (2001) a significant variation was present in number of days from emergence to maturity of leaves of anthurium. In the study the variety Pink recorded 44.40 days while the minimum duration was recorded in the variety Honeymoon Red (41.40 days). The leaves of variety Carrie (26.25 days) matured faster compared to other anthurium varieties and the maximum duration was noticed in Pompon Red x Dragon's Tongue (40.25 days).

Madhukumar (2010) noticed that the hybrid Pompon Red x Orange Glory (25.89 days) took the least number of days for leaf maturity while the genotype Agnihotri (36.6 days) took the maximum number of days. Variability study in exotic anthurium cultivars revealed that the time taken from emergence to maturity of leaves varied from 24.55 to 35.95 days. Cynthia variety took the maximum days for maturity of leaves while the minimum was observed in the variety Red Armour (Sheena, 2015).

Gopi (2016) briefed that the maximum number of days for maturity of leaves were observed in Liver Red and the genotype Boroque exhibited the lowest number days for maturity of leaves (24.4 days).

2.2.1.5 Number of Leaves Spadices^{-1} Plant^{-1} Year^{-1}

Cultivated anthuriums generally produce flowers all-round the year. An increase in number of leaves simultaneously results in increase of flower production in anthurium. Flowers are produced from the leaf axil at the rate of one flower from each leaf axil (Singh, 1987).

Mayadevi (2001), on studying 20 genotypes of anthurium reported that number of spadix produced ranged from 4.67 to 8. The variety Honeymoon Red registered the highest number of spadices. In a study conducted by Asish (2002) on anthurium

hybrids, the genotype KO x DT (7) produced the highest number of leaves and spadices while the minimum was observed for PR x DT and TR x MW (3).

Premna (2003) pointed out that the maximum production of spadices in the varieties Acropolis White, Tropical Red, OO x PR and PR x DT (6). The minimum production was observed in the variety Carrie. Pravin (2004) noticed the maximum number of spadices in the variety Liver Red (7) and the minimum in hybrids FR x MW (1). Madhukumar (2010) reported that variety Rembolina (7.42) accounted the highest number of spadices and the variety Chocos (4.55) showed the lowest number. Agasimani *et al.* (2010) disclosed that Esmeralda produced the maximum number of leaves per plant (5.20) and the variety Grace registered the minimum number of leaves per plant (3.05). Variations in leaf production among the anthurium cultivars occur due to their inherent genetic character.

Islam *et al.* (2013) stated that the cultivar Titiaca (7.2) recorded the highest number of flowers per plant compared to other anthurium cultivars. Sheena (2015) reported that the cultivar Paradise (8.75) produced the highest number of leaves while Red Armour and Anastacia cultivars produced the lowest number of leaves. In a variability study, Gopi (2016) affirmed that the variety Honeymoon Red (9) produced the highest number of leaves which was followed by Vezuvious Red (8.4), Tropical Peach (7.6), Gold Spark (7.2), Nitta Orange (7.2) and Hawaiian Orange (7.2).

2.2.1.6 Number of Suckers Plant^{-1}

Commercial anthurium varieties and hybrids have low suckering ability and propagation through these suckers are too slow (Mercy and Dale, 1994). Foliar application of Benzyl Adenine (BA) at 500 -1000 ppm or using Gibberellic Acid (GA_3) was found to have a pronounced effect on suckering ability. Abdussammed (1999) also confirmed the increased suckering ability by using growth regulators in anthurium. The

ability to produce sucker is considered to be an important trait for the selection of superior anthurium genotypes.

Mayadevi (2001) noticed that the variety Pink and Lady Jane had produced the maximum number of suckers (4) while Meringue White, Nitta Orange and Tropical Red exhibited lowest suckering. The variety Red Dragon (3.22) exhibited maximum suckering. Ravidas (2003) observed that the maximum number of suckers of 3.22 was produced by the variety Red Dragon and the minimum number of suckers by the genotype Agnihothri (2.33).

Pravin (2004), analyzed 14 genotypes of anthurium and concluded that the genotype Liver Red (4) produced the maximum number of suckers and the minimum number of suckers were found in the cultivars Acropolis White and Tropical Red. Agasimani *et al.* (2010) claimed that the variety Ivory showed the maximum suckering ability (4.14). The least sucker production was observed in the variety Jewel (0.49). Out of forty genotypes of anthurium studied by Madhukumar (2010), the cultivar Liver Red registered the maximum sucker production while Lady Jane and Esmeralda exhibited the minimum sucker production.

The variety Salmon Queen produced the maximum of seven suckers per year followed by Marjike and Hillary (Sheena, 2015). Cultivars Lima White (4.21) and Peach (3.60) recorded the maximum sucker production which was superior to Verdun Red and Linda. The cultivar De mole produced only two suckers per year (Anand *et al.*, 2013).

Gopi (2016) observed the highest number of sucker production in the variety Honeymoon Red (1.6) followed by Emperor (0.8), Lady Jane (0.8), and Dragon's Tongue. Few genotypes such as Agnihotri, Chocos, Esmeralda, Gold Spark, Hawaiian Orange, Honduras Red, Nitta Orange, Pistache, Tropical Red and Mauritius Orange failed to produce suckers.

Anand *et al.* (2017) reported the maximum number of sucker production in the cultivars Lumina (3.14) and Rosa, Titicaca and Angel (3.04), while Acropolis had the minimum number of suckers per plant (1.01). The suckering behavior varied among the cultivars as these characters are genetically controlled.

2.2.1.7 Colour of Petiole and Young Leaf

In *Anthurium andreanum*, petiole as well as young leaf colour ranges between brownish green and brown (Asish, 2002). A significant difference in young leaf colour and petiole colour was observed from brown to reddish brown to greenish brown to green and to light green (Madhukumar, 2010).

Anthurium cultivars such as Mozaik Fresh, Orange Glory, Paradise, Rosette, and Tropical Red had brownish green leaves while the leaves were reddish brown in Dragon's Tongue, Marjike and Liver Red cultivars (Sheena, 2015). All the varieties except Elizabeth, Paradise, Mozaik Fresh, Marjike, Tropical Red and Rosette had green petiole. A reddish brown petiole was observed only in Liver Red variety.

Gopi (2016) stated that colour of petiole and young leaves showed wide variation among the twenty five anthurium genotypes taken for the study. Petiole colour ranged from green to greenish brown and reddish brown while the colour of young leaves varied from green to light green and to reddish green and brown to reddish brown.

2.2.1.8 Pest and Disease Incidence

Bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* and anthracnose caused by *Colletotrichum gloeosporioides* are the major diseases that can have devastating effect on yield in anthurium. Blackening of leaf axil and stem is the symptom produced due to bacterial blight. Bacterial blight equally infect anthurium leaves and the inflorescence. Anthracnose can be identified by the presence of black circular spots on leaf and spadix (KAU, 2007 and Sheela, 2008).

Pests like aphids, scales, thrips and spider mites infect anthurium plants. Damage to anthurium foliage caused by snails and slugs was also reported in anthurium by Mercy and Dale (1994), KAU (2007) and Sheela (2008). Leaf eating caterpillar also feeds on anthurium leaves and cause damages as reported by Gopi (2016).

2.2.2 Floral Characters

2.2.2.1 Quantitative Traits

2.2.2.1.1 Days from Emergence to Maturity of Inflorescence

Mayadevi, (2001) reported that the number of days taken from emergence to maturity of inflorescence in anthurium hybrids ranged from 41 to 54 days. In case of varieties the maximum number of days from emergence to maturity of inflorescence was recorded in Honeymoon Red (50.60 days) while the variety Chilli Red (44.60 days) recorded the least number of days.

Ravidas (2003) claimed that the genotype Agnihotri (25.33 days) exhibited the maximum days from emergence to maturity of inflorescence and the minimum number of days was observed in the varieties Lima White and Candy Queen (19 days). Madhukumar (2010) analyzed 40 anthurium genotypes and concluded that the variety Liver red recorded 33.39 days from emergence to maturity of inflorescence. A variability study by Sheena (2015) concluded that the maximum days for inflorescence maturity was observed for the variety Cynthia (26.50 days) while the variety Marjike (20.75 days) had the least duration.

Gopi (2016) observed significant variation in duration of inflorescence maturity in anthurium cultivars. Days from emergence to maturity of inflorescence was the highest for the cultivar Agnihotri (35.2 days) and the lowest for the cultivar Can Can (28.4 days).

2.2.2.1.2 Spathe Size

United States Department of Agricultural Standards described that anthurium flowers can be graded according to the average length and width of spadix i.e., as miniature (under 8 cm), small (8-10 cm), medium (10-13 cm), large (13-15 cm) and extra-large (15 cm) (Singh, 1987). Mayadevi (2001) observed a wide variation in spathe size among 100 anthurium genotypes and reported an average spathe length of 10.80 cm and spathe width of 7.76 cm were recorded among the genotypes.

Cristiano *et al.* (2007) evaluated seven anthurium varieties and claimed that the variety Premier had the largest spathe compared to others. Shiva and Nair (2008), on studying the performance of 14 anthurium genotypes, concluded that the spathe size varied from 5.8 to 8.52 cm. The maximum spathe size was observed for Mauritious variety followed by the variety Honey. On analyzing 40 genotypes of anthurium, Madhukumar (2010) reported that the maximum spathe size was recorded in Acropolis White (123.43 cm^2) followed by Geisha white (118.18 cm^2) and Carrie (115.44 cm^2).

Agasimani *et al.* (2010) studied the performance of anthurium varieties and noticed that the variety Esmeralda had the maximum spathe length of 15.71 cm and the variety Ivory (7.54 cm) had the minimum spathe length. The maximum spathe breadth of 16.18 cm was registered in the variety Titicaca and the minimum spathe breadth was recorded in Aymara (7.04 cm) variety.

Islam *et al.* (2013) stated that the characters such as spathe length and breadth showed significant variation among the anthurium varieties. Variety Triticaca registered the maximum spathe length of 15.3 cm while the minimum spathe length of 6.3 cm was shown by the variety Ivory. The maximum spathe breadth of 13.5 cm was recorded in variety Titicaca and the minimum spathe breadth of 6.2 cm was noticed in the variety Jewel. Anand *et al.* (2013) evaluated the leading cut flower anthurium varieties and concluded that the variety Temptation has the highest spathe length (18.10

cm) followed by Peach (16.50 cm) and Ria Bamboo Red (14.70 cm). Mearague Red (7.50 cm) and Sunset Orange (8.20 cm) varieties exhibited the lowest spathe length among the studied varieties. Temptation variety also had the highest spathe width of 15.00 cm while the cultivar Mearague Red was found to have the least spathe width of 6.30 cm. Lima *et al.* (2014) reported that the *Anthurium andraeanum cv. 'Apalai'* produced large sized spathe with a length of 11.78 cm and width of 14.40 cm.

A variability study conducted by Gopi (2016) revealed that the spathe area ranged from 42.88 cm^2 to 101 cm^2. The maximum spathe size was exhibited by the variety Boroque (101 cm^2) and the minimum spathe size was reported in the variety Lady Jane (42.88 cm^2).

Anand *et al.* (2017) conducted a performance analysis in anthurium varieties and concluded that the cultivar Fantasia and Calisto has the highest spathe length (12.67 cm) followed by Lumina (12.50 cm). Spadix width ranged between 5.00 cm and 13.50 cm. Variety Fantasia (13.50 cm) recorded the highest spathe while the cultivar Marysia (5.00) cm had the least spathe width.

2.2.2.1.3 Spadix Length

Commercial markets prefer anthuriums with short slender candles. In a study conducted by Praneetha *et al.* (2002) on two *Anthurium scherzerianum* and eight *Anthurium andreanum* genotypes, the cultivar AA-2 exhibited the maximum spadix length of 13 cm. In a similar study Pravin (2004) identified the short candles in MO x KR (1) (3.83 cm) and PR x LR (1) (4.97 cm) hybrids.

Srinivasa and Reddy (2005) reported that among five cut flower cultivars of anthurium the maximum spadix length (6.57 cm) and girth (7.93 cm) were registered in the cultivar Honduras. Madhukumar (2010) identified that spadix length was the maximum for the hybrid OG x DT and the minimum for LR x PR. The variety

Esmeralda was found to have the maximum spadix length of 8.24 cm while the minimum was recorded in the variety Grace (3.35 cm) (Agasimani *et al.*, 2011a).

The variety Temptation showed the highest spadix length of 11.5 cm followed by Sweet Heart (9.93 cm) and the shortest spadix with a length of 5.67 cm was reported in Mearague Red (Anand *et al.*, 2013). Similarly Lima *et al.* (2014) reported that the spadix of *Anthurium andraeanum cv. 'Apalai'* measured 8.45cm. According to Sheena (2015) spadix was most lengthy in the variety Paradise (5.73 cm) and shortest in Anastasia (4.03 cm). In a similar study Islam *et al.* (2013) reported that the variety Titicaca had tallest spadix length of 8.1 cm and the shortest in the variety Ivory (4.5 cm).

The maximum spadix length was shown by the cultivar Chikoos (8.76 cm) followed by Cascade White (7.18 cm). The lowest mean spadix length was observed in the cultivar Honeymoon Red (2.2 cm) followed by Pistache (3.98 cm) (Gopi, 2016). Anand *et al.* (2017) identified that the spadix was the shortest in the cultivar Marysia (4.05 cm) and was the longest in the cultivar Fantasia (6.59 cm) followed by Midori (6.30 cm) and Calisto (6.08 cm).

2.2.2.1.4 Number of Flowers Spadix^{-1}

Anthurium flowers are bisexual in nature and are arranged in a series of spirals on the spadix (Croat and Bunting, 1978). Anthurium produces numerous number of flowers ranging from 150 to 350, arranged in acropetal succession on its candle (Mercy and Dale, 1994).

Mayadevi (2001) recorded the maximum number of flowers per spadix in the cultivar Pink (600) and the minimum flowers in Chilli Red (372). Among the hybrids, the cross Pink x Liver Red exhibited the minimum number of flowers per spadix (400) and the cross Pink x Kalympong Red and Honeymoon Red x Dragon's Tongue recorded the maximum number of flowers per spadix (600).

The hybrid Kalympong Red x Liver Red showed the maximum number of flowers per spadix of 518.67 while LR x PR and the variety Corolix recorded the minimum number of flowers (Madhukumar, 2010). Sheena (2015) briefed that the cultivar Hillary had the highest number of flowers (408.25) and the lowest number of flowers were reported in the cultivar Red Armour (164.33).

Gopi (2016) affirmed that the genotype Chikoos had the maximum number of flowers on the spadix (452.2) followed by Dragon's Tongue (446.6) and Arun Gold (414.8). Lesser number of flowers per spadix were recorded in the variety Esmeralda (246.2) followed by Chekas (251.6), Pistache (251.8) and Gold Spark (252).

2.2.2.1.5 Life of Spadix

In anthuriums, fertilized inflorescence exibit increased life (4-7 months) than that of unfertilized spathe (2 months) (Mercy and Dale, 1994). Senescence in anthurium is identified by yellowing of peduncle followed by withering of candle and the spathe. Increased longevity of spadix was noticed by Valsalakumari *et al.* (1998) by the foliar application of GA_3 at a concentration of 1000 ppm in the cultivar Agnihotri.

Renu (2000) studied the life of fertilized spadices and it ranged from 3.8 to 7.5 months. Premna (2003) compared the spadix life of various anthurium hybrids and varieties. The genotype PR x DT had the highest life span of 101.50 days and the lowest life span of 59.50 days was noticed in the cultivar Carrie.

On analyzing 14 genotypes of anthurium, Shiva and Nair (2008) concluded that the maximum life span varied widely among the genotypes. The varieties Wrinkled Orange and Honey showed the maximum life span compared to other cultivars. Another study by Madhukumar (2010) reported much higher variation in life of spadix among varieties and hybrids. The genotype PR x DT recorded the maximum spadix life span of 101.33 days while the variety Gold Spark had the minimum spadix life of 46.83 days. Spadix life was the maximum in Tropical Red (91.2 days) followed by

Liver Red (88.4 days) and was found to be the lowest in the variety Vezuvious Red (48.2 days) in a variability study conducted by Gopi (2016).

2.2.2.1.6 Days to Initiation of Female Phase

Anthurium inflorescence develops female flowers in the initial stage (Croat, 1980). The receptive female phase is identified by the presence of minute droplets of stigmatic fluid on the flower. Maturation of flower initiates from the base of the candle and progresses towards the apex. In an inter varietal hybridization study, Renu (2000) concluded that the mean number of days to initiation of female phase ranged from 3.60 days in Lady Jane Red variety to 6.80 days in the variety Mauritius Orange. Mayadevi (2001) analyzed 100 anthurium genotypes and reported that days to initiation of female phase varied from 3.60 to 6.20 days among anthurium hybrids.

Days to initiation of female phase ranged from 4.33 to 9.27 days among 14 genotypes of anthurium (Pravin, 2004). Mean value for days to initiation of female phase was found to be the maximum for the genotype Boroque (10.55) in a study conducted by Madhukumar (2010).

Sheena (2015) reported that the mean number of days to initiation of female phase ranged from 7.33 days (Elizebeth) to 12.67 days (Anastacia). Gopi (2016) conducted a variability study and reported that the maximum number days for female phase initiation was observed in the cultivar Boroque (10.8 days) followed by Merengue White (8.8 days) and Hawaiian Orange (8.2 days). Cultivar Pistache and Vezuvious Red (4.2 days) registered the lowest number of days for initiation of female phase.

2.2.2.1.7 Duration of Female Phase

Receptive female phase is identified by the viscous exudate on the stigmatic surface and lasts till the stigmatic surface is dried (Mercy and Dale, 1994).

Mayadevi (2001) reported that the number of days for female phase was the maximum in the Kalympong Red (13.4 days) variety and the minimum in the variety Pink (7.4 days). According to Premna (2003), among 14 genotypes of anthurium studied, the duration of female phase ranged from 5.50 to 9 days.

Gopi (2016) pointed out that the cultivar Liver Red exhibited the maximum duration for female phase of 9.4 days followed by Cascade White (8.2 days). The genotype Boroque (5.6 days) showed the minimum duration of female phase.

2.2.2.1.8 Duration of Interphase

Interphase is initiated after drying of stigmatic surface. On analyzing anthurium varieties, Liver Red had the longest interphase while the shortest interphase was recorded in Meringue White (Renu, 2000). Mayadevi (2001) opined that the duration of interphase ranged from 7.80 days in Chilli Red to 11.20 days in Pink variety among the parental genotypes and the duration ranged from 9.39 to 12.60 days in hybrids.

Among 50 anthurium genotypes studied by Asish (2002), interphase duration ranged from 2.00 to 11.67 days. Premna (2003) reported that the interphase duration was the minimum in the anthurium variety Carrie (4.50 days) and the maximum interphase duration of 9.25 days was observed in the hybrid Pompon Red x Dragon's Tongue. Duration of interphase was found to be the shortest in the variety Red Dragon (3.67 days) while the longest interphase of 20 days was exhibited by the variety Lima (Ravidas, 2003).

On analyzing 14 anthurium genotypes Pravin (2004) reported that the average number of days for interphase ranged from 2.33 to 6.83 days. Madhukumar (2010) stated that the variety Nitta Orange (9.22 days) had the longest number of days for interphase and the lowest mean number of days was noticed in the genotype Vezuvious Red (4.5 days). Anthurium variety Liver Red registered the longest interphase duration of 9.6 days followed by Chikoos (8 days).

The least number of days for interphase was accounted for the variety Vezuvious Red (3.4 days) followed by Pistache (4 days), Gold Spark (4 days), Tropical Peach, Cascade White and Can Can (4.2 days) (Gopi, 2016).

2.2.2.1.9 Duration of Male Phase

In anthurium, exertion of anther starts from the base and later it progresses towards the tip. Average days for male phase ranged from 5.4 days in Mauritius Orange variety to 10.4 days in Tropical Red (Renu, 2000). Mayadevi (2001) on studying 100 anthurium genotypes, reported that the duration of male phase ranged from 5 days to 7.20 days among these genotypes. Among fourteen genotypes of anthurium, Pravin (2004) observed that average duration of male phase ranged from 5.33 days to 10.83 days in the genotypes studied.

Madhukumar (2010) asserted that the maximum male phase duration was found in the hybrid genotype Tropical Red x Meringue White (10.89 days) followed by Acropolis white (10.36 days), Arun Gold (10.33 days) and Jewel cultivars. According to Gopi (2016), the duration of male phase was the longest in Arun Gold (9.8 days) variety followed by Chekas (9.2 days), Honduras Red (9.2 days), Cascade White (9 days), Dragon's Tongue (9 days), Chocos and Emperor (8.8 days). The lowest male phase duration was observed in Esmeralda (4.4 days) followed by Boroque (5.2 days) and Pistache (5.23 days).

2.2.2.1.10 Inclination of Candle with the Spathe

Mercy and Dale (1994) stated that ideal anthurium varieties should have angle of inclination of candle less than 45^0, which make them suitable for easy packing and increases their market value. Mayadevi (2001) reported that the angle of inclination of the spadix with the spathe ranged from 21^0 in Kalympong Red to 78.20^0 in Honeymoon Red. In case of hybrids, Pravin (2004) reported that the lowest candle to spathe angle was found in PR x MO (26.10^0) and highest in MO x KR (1) (70.07^0).

An evaluation of forty genotypes in anthurium by Madhukumar (2010) revealed that the inclination of the spadix with the spathe varied from 35.33^0 (Orange Glory) to 75.67^0 (Vezuvious Red). Islam *et al.* (2013) revealed that a superior variety called Aymara has the minimum angle of inclination of candle with the spathe (30°) while the variety Jewel was found to be inferior and exhibited the maximum angle of 60^0.

Sheena (2015) compared nine exotic anthurium cultivars and concluded that the highest angle of inclination of candle with spathe was identified in the variety Salmon Queen (80^0) followed by Mozaik Fresh (78.33^0), Marjike (78.25^0) and Cynthia (75^0) varieties. The minimum angle of inclination was reported in the cultivar Paradise (58.50^0). Angle of inclination that is ideal for market was found in the cultivars like Rosette (41^0), Tropical Red (41.33^0) and Orange Glory (44.67^0).

Angle of inclination of candle with the spathe was the maximum for the genotype Vezuvious Red (72.6) followed by Chikoos (70.4) and Gold Spark (67.8) varieties. The minimum inclination of candle with the spathe was identified in the variety Mauritius Orange followed by Honeymoon Red (44.8), Tropical Red (46) and Honduras Red (46.2) (Gopi, 2016).

2.2.2.1.11 Total Anthocyanin Content

Spathe colour in anthurium varies widely with the concentration of anthocyanin. Anthuriums with white colour lack anthocyanins and contain pigment called as flavone c-glycosides (Williams *et al.,* 1981). Variability study in anthurium conducted by Mayadevi (2001) briefed that the variety Liver Red showed the highest anthocyanin content (386.56 mg g^{-1}) and the lowest was recorded in the cultivar Pink (121.38 mg g^{-1}). In hybrids, the maximum anthocyanin content was observed in Kalympong Red x Chilly Red (330.95 mg g^{-1}) and the minimum in Honeymoon Red x Liver Red (146.03 mg g^{-1}). Mean anthocyanin content was found to be 234.86 mg g^{-1} on studying 50 anthurium genotypes (Asish, 2002).

Premna (2003) claimed that mean anthocyanin content was the lowest in the variety Acropolis White (10.09 mg g^{-1}) and it was the highest in Honduras (259.18 mg g^{-1}). Madhukumar (2010) studied forty anthurium genotypes and noticed that significant difference existed in the trait anthocyanin content and was reported to be in the ranging between 9.73 and 482.05 mg g^{-1}.

Sheena (2015) reported that the variety Red Armour had the highest anthocyanin content of 20.37 mg g^{-1} and the lowest anthocyanin content of 2.72 mg g^{-1} was noticed in the variety Cynthia. Gopi (2016) opined that anthocyanin content recorded the highest in the cultivar Liver Red (339.52 mg g^{-1}) followed by Dragon's Tongue (273.172 mg g^{-1}) and Honduras Red (242.44 mg g^{-1}). Lower anthocyanin content was reported in the cultivar Cascade White (24.01 mg g^{-1}) followed by Merengue White (24.78 mg g^{-1}), Pistache (26.23 mg g^{-1}), Gold Spark (26.48 mg g^{-1}) and Lucia Pink (32.67 mg g^{-1}).

2.2.2.1.12 Vase Life

The flowers were harvested after complete unfolding of the spathe (Kamemoto 1962). The higher sugar intake in the petal cells from the vase solution is known to enhance water uptake due to osmotic pull in cut flowers (Ho and Nicholus, 1975). The balance between the rate of water uptake and rate of transpiration have direct relation with turgidity and keeping quality of anthurium flowers (Mujaffar and Sankat, 1993).

In a variability study by Shriram (2008) the vase life of the variety Esmeralda (21days) was found to be the maximum and Ivory (10 days) had the minimum shelf life. Agasimani *et al.* (2011b) reported that vase life of flowers was significantly differing among the anthurium varieties. Anand *et al.* (2013) reported that among the anthurium varieties studied, the maximum vase life in water was observed in Honduras (24 days) followed by Temptation (23 days), Ria Bamboo Red (22 days), Verdun Red (22 days) and Peach (22 days). The minimum vase life in water was recorded in the

cultivar Lambada (14 days). Vase life of flower is said to be related to the inherent capacity of the cultivar.

Harishshivalingappa *et al.* (2013) claimed that significant difference exist between the effects of different holding solutions on post-harvest life of cut anthurium spikes. Vase life also differed among the varieties and cultivars. Flowers kept in distilled water recorded the minimum vase life of 10 days in the cultivar Marysia. Flowers held in distilled water also showed the minimum water loss (3.58 g per flower) on ninth day of vase life study.

Islam *et al.* (2013) identified that the vase life was the maximum in the variety Titicaca (16 days) and the minimum vase life was recorded in Caesar (10 days). All the other varieties studied were significantly different from each other. Sahare and Alka (2015) observed a vase life of 11.86 days in anthurium when the cut flowers were held in water. Anand *et al.* (2017) viewed that post-harvest life or vase life of cut flower is the ultimate requirement of any successful flower production technology. In anthuriums vase life was found to be a genetically controlled trait and was highly influenced by the size of flowers and stage of flower harvest.

2.2.2.1.13 Number of Inflorescence Year^{-1}

Renu (2000) claimed that the number of spadices per plant per year was the highest in Lady Jane (7.6) cultivar followed by Liver Red and Pompon Red. A varying degree of flower yield in anthurium cultivars was reported by Ehrenberger *et al.* (2003). According to Pravin (2004) the cultivar Liver Red exhibited an average of 7.00 spadices followed by Orange Glory and Acropolis White (6.33).

Agasimani *et al.* (2010) reported that among different anthurium varieties, the maximum number of flowers produced per plant per year was noticed in the variety Esmeralda (9.33) and the minimum in the variety Ivory (3.33). Madhukumar (2010) accounted the highest annual spadix production in the variety Rembolina (7.42) and

the least in the variety Chocos (4.83). Sheena (2015) reported the maximum flower yield in the genotype Salmon Queen (6.33) followed by Marjike (6.17) and Paradise (5.92).

Islam *et al.* (2013) opined that the variety Titicaca (7.2) produced the maximum number of flowers per plant, while the minimum flower production was recorded in the variety Ivory (4.1). According to Lima *et al.* (2014) cultivar *Anthurium andraeanum cv. Apalai* produced an average of 6.48 inflorescences per year.

Gopi (2016) analyzed the floral characters of several anthurium genotypes and concluded that the variety Hawaiian Orange (5) had the maximum flower yield followed by Cascade White (4.8) and Lady Jane (4.8). The lowest flower yield per year was reported in the variety Mauritius Orange and Lucia pink (1.2) followed by Agnihotri (1.6), Honeymoon Red (1.8), Tropical Peach (2), Merengue White (2.2) and Nitta Orange (2.4).

2.2.2.2 Qualitative Traits

2.2.2.2.1 Spathe Colour

Anthuriums occur in wide range of spathe colours including white, red, orange, pink and coral (Kamemoto *et al.*, 1988). Anthocyanins are derived from pelargonidin and cyanidin are the main colour giving pigments in the anthurium spathe (Iwata *et al.*, 1979). Coral and orange spathe colour occur due to presence of pelargonidin 3-rutinoside while pink and red spathe colour occur due to combined effect of pelargonidin 3-rutinoside and cyanidin 3-rutinoside. Anthocyanins are absent in white spathe and white colour is due to colourless flavonones and glycosides.

Spathe colour is regulated by gene action where the two genes, M and O, are responsible for anthocyanin production and recessive epistasis of the O locus exists over the M locus in anthurium. M gene controls cyanidin 3-rutinoside production while

O gene controls pelargonidin 3-rutinoside production. These two genes regulate the production of pink, orange, red, coral and white colouration of the spathe. Presence of both M and O gene result in pink and red colour. If both the genes are in double heterozygous condition the spathe colour will be pink. Double recessive 'mmoo' condition or occurrence of 'OO' in combination with M gene can result in white spathe colour. Orange and white breed true (Marutani *et al.* 1988). Mercy and Dale (1994) reported that as the age of the plant increases, the spathe colour gradually decreases. Increased fertilization can lead to production of green spathe colour and the spathe becomes photosynthetic in nature.

Renu (2000) observed significant variation in spathe colour from deep maroon to white. Anthuriums showed significant variation in red coloured spathe ranging from dark red (Chilli Red) to red (Honeymoon Red) (Mayadevi, 2001). Deep maroon to white coloured spathe colour was observed in a study conducted by Asish (2002). Asish and Mayadevi (2001) claimed that the higher anthocyanin content in the spathe resulted in deep maroon colour while lower concentration resulted in pink coloured spathe. In addition to these colour variants, Madhukumar (2010) also observed double coloured spathe.

A variability study on the twenty five anthurium genotypes by Gopi (2016) revealed that anthurium varieties produced deep maroon, dark red, bright red, red, pink, peach, green, bright orange, orange and chocolate brown coloured spathe.

Anand *et al.* (2017) studied various anthurium cultivars and reported that the genotype Acropolis (pure white), Angel (white), Calisto (red), Cheers (pink), DO 32 (deep orange), Fantasia (greenish), Fire (red), Lumina (pale white), Marysia (creamy yellow), Midori (green), Rosa (whitish pink), Titicaca (white with green margins) and Xavia (red) showed significant difference in spathe colour.

2.2.2.2.2 Spadix Colour

Renu (2000) analyzed the spadix colour in ten varieties of *A. andreanum* and observed colour variations such as yellow, light yellow, pink, light pink, green and light green. Similarly Asish (2002) observed spadix colours like yellow, yellowish white, pink, reddish pink, light pink, pinkish yellow, pinkish white and cream.

Madhukumar (2010) documented different spadix colours in anthrium varieties and the colours ranged from light yellow, yellow, pink, maroon, yellowish white and greenish yellow to creamy white in various varieties. Wide variation in spadix colour according to the cultivars was also reported by Sheena (2015).

Anand *et al.* (2017) studied various anthurium cultivars and reported that the genotype Acropolis (creamy yellow), Angel (whitish and greenish tinge), Calisto (whitish and greenish tinge at the tip of spadix), Cheers (Peach with green), DO 32 (creamy yellow), Fantasia (green), Fire (creamy yellow), Lumina (Violet), Marysia (whitish and greenish tinge at the tip of spadix), Midori (green), Rosa (pinkish with yellowish tinge at the tip of spadix), Titicaca (green) and Xavia (red) exhibited significant difference in spadix colour.

2.2.2.2.3 Type of Inflorescence Axis

Mercy and Dale (1994) stated that short and straight inflorescence axis is the characteristic feature of superior anthurium varieties. Mayadevi (2001) observed difference in length of inflorescence axis and categorized it as long, straight and very strong. Pravin (2004) opined that the varieties like Liver Red, Orange Glory, Acropolis White and Tropical Red had the type of inflorescence axis that was suitable for commercial cultivation.

On analyzing forty anthurium genotypes Madhukumar (2010) concluded that wide range of variation in the type of inflorescence which ranged from long thick

straight, long thick curved, long thin straight, long thin curved, medium thick straight, medium thick curved, medium thin curved, short thick straight, short thin straight, short thick curved and short thin curved. Sheena (2015) reported variation in type of inflorescence axis in anthurium and categorized it as short to long, thin to thick and curved to straight.

In a study conducted by Gopi (2016) inflorescence axis varied from thick straight, long thick curved, long thin curved, short thick straight, medium thick straight and medium thin curved. The ideal type of straight long inflorescence axis was observed in Liver Red, Tropical Red, Tropical Peach, Mauritius Orange, Honduras Red and Lady Jane cultivars.

2.2.2.2.4 Pollen Emergence Pattern

Renu (2000) viewed that anther dehiscence occur during early morning between 8 to 10 A.M while no pollen was noticed in the varieties like Nitta Orange, Pompon Red and Midori Green. Pollen emergence followed a regular pattern throughout the year except in the variety Meringue White.

Madhukumar (2010) reported that pollen emergence was the maximum in October to December months and it was much lower in March to June season. Sheena (2015) observed the maximum pollen emergence in the months of October to January and the minimum in the months from March to May.

Similarly, Gopi (2016) stated that there was high pollen emergence in November to February months and low pollen emergence was observed during March to June months. The increase in atmospheric temperature during summer months had a retarding effect on pollen emergence.

2.2.2.2.5 Pollen Shape

Variable texture and shape of pollen was identified in *A. andreanum* in a study conducted by Croat (1980). According to Madhukumar (2010), among 40 anthurium varieties studied, round pollen was the most predominantly observed and few were oval in shape.

Sheena (2015) observed that the pollen shape varied from round to oval in all the varieties studied. Similar reports on occurrence of round to oval pollen were also made by Gopi (2016) on studying 25 anthurium genotypes.

2.2.2.2.6 Pollen Colour

Croat (1980) recorded that there was a wide range of pollen colour ranging from orange, yellow, purple and white. The colour of the pollen fades as the age of the flower increases.

Gopi (2016) reported that pollen colour ranged from cream to white among 25 genotypes of anthurium.

2.2.3 Statistical Analysis

2.2.3.1 Variability analysis

Variability in a genotype is the prerequisite for any genetic improvement programme. In the development of superior anthurium hybrids, variability studies can act as basis for selection. Allard (1960) stated that high variability in a crop indicated the scope for improvement and selection. Renu (2000) studied 10 *Anthurium andreanum* varieties and reported high PCV and high GCV for the characters plant height, position of candle, days to initiation of female phase, number of days in female phase and spathe size.

On studying 100 genotypes of anthurium, Mayadevi (2001) concluded that among the characters studied, the highest PCV and GCV were observed for the characters such as number of suckers per plant followed by leaf area, inclination of candle with the spadix and width of leaf blade. The minimum PCV and GCV were observed for the character number of spadices per plant per year. High variability was accounted for the traits total anthocyanin content followed by pollen fertility, inclination of candle to the spathe, number of days for interphase and leaf area (Asish, 2002). Variability studies conducted by Premna (2003) revealed that high PCV along with high GCV were present for number of suckers per plant, inclination of candle and anthocyanin content.

According to Pravin (2004) high GCV and PCV were observed for the characters such as number of suckers per plant, pollen fertility and duration of male phase. The highest variability in anthurium cultivars were observed for the character anthocyanin content followed by pollen fertility, leaf area, spathe size and spadix length (Madhukumar, 2010).

Sheena (2015) opined that the character leaf area has the highest GCV and PCV of 30.21 per cent and 31.79 per cent. High variability was also observed for the traits such as number of flowers spadix^{-1}, leaf area and plant height. Tamuli *et al.* (2015) opined that Phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all vegetative and floral characters indicating the environmental influence on these characters.

Similarly, variability studies by Gopi (2016) expressed high PCV, coupled with high GCV for the characters namely pollen fertility, number of flowers year^{-1}, anthocyanin content, suckers plant^{-1} and leaf area. These characters could be improved by direct selection. The lower PCV and GCV were reported for the characters such as days from emergence to maturity of leaves, days from emergence to maturity of inflorescence and duration of female phase. The maximum difference between PCV

and GCV was observed in the characters like number of suckers plant^{-1}, leaf area, plant height, number of leaves spadices^{-1} plant^{-1} year^{-1} and this indicated the influence of environment on these characters.

2.2.3.2 Heritability and Genetic Advance

Heritability and genetic advance are important parameters for selection. Heritability along with the estimates of genetic advance are helpful in predicting the gain under selection than the estimate of heritability alone. High heritability coupled with high genetic advance indicated that the respective character is governed by additive gene action and phenotypic selection is advised for the improvement of those characters. The magnitude of heritability also plays a key role in crop improvement programmes (Allard, 1960).

In anthuriums, vegetative characters such as plant height, number of suckers per plant, length of leaf blade, width of leaf blade, leaf area and floral characters such as spathe length, spathe width, spadix length and inclination of spadix exhibited high heritability along with high genetic advance (Mayadevi, 2001).

Asish (2002) pointed out that the character anthocyanin content (99.73 per cent) had the highest heritability of 99.73 per cent whereas the lowest heritability of 39.34 per cent was reported for the character number of spadices plant^{-1} year^{-1}. Genetic advance was the highest for the character anthocyanin content (118.98 per cent) followed by pollen fertility (86.55 per cent). High heritability coupled with high genetic advance was reported for the characters like leaf area, inclination of spadix, life of spadix, anthocyanin content and pollen fertility (Premna, 2003).

Madhukumar (2010) observed high heritability and high genetic advance for the characters total anthocyanin content and pollen fertility. The characters like plant spread, length of leaves, stalk length and spathe width indicated high heritability along with high genetic advance in a study conducted by Anand *et al.* (2013). So these

characters in anthurium were controlled by additive gene action and thereby further improvement can be brought by selection.

According to Sheena (2015) the characters like number of leaves per spadices per plant per year, internodal length, days from emergence to maturity of leaves, leaf area, plant height, number of true flowers per spadix, inclination of spadix with the spathe, days from emergence to maturity of spathe, days to initiation of female phase, duration of female phase and length of leaf stalk exhibited high heritability in anthurium.

In the variability study by Tamuli *et al.* (2015) high heritability coupled with high genetic advance was revealed in characters namely plant spread, increase in spathe size at third day after harvest, leaf area, number of suckers, plant height, fresh weight of the cut flower at senescence, water uptake at third day after harvest and at senescence, total chlorophyll and anthocyanin content in spathe. High heritability with low genetic advance was shown by the characters spadix length, spadix diameter, spadix to spathe angle, days taken from unfurl to full bloom, flower stalk girth and vase life. This indicated that these characters were controlled by non-additive gene action and selection with adequate progeny testing need to be followed by the breeder to improve these traits. Gopi (2016) stated that in anthurium, all the characters except days from emergence to maturity of inflorescence, duration of female phase, days from emergence to maturity of leaves and number of suckers plant^{-1} had high heritability along with high genetic advance and selection can be practiced for improvement of these characters.

Gopi *et al.* (2016) observed high heritability and high genetic advance for the characters leaf area, number of flowers per spadix, spathe size, spadix length, days from emergence to maturity of inflorescence, life of spadix, inclination of candle with the spathe, days to initiation of female phase and duration of interphase.

2.2.3.3 Correlation Analysis

The degree and direction of association between two or more variables is called correlation. Correlation studies are effective basis for phenotypic selection of plants. Yield being a polygenic trait, direct selection may not be effective and indirect selection need to be practiced for crop improvement. In case of anthurium, spadix life was found to have a positive correlation with spadix length and leaf area (Asish, 2002).

According to Premna (2003), the characters such as leaf area, days from emergence to maturity of leaves, internodal length, number of leaves per plant, number of spadices per plant, number of days from emergence to maturity of inflorescence, spadix length, number of days for initiation of female phase, duration of female phase, duration of inter phase, duration of male phase, inclination of spadix and pollen fertility had a positive phenotypic and genotypic correlation with the character, life of spadix in anthurium.

Shiva and Nair (2008) observed positive and significant association of the trait number of suckers per plant with leaf fresh weight, plant spread and number of leaves per plant. The flower yield per plant registered a positive correlation with other characters such as life of flower on plant, number of coils per spadix, spathe size and peduncle length.

Plant height showed a positive genotypic correlation with anthocyanin content, number of flowers per spadix, life of spadix, spadix length, leaf area and internodal length (Madhukumar, 2010). Anand *et al.* (2013) reported that, in anthurium a negative correlation exist between the traits number of suckers and number of leaves with the trait number of spikes per plant.

Correlation studies by Gopi (2016) on 25 anthurium genotypes revealed that plant height had significant and positive genotypic correlation with all the characters except pollen fertility. Number of flowers year^{-1} showed positive significant genotypic

correlation with leaf area and spathe size. Environmental correlation was found to be absent in most of the characters. In genotypic correlation studies by Anand *et al.* (2017), a positive and significant correlation was identified between the traits leaf length, leaf breadth, number of leaves, plant height, plant spread, stalk length, petiole length, spathe length, spathe width and number of suckers with number of spikes per plant.

2.2.3.4 Path Analysis

Path coefficient is a standardized partial regression coefficient that measures the direct influence of one variable on another and categorizes the correlation coefficients into direct and indirect component effects (Dewey and Lu, 1959). This analysis helps in indirect selection of traits for yield improvement. Shiva and Nair (2008) opined that flower yield per plant in anthurium had high positive direct effect with time taken for flowering. Sucker yield in anthurium had positive and direct effect on other characters such as number of leaves per plant, leaf fresh weight and leaf area.

According to Madhukumar (2010) the dependent character i.e., number of flowers per spadix was found to have the maximum direct effect with spadix length followed by plant height, leaf size, life of spadix, days to female phase initiation, pollen size, pollen fertility, spathe size, anthocyanin content, internodal length, days to inter phase and days from emergence to maturity of inflorescence. Path co-efficient analysis conducted by Anand *et al.* (2013) revealed that the traits such as leaf diameter, stalk length, vase life, spathe length and number of leaves had direct positive effect on flower yield of anthurium while plant spread showed the highest indirect effect.

Anand *et al.* (2017) reported that flower yield in anthurium had a positive and direct relation with characters such as number of suckers, leaf length, leaf breadth, number of leaves, spathe length, petiole length and plant height. The highest direct effect was accounted for the character leaf breadth (0.925) followed by plant height

(0.850) and petiole length (0.330) and these characters emerged out as component traits for yield in anthurium.

2.3. *IN VITRO* MULTIPLICATION

2.3.1 Somatic Organogenesis

Organogenesis is a process of differentiation by which plant organs *viz.*, bud, shoots, stem, flower, roots etc. are formed from unusual points of an organized explant where a preformed meristematic region is absent. Organogenesis is more common than somatic embryogenesis and has far more potential for mass clonal propagation of plants.

In direct somatic organogenesis, adventitious buds arise directly from the epidermal or sub epidermal layers of explant. In indirect somatic organogenesis, the intact tissue explants are made to dedifferentiate to yield a mass of undifferentiated parenchymatous cells called callus. The callus in turn gives rise to shoot and root initials (George and Sherrington, 1984).

Anthurium has been most commonly propagated through indirect organogenesis using various explants, such as leaves (Atak and Çelik, 2009), axillary buds (Kunisaki, 1980), roots (Chen *et al.*, 1997), petioles (Yu *et al.,* 2009), seeds (Maira *et al.*, 2010), fruits (dos Santos *et al.,* 2005), seeds (Maira *et al.*, 2010), or anthers (Winarto *et al.*, 2010).

2.3.2 Factors affecting *in vitro* culture

2.3.2.1 Basal medium

The morphogenetic response of plant issues mainly depend upon the major components in the medium such as carbon sources, nutrients, growth regulators and

the gelling agents. Sreelatha (1992) recorded the best callusing response in modified Murashige and Skoog medium with reduced salt concentration for anthuriums.

Lan *et al.* (2003) compared the response of various basal media such as N6, KC, Pierik and half MS on anthurium cultures and concluded that half MS, N6 and Pierik successfully generated calli from leaf explants while half MS, N6 and KC were successful in case of petiole explants. Jeshima (2007) reported that in *Anthurium andreanum* callus was observed in modified MS medium with reduced major salt composition. A gradient in callus induction was reported by Cui *et al.* (2007) and the callus formation frequency followed the order half MS > MS > N6 > B5 > White's medium. The organic and inorganic constituents in the medium mainly influenced the response of inoculated plant parts during *in vitro* culture and thereby determines the success of plant tissue culture (George and Debergh, 2008).

For anthurium bud induction, half MS medium supplemented with 2.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA were found to be better than quarter strength MS and full MS medium with the same concentrations of plant growth regulators. Similar responses were observed for three anthurium cultivars namely Abason, Alabama and Mississippi (Duan *et al.*, 2009).

Gu *et al.* (2012) cultured *Anthurium andraeanum* 'Alabama' and 'Sierra' leaf explants on modified Murashige and Skoog (MS) basal medium supplemented with thidiazuron. Thokchom and Maitra (2017) successfully propagated *Anthurium andraeanum* Lind. cv. Jewel using MS medium as basal medium, supplemented with various plant growth regulators.

2.3.2.2 Genotype

Geier (1990) reported that the most critical factor in anthurium tissue culture is genotype. Among the genotypes, callus induction rate was found to be variable when young parts of mature plants and embryos were used as starting material for *in vitro*

culture (Pierik, 1975). Shoot regeneration capacity from leaf lamina of anthurium was studied by Yang-Yuan Hsin *et al.* (2002) and reported significant variation among all the studied genotypes. Similarly, shoot regeneration capacity of *A. andreanum* cv. Tinora Red was found to be more compared to the cultivar Senator (Martin *et al.*, 2003). George and Debergh (2008) also identified genotype dependent responses of anthurium with the culture environment. Various scientists reported wide variations in duration from callusing to plantlet regeneration in anthurium.

Vargas *et al.* (2004) reported that plantlet regeneration from seed derived callus occurred in six weeks, 15 weeks for cultivar Flamingo (Viégas *et al.*, 2007), 10 weeks for leaf segment derived callus in the cultivar Agnihothri (Bejoy *et al.*, 2008), 24 weeks for Valentino cultivar (Yu *et al.*, 2009) and almost 12 months for the cultivar Local Pink (Maitra *et al.*, 2012).

Acropolis, Esmeralda and Tropical Red varieties of anthurium were analyzed by Prakash *et al.* (2017). Number of shoot buds produced per explants varied from 30 to 39 per explant among the genotypes. Average callus multiplication ratio ranged from 1.81 to 2.31 while shoot multiplication ratio ranged from 1.45 to 2.46 in various cultivars. The cultivars also exhibited varying shoot height ranging from 5.31 to 6.98 cm.

2.3.2.3 Explant

In *in vitro* culture the age of explant, kind of explant, explant size, age and manner of culture had direct effect on successful response (George and Sherrington, 1984). Callus induction and plant regeneration can occur from both immature and mature organs but mitotically active cells show better response.

The plastic nature of immature plant parts suits *in vitro* operations and successful regeneration occurs compared to mature organs. Immature petioles, young leaves,

spadix, spathe and inflorescence stalks can be used as explants for successful *in vitro* responses in anthurium (Geier, 1990).

According to Martin *et al.* (2003) explants with young brown lamina were superior to young green leaf lamina. Puchooa (2005) reported that proximal part of tender leaf lamina produces the maximum shooting response compared to the mid and distal part of leaf lamina.

Sathyanarayana (2007) opined that newly formed young leaf tissues are easy to surface disinfect and establish clean cultures compared to mature leaf tissues. Bejoy *et al.* (2008) used pale greenish brown young leaf lamina, 5-10 days after unfolding of leaf, collected from healthy and mature plants as explant and successfully generated callus in *Anthurium andreanum* Hort. cv. Agnihotri. Leaf lamina explant of 1-1.5 cm^2 were used for inoculation in the culture medium.

Agasimani *et al* (2011b) pointed out that the plants have variable hormonal levels throughout the plant system. So, as the location of explant on the plant varies the endogenous hormonal levels in the tissues varies, eventually resulting in varying responses. Zhou *et al.* (2012) analyzed and ranked the explants based on callus formation ability: leaves > petioles > spathes or spadices > lateral buds. Cui *et al.* (2007) noted a trend in callus formation: young leaves > middle-aged leaves > old leaves and leaves with a margin > leaves without a margin.

Thokchom and Maitra (2017) reported that newly emerging young brown coloured leaves induced callus in *Anthurium andreanum* cv. Jewel. According to Prakash *et al.* (2017), segments of leaf lamina, petiole with leaves of varying stages i.e., brown and green, in the same cultivar showed varying morphogenetic responses. Bhavana (2018) developed a regenerative protocol for anthurium using two to four days old leaf lamina explants.

2.3.2.4 Surface Sterilization

Fungal and bacterial contaminations are serious problems that are to be tackled by a tissue culturist. Surface sterilizing agents *viz.*, sodium hypochlorite is widely used for this purpose but sometimes efficiency of using single surface sterilant is doubtful (Hu and Wang, 1983). Sometimes stronger sterilizing agents such as mercuric chloride are used alone or in combination with sodium hypochlorite to obtain contamination free cultures in humid tropical countries where the presence of microbial spores in the environment is high throughout the year. Mahanta and Paswan (2001) successfully surface sterilized axillary bud explants of anthurium by disinfecting in 70 per cent ethanol for one minute followed by 0.1 per cent mercuric chloride for two minutes.

According to Bejoy *et al.* (2008) anthurium leaf sterilized by soaking in 0.1 per cent mercuric chloride for 7-12 minutes followed by rinsing three times with sterile distilled water gave sterile cultures. Gantait *et al.* (2008) cultured anthurium from shoot tips. Initially the shoot tips were disinfected using antifungal agent cetrimide for five minutes followed by five percent sodium hypochlorite and 0.1 per cent mercuric chloride treatment. Surface sterilization of anthurium leaf explants with 70 per cent ethanol for one minute followed by disinfectant treatment with 1.5 per cent sodium hypochlorite was successful in producing contamination free cultures (Jahan *et al.*, 2009). Soaking of explant with surfactant like 0.01 per cent Tween-20 before sterilization can also result in reduced contamination rate.

Atak and Celik (2009) identified that contamination caused by fungus as well as endogenous and exogenous bacteria can be controlled by surface sterilization of explant for one minute in 70 per cent ethanol followed by 30 minutes soaking in gentamicin solution for three minutes and then again soaking in 20 per cent (v/v) commercial bleach for 12 minutes.

Gu *et al.* (2012) proposed that the leaf explants need to be washed in running tap water for 15 minutes prior to surface sterilization treatments. The leaves were then treated with 70 per cent ethanol for 30 seconds and then soaked in a 20 per cent clorox solution (1.2% sodium hypochlorite) for 20 minutes. Then the clorox solution was poured off and leaves were rinsed thoroughly with sterile distilled water.

Thokchom and Maitra (2017) conducted an experiment to standardize the surface sterilization technique in immature bronze coloured leaf explants of *Anthurium andraeanum* cv. Jewel. The leaf explants treated with 0.1 per cent mercuric chloride (three minutes) + 70 per cent ethyl alcohol (30 seconds) resulted in the lowest percentage of contaminated cultures (3.67 per cent), the lowest explant mortality of 11.33 per cent and the highest survival of 85.67 per cent.

In the disinfection protocol followed by Bhavana (2018), the young leaves were swabbed with 70 per cent ethanol followed by sterile double-distilled water for five minutes and then treated with one per cent bavistin for 15 minutes, 70 per cent ethanol for 30 seconds, gentamycin for 30 minutes, and later with one per cent (w/v) sodium hypochlorite for 12 minutes. After each treatment the explants were thoroughly washed five times with sterile distilled water.

2.3.2.5 Culture Conditions

Maintenance of photoperiod and temperature is critical for *in vitro* response in anthurium. In case of effect of light on anthurium culture, reports show mixed results. However, most researchers opined that callus induction and subsequent growth is favored under continuous darkness at temperature around 25^0 C. According to Lan *et al.* (2003) various light treatments had no effect on callus induction from petiole explants. Treatments with 24 hours and 10 hours per day light showed an increased bud differentiation response compared to no light treatment.

According to Xia *et al.* (2005) dark time period had a conductive effect on callus formation in anthurium. The callusing response was found to be 87.6 per cent for the variety Pink Champion and 76.7 per cent for the variety Arizona. Under 2000 lux light condition callusing significantly decreased to 12.2 per cent for Arizona and 18.8 per cent for Pink Champion. Jiang *et al.* (2006) opined that callus induction in the dark was significantly higher compared to the light conditions and the callusing was significantly inhibited at 2000 lux light intensity.

Bejoy *et al.* (2008) stated that callus establishment occurred when the inoculated explants were incubated in dark condition. Explants incubated under lighted condition failed to develop callus and turned brown in two to three weeks. For shoot regeneration from callus, a photoperiod of 16 hours light and eight hours dark conditions were found to be optimum. Chen *et al.* (2013) studied the effect of light quality on *in vitro* response in anthurium. Light-emitting diodes promoted plantlet growth and an improvement in growth and quality of plantlets were observed under the combination of 50 per cent blue light and 50 per cent red light.

Bhavana (2018) reported that for anthurium the most ambient culture conditions include a temperature of 25±2.0°C and a relative humidity of 70 per cent. Cultures after inoculation were incubated under cool and dark conditions. Similar findings were also reported by Bhattacharya *et al.* (2015).

2.3.2.6 Media Components

2.3.2.6.1 Ammonium Nitrate

Pierik *et al.* (1979) compared the influence of three different inorganic nitrogen sources on *in vitro* response of anthurium. Among various concentrations a reduced ammonium nitrate concentration of 260 mg L^{-1} was found optimal for inducing callus. The study also concluded that increased response was due to low levels of ammonium ions and not due to reduction in nitrate ions in the medium.

Cai (2002) reported the highest callus formation frequency of 89 per cent on modified Nitsch medium (200 mg L^{-1} ammonium nitrate) containing 0.1 mg L^{-1} 2,4-D and 1.0 mg L^{-1} BA. Nitsch medium (720 mg L^{-1} ammonium nitrate) supplemented with 0.5 mg L^{-1} BA was the most suitable for callus multiplication and shoot induction while Nitsch medium (720 mg L^{-1} ammonium nitrate) without any plant growth regulators was suitable for rooting.

On comparing the effect of various concentrations of ammonium nitrate in MS media, Xia *et al.* (2005) concluded that modified MS with 410 mg L^{-1} ammonium nitrate concentration exhibited the highest callus formation frequency compared to MS medium with 1650 mg L^{-1} ammonium nitrate. Atak and Celik (2009) and Farsi *et al.* (2012) pointed out that 250 mg L^{-1} ammonium nitrate has a positive influence on shoot regeneration in anthurium.

Winarto (2013) reported that anthurium anther wall explants induced callus in half-strength WT medium supplemented with 700 mg L^{-1} ammonium nitrate. Reduced ammonium nitrate concentration of 205 mg L^{-1} in half-strength NWT medium was found optimal for callus regeneration. By reduced use of ammonium nitrate, 55 to 70 per cent increase in callus formation and successful shooting with 3.2 to 5.3 shoots per callus cluster was attained.

Bhavana (2018) also recommended the use of lower level of ammonium nitrate in modified MS medium for callus induction and regeneration of plantlets in all the anthurium genotypes taken up for the study.

2.3.2.6.2 Activated Charcoal

Growth inhibitors that are produced in the culture medium can become a hindrance to *in vitro* culture response. Activated charcoal is an antioxidant that has the capacity to absorb growth inhibitors such as polyphenols from the culture medium. In *Anthurium andreanum* cultivar Jolanba, addition of activated charcoal in the culture

medium resulted in robust growth of aseptic plantlets (Zhao-Yunpeng *et al.*, 2004). Activated charcoal has the capacity to modify the culture conditions by absorbing the dissolved solids and gases (George and Debergh, 2008).

Thomas (2008) reported that activated charcoal adsorbs various secreted compounds from cultured tissues such as phenols, prevents unwanted callus growth, stimulates root generation and alters the pH changes of the medium to an optimum range that is favourable for morphogenesis. Atak and Çelik (2009) reported that presence of activated charcoal in the medium promoted root growth in anthurium. Similar reports were also given by Gantait and Mandal (2010).

Bakhsi-Khaniki *et al.* (2011) recommended the use of 0.1 per cent activated charcoal along with MS medium in callusing, shooting and root initiation medium. Asaduzzaman *et al.* (2012) on studying the effect of activated charcoal on *in vitro* cultures concluded that activated charcoal causes rigorous decrease in organic supplements in the medium such as plant growth regulators and polyphenol exudations.

2.3.2.6.3 Carbon Source

Carbohydrates such as sucrose and glucose serves as carbon source in *in vitro* cultures and they act as the chief energy source and as an osmoticum. Weiming *et al.* (2004) observed enhanced shoot growth with increased content of soluble sugars in the medium.

Xia *et al.* (2005) compared the effect of different levels of glucose and sucrose and reported that three per cent glucose had the greatest inductive effect on callus formation. Yao *et al.* (2006) indicated a better callusing response by using three per cent sucrose in the medium.

Farsi *et al.* (2012) recommended the use of 0.3 per cent sucrose in modified MS medium for ensuring callus induction from anthurium explants. Thokchom and Maitra

(2017) stated that MS medium supplemented with 30 g L^{-1} sucrose along with plant growth regulators was required for anthurium callus induction.

2.3.2.6.4 Effect of Growth Regulators

The success of *in vitro* organogenesis in anthurium depend upon the concentration of growth regulators and additives in the medium. In general, high auxin concentration and low cytokinin concentration in the medium favors callus induction (Skoog and Miller, 1957).

High cytokinin concentration coupled with low auxin concentration in the culture medium result in shoot induction and regeneration. Root initiation was favored by a low cytokinin concentration along with high auxin concentration or by using auxin alone in the medium. In anthurium a wide variety of plant growth regulators are used either alone or in combinations based on the type of explant used, varieties used in the study, stage of culture and other organogenic processes.

2.3.2.6.4.1 Callus induction and proliferation

Yuan *et al.* (2004) stated that the callus induction frequency in *Anthurium andreanum* leaf explants increased as the concentration of BA in the medium increased from 0.2 to 1.0 mgL^{-1}. Cui *et al.* (2007) opined that single plant growth regulator was unable to produce callus and a combination of auxins and cytokinins are required for induction of callusing from anthurium leaf explants. In agreement to this, Atak and Celik (2009) found that half-strength MS basal salt provided with 0.6 mg L^{-1} 2,4-D and 1.0 mg L^{-1} BA was able to induce calli from anthurium leaf explants.

Bejoy *et al.* (2008) observed callusing response four to five weeks after culture, along the cut edges of leaf lamina, mainly from midrib and vein region. The maximum callusing response of 35.30 per cent was attained in half MS medium supplemented

with 1.0 mg L^{-1} BAP. 1.0 mg L^{-1} BAP along with 0.5 mg L^{-1} 2,4-D was found to be the best for callus development and differentiation.

However, Yang *et al.* (2008) and Liu *et al.* (2009) analyzed the organogenic response in various anthurium cultivars and concluded that the optimum BA concentration required for callus induction and differentiation was 0.5 mg L^{-1}. Wu (2010) observed improved callus proliferation of *Anthurium andraeanum* when concentration of BA ranged between 1.0 to 3.0 mg L^{-1} and the concentration of 2.0 mg L^{-1} BA showed the highest response.

Thokchom and Maitra (2017) reported that yellowish brown nodular calli was induced in MS basal medium when supplemented with 0.5 mg L^{-1} BAP + 3.0 mg L^{-1} 2,4-D + 0.4 mg L^{-1} thidiazuron in a period of 37.68 days. The highest callusing percentage and induction of dark green and compact callus was observed in MS medium supplemented with 2 mg L^{-1} NAA.

Prakash *et al.* (2017) studied the *in vitro* response in three anthurium cultivars namely Acropolis, Esmeralda and Tropical Red. Compact callus production was observed after 30 to 35 days from petiole explants and after 40 to 45 days from leaf lamina with mid rib. Average callus induction ratio was the highest in anthurium cultivar Esmeralda (4.40 ± 0.27) on MS + 0.2 mg L^{-1} 2,4-D +1.0 mg L^{-1} BAP. Average callus multiplication ratio on MS + 0.2 mg L^{-1} 2,4-D +1.0 mg L^{-1} BAP varied from 2.61 to 4.40.

Bhavana (2018) opined that half strength modified MS medium having 250 mg L^{-1} ammonium nitrate and 0.1 per cent EDTA sodium salt supplemented with 1.4 mg L^{-1} 2,4,-D + 4.4 mg L^{-1} BAP was found suitable for callus induction in anthurium. Callus initiation occurred at the corners of leaf explant within 15 days of inoculation.

2.3.2.6.4.2 Shoot Regeneration

Multiplied callus generates shoots in response to plant growth regulators in the culture medium. Shoot initiation is followed by shoot proliferation. Cytokinins are utilized to overcome the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils. A high frequency of shoot regeneration was noticed by Yakandawala *et al.* (2000) in *Anthurium andreanum* variety Avo Nette when cultured in modified MS medium supplemented with 0.5 mg L^{-1} BA. In *Anthurium andreanum* cv. Mauritius 10 month old cultures initiated shoots in basal MS medium (Prakash *et al.*, 2001).

Shoot primordial initiation of *Anthurium andreanum* cv. Mauritius orange was noticed on ten month old callus cultures when transferred to MS basal medium (Prakash *et al.*, 2001). A shoot regeneration frequency of 20 to 50 shootlets per explant was attained by Dhananjaya and Sulladmath (2003) on culturing anthurium from petiole on MS medium containing 6 mg L^{-1} kinetin and 2 mg L^{-1} IBA. *Anthurium andreanum* cv. Agnihotri mass multiplication was achieved by inoculation of axillary buds on modified MS medium provided with 0.8 mg L^{-1} BA, 0.5 mg L^{-1} vitamin B_5, 0.1 mg L^{-1} IAA, 200 mg L^{-1} PVP and 150 ml L^{-1} coconut water. An average of 4.66 shoots were produced per explant (Mohanta and Paswan, 2001).

According to Martin *et al.* (2003) callus cultured on half strength MS medium supplemented with 1.1 mg L^{-1} BA, 1.14 mg L^{-1} IBA and 0.46 mg L^{-1} kinetin were the most effective medium for shoot formation. An improved bud induction was noticed when a combination of 0.1 to 1.0 mg L^{-1} 2, 4-D and 0.5 mg L^{-1} thidiazuron was used. Similar response was also observed when thidiazuron concentration in the medium was reduced to 0.01 mg L^{-1}.

Bejoy *et al.* (2008) subcultured anthurium callus on an auxin free medium and observed shoot buds that appeared as light brown or pink coloured protuberances, three

to four weeks after culturing. Initially each subcultured callus produced an average of 6.7 shoots on basal medium supplemented with 0.3 mg L^{-1} BAP. Further increase in BAP level did not show any significant improvement in shooting response. After 60 days of culture in the same medium, callus exhibited an increased shooting response of 9.7 shoots per explant.

Liendo and Mogollon (2009) observed the formation of an average of 4.17 shoots per explant, when MS medium was supplemented with 1.0 mg L^{-1} 6-BAP. Callus subcultured on MS media provided with 1.0 mg L^{-1} BA + 0.1 mg L^{-1} 2,4-D produced 2.08 shoots from each of the callus clumps (Bakhsi-Khaniki *et al.,* 2011). In a micropropagation study on anthurium attempted by Paola *et al.* (2014), multiple shooting was observed on half strength MS medium fortified with 1.0 mg L^{-1} BAP and a shoot multiplication rate of 23.7 shoots per explant was attained in the same culture medium.

Thokchom and Maitra (2017) reported that the least duration of 33.5 days from callus to shoot induction was observed in MS basal medium fortified with 3 mg L^{-1} BAP and 0.5 mg L^{-1} NAA. The highest shoot regeneration percentage of 98.89% was identified on MS media fortified with 2 mg L^{-1} BAP and 0.5 mg L^{-1} NAA. The maximum number of shoots per callus (5.83 at first subculturing and 22.60 at second subculturing) was produced on MS media supplemented with 2 mg L^{-1} BA. Bhavana (2018) reported higher level of shoot induction from anthurium callus by using 0.3 mg L^{-1} BAP in the medium.

2.3.2.6.4.3 Rooting

Rooting response generally occur after shoot regeneration in anthurium. Mohanta and Paswan (2001) reported that the highest rooting of 80 per cent was observed in basal MS medium fortified with 1.0 mg L^{-1} IAA. Zhang *et al.* (2001) identified *in vitro* rooting from subcultured anthurium callus on MS medium provided with 0.1 mg L^{-1}

IBA or 0.1 mg L^{-1} NAA. The survival rate of 85 per cent was recorded in the regenerated plantlets.

Dhananjaya and Sulladmath (2003) studied and concluded that MS medium supplemented with 1.5 mg L^{-1} IBA and 5 mg L^{-1} kinetin was good for optimum root production *in vitro*. Alex (2006) reported that in *Anthurium andreanum* Linden no separate rooting medium was necessary as considerable rooting occurred along with shooting.

Bejoy *et al.* (2008) reported that rooting occurred simultaneously from 40 per cent of the developed shoots and made multiplication cost effective and easy. Half MS medium supplemented with 0.5 mg L^{-1} NAA induced 98 per cent rooting within six weeks of culture. Rhizogenic response was found to be lower in IBA (75 per cent) and IAA (65 per cent).

According to Liendo and Mogollon (2009) anthurium cultures do not require any phytohormones for root development. Similarly, Paola *et al.* (2014) opined that one specific medium for rooting was not necessary during anthuriun *in vitro* culture.

Thokchom and Maitra (2017) compared the effect of various auxins on *in vitro* rooting of anthurium and found that all the auxin treatments such as half strength MS medium supplemented with 1.0 mg L^{-1} IBA, 1.0 mg L^{-1} NAA and 2.0 mg L^{-1} IBA induced roots. Half strength MS supplemented with 2.0 mg L^{-1} NAA produced roots in 33.55 days while other treatments showed a much longer duration. Number of roots was found to be the highest (5.83) in half strength MS supplemented with 1.0 mg L^{-1} NAA. Bhavana (2018) reported that the highest root initiation occurred in the shooting medium itself which was supplemented with 1.3 µM 6-BAP. A lower root initiation was recorded in the absence of 6-BAP.

2.4 *EX VITRO* ESTABLISHMENT

Tissue cultured plants show poor adaptation to *ex vitro* environment. They are susceptible to temperature variations, low relative humidity and higher light levels (Wainwright, 1988). So temperature, light and relative humidity are the main three factors to be regulated during acclimatization. In anthurium, *in vitro* tissue cultured plantlets that have attained at the least 2.5 to 3.0 cm length, three to four leaves and more than two roots can be taken for hardening (Ajithkumar and Nair, 1998).

Paswan and Mahanta (2001) obtained a plantlet survival rate of 60 per cent when *in vitro* raised plants were transferred into *in vivo* conditions in plastic pots containing Solrite and Perlite (10:1) mixture. According to Dhananjaya and Sulladmath (2003) hardened plants showed a survival rate of 80 per cent in the hardening medium constituted with coffee cherry husk: FYM: soil: sand in the ratio 2:1:1:1. Plantlets were transferred for *ex vitro* hardening at four to six leaf stage with three to five roots.

Winarto and da Silva (2012) opined that anthurium can be successfully hardened in a medium containing burned-rice husk, raw rice husk and organic manure in 2:2:1 ratio. Thokchom and Maitra (2017) compared various hardening medium for anthurium plantlets and observed the maximum survival rate of 96.17 per cent in coconut husk medium. Greenhouse condition with 70 per cent relative humidity, temperature of 27±1°C with a light density of 4000 lux was ambient for acclimatization.

Prakash *et al.* (2017) hardened the *in vitro* rooted plantlets in primary and secondary hardening units. Primary hardening was done in a medium containing coco-peat and soil-rite mixture in 1:1 ratio and misting facilities. In primary hardening, plantlets showed a survival rate of 91.93 percent. Secondary hardening was done on coir-pith bed under ventilated polyhouse and attained a survival rate of 95.63 per cent. Finally the hardened plantlets were transferred into polythene covers.

Bhavana (2018) planted the *in vitro* developed plantlets directly into jiffy plugs and later hardened *ex vitro* in plant growth chambers. The survival rate of 80 per cent was recorded after 30 days of hardening.

Materials and Methods

3. MATERIALS AND METHODS

The present study was undertaken to assess the variability among *Anthurium andreanum* Linden hybrids so as to identify suitable hybrids with commercial qualities and standardize the protocol for *in vitro* mass multiplication of these selected hybrids. The investigation was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2017-19. The research programme involved two experiments *viz.,*

Experiment I: Genetic variability analysis of 20 *Anthurium andreanum* Linden hybrids.

Experiment II: *In vitro* mass multiplication of selected *Anthurium andreanum* Linden hybrids.

Materials and methods of both the experiments are presented below under separate subheads.

3.1 EXPERIMENT I

3.1.1 Materials

The 20 different hybrid genotypes of anthurium showing variations in spathe colour, shape and size and other commercially important morphological characters, generated through hybridization in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani were utilized for the present study (Plate 1). Commercially superior and high yielding hybrids selected from these hybrid genotypes were used for further *in vitro* regeneration studies. The hybrid genotypes used in the study are listed below.

1. Liver Red X Dragon's Tongue (LR x DT)
2. Honeymoon Red X Miniature Red (HR x MR)
3. Lady Jane X Orange Glory (LJ x OG)
4. Orange Glory X Nitta Orange (OG x NO)
5. Honeymoon Red X Kalympong Red (HR x KR)
6. Hounduras Red X Kalympong Red (HoR x KR)
7. Honeymoon Red X Lady Jane (HR x LJ)
8. Honeymoon Red X Dragon's Tongue (HR x DT)
9. Pompon Red X Honeymoon Red (PR x HR)
10. Honeymoon Red X Pink (HR x P)
11. Pink X Liver Red (P x LR)
12. Ceylon Red X Kalympong Red (CR x KR)
13. Honeymoon Red X Liver Red (HR x LR)
14. Pompon Red X Dragon's Tongue (PR x DT)
15. Liver Red X Orange Glory (LR x OG)
16. Pompon Red X Liver Red (PR x LR)
17. Dragon's Tongue X Honeymoon Red (DT x HR)
18. Kalympong Red X Liver Red (KR x LR)
19. Pompon Red X Dragon's Tongue (PR x DT (1))
20. Liver Red X Orange Glory (LR x OG)

3.1.2 Methods

3.1.2.1 Evaluation of Hybrid Genotypes

The selected plants were raised in pot culture experiment under completely randomized design with five replications (Plate 2). Six commercially superior hybrid genotypes were identified based on observations of morphological and floral characters.

3.1.2.2 Plant Protection

1. For the control of blight or anthracnose by *Colletotrichum gloeosporioides* regular application of any one of the following chemicals were used.

A. Kocide 2000 @ 1.5 g l^{-1}

B. Nativo @ 0.5 g l^{-1}

C. Bavistin 50 per cent WP @ 2g l^{-1}

D. Indofil M-45 2 g l^{-1}

2. Dipping the roots of plants in Indofil M-45 at the time of planting helped to avoid soil borne diseases.

3. *Pseudomonas fluorescens* @ 2 per cent was applied as prophylactic measure against bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* at weekly intervals.

4. Need based application of Metacid (2g l^{-1}) or Nuvacron (2g l^{-1}) were used to control leaf feeding caterpillars and grass hoppers. Mites were controlled using Kelthane (2ml l^{-1}).

5. Snails and slugs were controlled by hand picking and also with the application of salt along the borders of the green house.

6. For the control of bacterial fungal attack, Bacteriomycin was sprayed in the whole field at a concentration of 6g per 20 litres of water at fortnightly intervals.

3.1.3 Morphological Studies

The plant materials with stabilized vegetative and floral characters were used for taking all the observations. Observations on the following eight qualitative

traits (vegetative and floral characters) and 19 quantitative traits (vegetative and floral characters) were recorded and their mean values were worked out.

3.1.3.1 Vegetative Characters

3.1.3.1.1 Plant Height

Plant height in centimeters was measured from the base of the plant to the tip of the top most leaf on the plant.

3.1.3.1.2 Leaf Area

Leaf area was measured using graphical method. The outline of the third leaf from top of the plant was drawn on a graph paper and estimation of leaf area was made in centimeter squares. The third leaf was chosen as it will be fully unfurled and would have achieved its full growth and spread of the leaf blade.

3.1.3.1.3 Internode Length

The distance between two nodes was measured from the basal part of the plant and the observations were recorded in centimeters.

3.1.3.1.4 Days from Emergence to Maturity of Leaves

Days from the emergence of the leaf to the maturity of leaves were recorded.

3.1.3.1.5 Number of Leaves Spadices $^{-1}$ Plant^{-1} Year^{-1}

The number of leaves spadices $^{-1}$ plant^{-1} year^{-1} produced during a period of one year was observed and recorded.

3.1.3.1.6 Number of Suckers Plant^{-1}

The potential of the mature mother plant to produce new suckers from the base was studied and the number of suckers produced from the mother plant during a period of one year was recorded.

3.1.3.1.7 Colour of Petiole and Young Leaf

The colour of petiole and young leaf of each hybrid genotype was recorded by visual observation when the leaves were not fully unfurled.

3.1.3.1.8 Pest and Disease Incidence

The pest and disease incidence among the genotypes during the period of two years were observed.

3.1.3.2 Floral Characters

3.1.3.2.1 Quantitative Traits

3.1.3.2.1.1 Days from Emergence to Maturity of Inflorescence

The duration from the emergence of inflorescence to its full maturity was documented.

3.1.3.2.1.2 Spathe Size

The spathe size of each variety was recorded by using standard graph sheet method and the size was recorded in centimeter squares.

3.1.3.2.1.3 Spadix Length

Candle length was measured in centimeters from the base of the candle to its tip.

3.1.3.2.1.4 Number of Flowers Spadix^{-1}

The total number of flowers arranged spirally in acropetal succession from the base to the tip of the candle was counted and documented.

3.1.3.2.1.5 Life of Spadix

The number of days from first day of emergence of inflorescence to the time of its yellowing, withering of spathe and shriveling of candle was recorded as life of spadix.

3.1.3.2.1.6 Days to Initiation of Female Phase

The number of days from the emergence of the spathe to first emergence of mature stigmas of the basal flowers of the candle, identified by the presence of honey dew or stigmatic droplets was observed and recorded as the days to initiation of female phase.

3.1.3.2.1.7 Duration of Female Phase

The number of days from initiation of female phase to completion of female phase was identified by drying of stigmatic surface and recorded as duration of female phase.

3.1.3.2.1.8 Duration of Interphase

The duration between the end of female phase and the emergence of anthers from the basal flowers, indicating the start of male phase, was recorded as the days of interphase.

3.1.3.2.1.9 Duration of Male Phase

The number of days from the emergence of the first anthers on the candle to the emergence of its last anthers was recorded as male phase duration.

3.1.3.2.1.10 Inclination of Candle with the Spathe

The angle between the base of the candle to the plane of the subtending spathe was measured with a protractor and documented as inclination angle between candle and spathe.

3.1.3.2.1.11 Anthocyanin Content

Estimation of anthocyanin content in the spathe was done as per the method described by Rangana (1977). The initial step was alcoholic extraction of the plant material i.e. spathe. For this one gram of the spathe sample from each hybrid genotype was extracted with ethanolic hydrochloric acid, filtered through a Buchner funnel using Whatman No. 1 filter paper and the filtrate was then diluted with ethanolic hydrochloric acid to 50 ml to yield optical density values within the optimum range of the spectrophotometer at 535 nm. The anthocyanin content was calculated using the following formula and the quantity of anthocyanin in the sample was expressed as milligram per 100 gm of the sample.

Total OD (optical density) per 100 g of sample (X) = [(Absorbance at 535nm) x (Volume made up of the extract used for colour development) x (Total volume) x 100] ÷ [Volume (ml of the extract) used x Weight of sample taken]

The absorbance of a solution containing 1 mg per ml is equal to 98.2 (constant).

Therefore, total anthocyanin in mg per 100 g of the sample = X/98.2

3.1.3.2.1.12 Vase Life

The flowers were harvested after complete unfolding of the spathe (Kamemoto 1962). Five flowers were randomly selected from each hybrid genotype and placed in a 250 ml conical flask containing 100 ml tap water. Flower stalk ends were cut and tap water was changed every alternate days. The number of days until the spathe started wilting and the spadix started necrosis was recorded as the vase life of the genotype.

3.1.3.2.1.13 Number of Flowers Year^{-1}

The number of anthurium flowers produced from a mature plant for a period of one year was documented.

3.3.3.2.2 Qualitative Traits

3.1.3.2.2.1 Spathe Colour

The spathe colour of each hybrid genotype was recorded by visual observation.

3.1.3.2.2.2 Spadix Colour

Spadix (candle) colour of the hybrid flowers was identified by direct visual observation.

3.1.3.2.2.3 Type of Inflorescence Axis

Length, nature and strength of inflorescence axis in each hybrid genotype were observed and recorded by direct visual observation.

3.1.3.2.2.4 Pollen Emergence Pattern

Pattern of pollen emergence in all the hybrid genotypes were observed for a period of one year and the seasonal influence on pollen emergence was documented.

3.1.3.2.2.5 Pollen Shape

Initially the anthers were collected and were tapped on glass slide so as to break the anther wall and bring the pollen out. Few drops of water was added to keep the pollen moist. The pollen shape of each hybrid genotype was recorded by direct observation using microscope without staining the pollen.

3.1.3.2.2.6 Pollen Colour

Pollen colour of each hybrid genotype is observed along with the pollen shape by visual observation using microscope.

3.1.4 Statistical Analysis

3.1.4.1 Analysis of Variance

ANOVA with two characters X and Y measured in 'g' genotypes raised in completely randomized design with 'r' replications is as follows

Source	df	Mean square		
		X	Y	XY
Between genotypes	(g-1)	G_{xx}	G_{yy}	G_{xy}
Error	(r-1) (g-1)	E_{xx}	E_{yy}	E_{xy}

3.1.4.2 Coefficient of Variation

Genotypic and phenotypic coefficients of variation were estimated using the formula proposed by Singh and Chowdhury (1977). The phenotypic and genotypic coefficients of variation (PCV and GCV) for a trait X were estimated using the following formulae.

$$GCV = \frac{\sigma_{gx}}{\overline{X}} \times 100$$

$$PCV = \frac{\sigma_{px}}{\overline{X}} \times 100$$

Where,

σ_{gx} = genotypic standard deviation

σ_{px} = phenotypic standard deviation

$\overline{X}$ = mean of the character under study

3.1.4.3 Heritability and Genetic Advance

Heritability (H^2) in broad sense was estimated as the proportion of heritable component of variation. Broad sense heritability for each character was calculated as a percentage based on the formula given by Jain (1982).

$$\text{Heritability coefficient (in broad sense), } H^2 = \frac{\sigma_{gx}^2}{\sigma_{px}^2} \times 100$$

Where,

σ_{gx}^2 = genotypic variance of the character X

σ_{px}^2 = phenotypic variance of the character X

According to classification of Heritability by Allard (1960),

< 30 percent - Low heritability

30-60 percent - Medium heritability

>60 percent - High heritability

$$\text{Genetic advance as percentage of mean (GA)} = \frac{K\, H^2\, \sigma_{px}}{\overline{X}} \times 100$$

Where,

k = Selection differential whose value is 2.06 if 5% selection is to be practiced (Miller *et. al.*, 1958).

H^2 = Heritability in broad sense

σ_{px} = Phenotypic standard deviation

$\overline{X}$ = Mean of the character over all varieties

Robinson *et al.* (1949) classified Genetic advance as percentage of mean into three categories i.e.

< 20 % - Low genetic advance

> 20 % - High genetic advance

3.1.4.4 Correlation Analysis

The correlation coefficients namely phenotypic, genotypic and environmental correlation coefficients between two characters denoted as 'x' and 'y' were worked out as follows:

$$\text{Genotype correlation } (r_{gxy}) = \frac{\sigma_{gxy}}{\sigma_{gx} \times \sigma_{gy}}$$

$$\text{Phenotypic correlation } (r_{pxy}) = \frac{\sigma_{pxy}}{\sigma_{px} \times \sigma_{py}}$$

$$\text{Environmental correlation } (r_{exy}) = \frac{\sigma_{exy}}{\sigma_{ex} \times \sigma_{ey}}$$

Where,

σ_{gxy}, σ_{pxy} and σ_{exy} are the genotypic, phenotypic and environmental co-variances between the characters 'X' and 'Y'.

σ_{gx}, σ_{px} and σ_{ex} are the genotypic, phenotypic and environmental standard deviations for the character 'X'.

σ_{gy}, σ_{py} and σ_{ey} are the genotypic, phenotypic and environmental standard deviations for the character 'Y'.

3.1.4.5 Path Analysis

The direct and indirect effect of each component characters on flower yield of hybrid genotypes were estimated through path coefficient analysis (Wright, 1954; Dewey and Lu, 1959).

3.2 EXPERIMENT II

Six superior hybrid genotypes of *Anthurium andreanum* Linden selected after genetic variability analysis, showing variations in spathe colour, high flower yield and other commercially valuable morphological and floral characters were utilized for the *in vitro* multiplication studies. The study was aimed at development of effective protocol for *in vitro* propagation in selected hybrids of *Anthurium andreanum* Linden in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 2017-2019.

The materials and methods followed for the standardization of *in vitro* propagation *via* indirect somatic organogenesis is elaborated below.

3.2.1 Explants

Anthurium explants were taken from healthy, disease free and actively growing mature mother plants. Young leaf lamina of the plants at the transition stage from pale brown to pale green stage were used as explant source for the culture (Plate 3).

3.2.2 Collection and Preparation of Explants

Young leaves with pale brown to pale green colour, collected 5-10 days after unfurling was taken as explant. These tender leaves were collected by excision with their petioles in ice boxes. The cut end of the petiole were kept immersed in water inside the ice box. In the laboratory, petioles of the leaf were removed with a sharp blade and the leaves were washed thoroughly in running tap water. The leaf lamina was washed in distilled water containing few drops of a wetting agent *viz.*, 'labolene'. They were further washed in distilled water to remove the traces of 'labolene' from the leaf lamina. Culturing was done at the earliest and before exceeding six hours after explant collection.

The young leaf explants are covered with polythene covers before unfurling to reduce the pest and disease infestation. Collection of explants was done throughout the year; but the rate of microbial contamination in cultures was higher in monsoon season. When the explants were collected during monsoon season and immediately after rainfall, they were treated with a systemic fungicide Bavistin (0.1%) for 20 minutes followed by repeated washing with distilled water prior to surface sterilization.

3.2.3 Surface Sterilization

Surface sterilization of explants was carried out inside a laminar air flow chamber. The leaves were initially rinsed thoroughly in sterile double distilled water.

The whole leaf explants were then cut into smaller pieces of 5-6 cm^2 with a sharp blade for easy handling during sterilization. The cut explants were treated with surface sterilization agents with continuous shaking for different durations as per Table 1. Each treatment was replicated eight times. After each surface sterilization, the explants were rinsed thrice with sterile double distilled water and 1-1.5 cm^2 sized explants were inoculated on half strength MS medium to study the explant survival.

Table 1. Treatments to assess the effect of surface sterilization

S.No.	Treatment No.	Treatments	Duration
1.	TS_1	0.1 % Mercuric chloride	5 minutes
2.	TS_2	0.1 % Mercuric chloride	7 minutes
3.	TS_3	5% Sodium hypochlorite	12 minutes
4.	TS_4	5 % Sodium hypochlorite	15 minutes
5	TS_5	5 % Sodium hypochlorite 0.1 % Mercuric chloride	10 minute 5 minutes

3.2.4 Media

The basal media used for the study was half strength Modified MS medium (Murashige and Skoog, 1962) prepared as per Annexure 1. Analytical grade chemicals obtained from Sisco Research Laboratory (SRL, Bombay), Merk

(Bombay), Sigma (U.S.A) and Hymedia were used for the preparation of the culture media. Standard procedure was followed for the preparation of media (Thorpe, 1980).

Stock solutions of major and minor nutrients were prepared by dissolving the required quantity of chemicals in exact volume of double glass-distilled water. Plant growth regulators were first dissolved in 1N NaOH or 95 per cent Ethanol and the volume was made up with double glass-distilled water and the stock solutions were stored under refrigerated condition at 4°C.

The stock solutions of plant growth substances were stored only for a period of one week whereas the other stock solutions were stored up to a period of one month. All the glasswares used for the preparation of the culture media were washed with tap water containing a few drops of labolene and rinsed with double glass-distilled water. Specific quantities of stock solutions were pipetted into a 1000 ml beaker. Sucrose was freshly added and dissolved. For specific treatments, other additives were directly added while preparing the media. Then volume was made up to 950 ml using double glass-distilled water. After adding all the media components, except agar, the pH was adjusted between 5.5 and 5.7 using 0.1 N NaOH and 0.1 N HCl with an electronic pH meter. Then 6.0 g L^{-1} of agar was added to the medium and the final volume was made up to 1000 ml using double glass-distilled water. The beaker containing media was then kept for boiling in a microwave oven with intermittent stirring using a glass rod till the agar melted and a clear solution was obtained.

The boiled medium was then quickly poured into the pre-sterilized tissue culture bottles using a glass funnel and the mouth of the tissue culture bottles were closed tightly just after pouring the medium. Tissue culture bottles were then autoclaved at 121 °C and 1.06 Kg cm^{-2} pressure for 20 minutes. After autoclaving, the culture bottles were allowed to cool by placing them on a level plane, the mouth of the culture bottles were wiped with 70 per cent ethanol and transferred to the culture

room. The culture bottles were kept for five to seven days and the culture bottles showing media contamination could be removed.

3.2.5 Inoculation

The inoculation operations were carried out inside laminar air flow chamber. The glass wares (petri plates and beakers) and tools (blades, scalpels and forceps) that were required for inoculation were thoroughly washed, rinsed with double glass-distilled water, wrapped in plastic film and kept inside autoclavable poly propylene cover and autoclaved at 121°C and 1.06 Kg cm^{-2} pressure for 40 minutes.

After surface sterilization the explants were rinsed thrice with sterile double distilled water and the sterile leaf explants were given fresh and sharp cut along the margins to remove the portions which had turned brown due to sterilization. These sterile leaf explants were further cut in to into square pieces of 1-1.5 cm^2 on sterile petri plates using sharp blades. Leaf explants with and without midrib or major veins were used for inoculation. The water adhering to the explant was removed using sterilized blotting paper placed over a sterile petri dish. During inoculation the culture bottles were opened and closed in front of the spirit lamp flame and the explants were placed on the medium. The inoculated culture bottles were incubated in the culture room.

3.2.6 Incubation

The inoculated culture bottles were incubated at 25 °C in dark for callus induction and in light for shoot regeneration. Subculturing was done every three to four weeks. The explants were inoculated initially in a callusing medium and subsequently in callus multiplication and shooting medium. The neck of the culture bottles were wiped with 70 per cent ethanol at two days interval to avoid the entry of contaminants into the culture bottle.

3.2.7 Somatic Organogenesis

The six hybrids namely, HR X MR, LJ X OG, OG X NO, HoR X KR, PR X HR and HR X LR were used for the study. The leaf explants of these six hybrids were subjected to different treatments for callus induction (Table 2). The basal medium used was half strength Modified MS medium with quarter strength of ammonium nitrate (200 mgl^{-1}) as reported by Nirmala and Singh (1993) and Singh (1994). The treatments were replicated eight times and the influence of these treatments on callus initiation were studied. The number of cultures initiating callus in these six hybrids in the different treatments were recorded.

The calli obtained in all the six hybrids were subcultured in the same medium for a period of two months for callus multiplication. These were then subjected to different treatments for plantlet regeneration (Table 3).

3.2.7.1 Callus Induction

The leaf explants were cultured on half strength Modified MS medium with quarter strength of ammonium nitrate (200 mg L^{-1}) supplemented with different hormone concentrations of BA (0.5-1.5 mg L^{-1}) and 2,4-D (0.5-1.0 mg L^{-1}) to get maximum callus induction (Table 2). For callus induction the culture bottles were kept in dark at 25 °C and subcultured every third week. Observations on survival of explant at callus initiation stage, callus induction percentage and days to callus induction were recorded.

3.2.7.2 Callus Multiplication

The calli induced in all the six hybrids were subcultured in the same callusing medium for a period of two months for callus multiplication. The cultures were maintained in dark condition during callus multiplication.

Table 2. Treatments to assess the effect of media and hormones on callus induction

S. No.	Treatment No.	Treatments
1.	TC_1	½ MS + 0.5 mg L^{-1} BA
2.	TC_2	½ MS + 1.0 mg L^{-1} BA
3.	TC_3	½ MS + 0.5 mg L^{-1} BA + 0.5 mg L^{-1} 2,4-D
4.	TC_4	½ MS + 1.0 mg L^{-1} BA + 0.5 mg L^{-1} 2,4-D
5.	TC_5	½ MS + 1 mg L^{-1} BA + 1 mg L^{-1} 2,4-D

3.2.7.3 Regeneration of Shoot and Root

To obtain plantlets, the cultures after callus multiplication were subjected to different treatments (Table 3) for shoot regeneration. Cultures were provided with white light (3000 lux) with a photoperiod of 16 hours light and 8 hours dark photoperiod and incubated at 25°C. Observations on days to regeneration, days to emergence of first leaf and days to shoot and root generation were recorded. Shoot initiation percentage and root initiation percentage were calculated after three months of culturing.

Table 3. Treatments to evaluate the influence of media and hormones on regeneration of shoot and root.

S. No.	Treatment No.	Treatments
1.	TR_1	½ MS + 0.1 mg L^{-1} BA
2.	TR_2	½ MS + 0.3 mg L^{-1} BA
3.	TR_3	½ MS + 0.5 mg L^{-1} BA
4.	TR_4	½ MS + 0.8 mg L^{-1} BA
5.	TR_5	½ MS + 1.0 mg L^{-1} BA

3.2.7.4 Shoot Proliferation and Rooting

No separate treatments were tried for shoot proliferation and rooting as substantial proliferation and rooting occurred in the shoot initiation media itself.

3.2.8 Hardening and Acclimatization

Plantlets of two to three centimeter length were removed from the culture bottles, treated with 0.2% Bavistin 50 WP solution for 20 minutes and then drained to remove excess moisture. The rooted plantlets were planted in a tray containing sterilized fine river sand and charcoal in the ratio 3:1 and kept in mist chamber with sufficient nutrient supply in the form of fertilizer mixture N:P:K 19: 19: 19 as placement application and foliar spray of 1 % solution.

3.2.9 Statistical Analysis

Completely Randomized Design (CRD) was followed for statistical analysis wherever necessary as per Panse and Sukhathme (1985).

Results

4. RESULTS

The results of the two experiments of the present study are presented below, in two sections.

1. Genetic variability analysis of twenty *Anthurium andreanum* Linden hybrids.

2. *In vitro* mass multiplication of selected *Anthurium andreanum* Linden hybrids.

4.1 GENETIC VARIABILITY ANALYSIS OF TWENTY *ANTHURIUM ANDREANUM* LINDEN HYBRIDS

Qualitative and quantitative traits of twenty *Anthurium andreanum* Linden hybrids were recorded. The vegetative as well as the floral qualitative characters of the hybrids were evaluated under greenhouse conditions. Each genotype was replicated five times. The statistical analysis of the recorded data was carried out and the results of the study are presented under the following subheads.

4.1.1 Evaluation of hybrid anthurium genotypes

4.1.2 Estimation of variability components i.e., PCV and GCV

4.1.3 Estimation of Heritability and Genetic Advance

4.1.4 Correlation Analysis

4.1.5 Path Analysis

4.1.1 Evaluation of hybrid anthurium genotypes

The vegetative and floral characters recorded among twenty anthurium hybrids were subjected to analysis of variance (Table 4) and significant variations were observed among all the hybrids. The mean performances of the 20 hybrids for 19 (morphological, floral and qualitative) characters studied are represented in Table 5.

Table 4. Analysis of variance of vegetative and floral characters in *Anthurium andreanum* hybrids

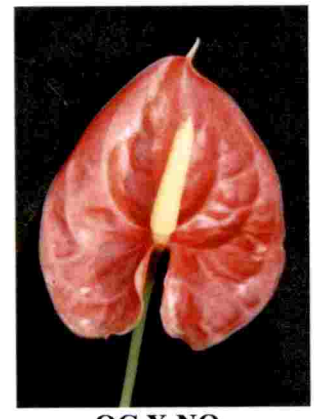
Sl. No.	Characters	Mean square	
		Genotype	Error
1	Plant height (cm)	233.284**	13.079
2	Leaf area (cm^2)	27901.166**	653.811
3	Internode length (cm)	0.216**	0.036
4	Days from emergence to maturity of leaves	48.027**	5.765
5	Number of leaves spadices^{-1} plant^{-1} year^{-1}	40.792**	2.115
6	Number of suckers plant^{-1}	2.234**	0.500
7	Days from emergence to maturity of inflorescence	35.227**	3.760
8	Spathe size (cm^2)	3665.580**	94.493
9	Spadix length (cm)	7.527**	0.316
10	Number of flowers spadix^{-1}	72907.610**	731.105
11	Life of spadix (days)	383.747**	23.560
12	Days to initiation of female phase	7.290**	0.775
13	Duration of female phase	9.648**	1.220
14	Duration of interphase	9.535**	1.115
15	Duration of male phase	7.432**	1.085
16	Inclination of candle with spathe (degrees)	1207.480**	19.410
17	Anthocyanin content (mg /g)	44,565.420**	51.469
18	Vase life	99.568**	1.275
19	Number of inflorescence year^{-1}	6.322**	0.630

*Significant at five per cent level ** Significant at one per cent level

LR X DT

HR X MR

LJ X OG



OG X NO

HR X KR

HoR X KR

Plate 1. Different genotypes of *Anthurium andreanum* Linden hybrids used for the study

HR X LJ

HR X DT

PR X HR

HR X P

P X LR

CR X KR

Plate 1. (Continued) Different genotypes of *Anthurium andreanum* Linden hybrids used for the study

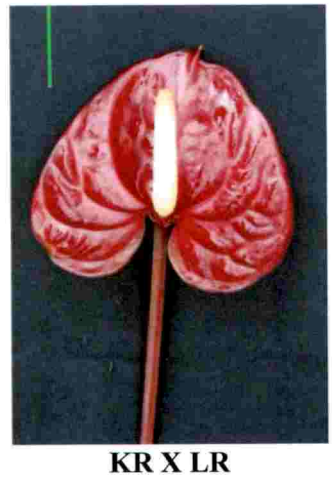
HR X LR

PR X DT

LR X OG

PR X LR

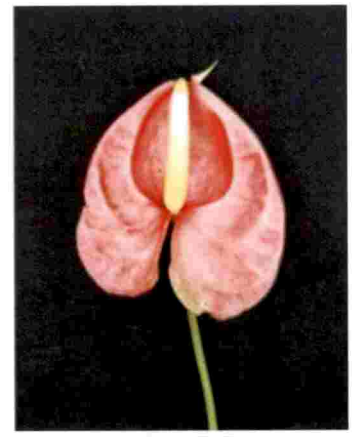
DT X HR



KR X LR

Plate 1. (Continued) Different genotypes of *Anthurium andreanum* Linden hybrids used for the study

PR X DT (1)



OG X DT

Plate 1. (Continued) Different genotypes of *Anthurium andreanum* Linden hybrids used for the study

Plate 2. General view of experimental field

4.1.1.1 Morphological Characters

4.1.1.1.1 Plant Height

The genotype HR x KR recorded the highest plant height (62.2 cm) followed by HoR x KR (51.9 cm), OG x NO (50.8), HR x DT (50.7 cm) and HR x LJ (50.3 cm). The lowest mean plant height was exhibited by the hybrid LR x OG (33.5 cm) which was on par with LR x DT (37.4 cm) and HR x LR (34.7 cm).

4.1.1.1.2 Leaf Area

Leaf area of the hybrid HoR x KR (410.85 cm^2) was found to be the maximum followed by HR x KR (366.55 cm^2), LR x DT (291.80 cm^2), OG x NO (282.60 cm^2) and HR x DT (268.70 cm^2). Leaf area was the minimum for the hybrid PR x HR (120.20 cm^2) which was on par with PR x DT (1) (152.10 cm^2).

4.1.1.1.3 Internode Length

Internode length was noticed to be the highest in the hybrid HR x KR (1.60) which was on par with the hybrids HoR x KR (1.58) and HR x P (1.44). The lowest internodal length was observed in the genotype HR x MR (0.70) which was found to be on par with PR x DT (1) (0.92).

4.1.1.1.4 Days from Emergence to Maturity of Leaves

The maximum number of days from emergence to maturity of leaves was recorded in the hybrid HR x KR (31.4 days) which was on par with OG x DT (30.2 days), DT x HR (29.0 days), KR x LR (29.0 days), PR x LR (28.8 days), HR x P (28.8 days) and HoR x KR (28.6 days). Hybrid LR x DT recorded the lowest number of days (18.8) for leaf maturity.

4.1.1.1.5 Number of leaves spadices^{-1} plant^{-1} year^{-1}

The highest number of leaves spadices^{-1} plant^{-1} year^{-1} was recorded in the hybrid HR x MR (15.8) followed by HR x LJ (10.6), LR x OG (7.8), HoR x KR (6.2) and OG x NO (6). The hybrids HR x P (3.6) and LR x OG (3.6) had the lowest number of leaves and the value was on par with LR x DT (5.4), PR x HR

(5.4), PR x DT (1) (5.2), HR x LR (5.2), HR x DT (5.2), HR x KR (4.8), PR x LR (4.8), DT x HR(4.8), KR x LR (4.4), OG x DT (4.4), CR x KR (4.2), P x LR (4) and PR x DT (3.8).

4.1.1.1.6 Number of Suckers Plant^{-1}

The maximum number of suckers plant^{-1} was noticed in the hybrid OG x NO (2.2) which was on par with HR x MR (1.8). The hybrids HR x LJ, HR x P and PR x DT (1) produced no suckers and were on par with OG x DT(1), LJ x OG (0.8), LR x DT (0.6), HoR x KR (0.6), PR x HR (0.6), KR x LR (0.6), HR x KR (0.4), HR x DT (0.4), HR x LR (0.4), P x LR (0.2), PR x LR (0.2) and DT x HR (0.2).

4.1.1.1.7 Colour of Petiole and Young Leaf

A wide variation was observed among the hybrids regarding the character colour of petiole and colour of young leaf. Green, greenish brown, brownish green and brown coloured petiole and young leaves were observed among the hybrids studied.

4.1.1.1.8 Pest and Disease Incidence

No major pest incidence was noticed in the experimental field during the period of study. Incidence of sucking pests was less due to application of pesticides at monthly intervals. Snails and leaf eating caterpillars were also rarely observed in field during the study.

The major diseases that infected the experimental plot were anthracnose and bacterial leaf blight. Symptoms of bacterial leaf blight occurred both on leaves and flowers. All anthuriums irrespective of genotype was infected with these diseases. Bacterial leaf blight was controlled by the application of Bacteriomycin 6 g per 20 litres of water at fortnightly intervals. Control over anthracnose was achieved by foliar application of Nativo at the concentration 0.5 g L^{-1} in weekly intervals. Anthracnose disease was also controlled by using Mancozeb 3 per cent spray at fortnightly intervals.

Table 5a. Mean performance of vegetative characters in *Anthurium andreanum* hybrids

Sl. No.	Genotypes	Plant height (cm)	Leaf area (cm^2)	Internode length (cm)	Days from emergence to maturity of leaves	Number of leaves spadices^{-1} plant^{-1} year^{-1}	Number of suckers plant^{-1}
1	LR x DT	37.4	291.80	1.22	18.8	5.4	0.6
2	HR x MR	39.6	189.85	0.70	22.0	15.8	1.8
3	LJ x OG	46.9	210.45	1.10	21.0	7.8	0.8
4	OG x NO	50.8	282.60	1.28	26.0	6.0	2.2
5	HR x KR	62.2	366.55	1.60	31.4	4.8	0.4
6	HoR x KR	51.9	410.85	1.58	28.6	6.2	0.6
7	HR x LJ	50.3	261.05	1.04	27.4	10.6	0
8	HR x DT	50.7	268.70	1.02	27.8	5.2	0.4
9	PR x HR	42.8	120.20	1.22	27.2	5.4	0.6
10	HR x P	49.7	167.45	1.44	28.8	3.6	0
11	P x LR	39.3	183.60	1.22	27.0	4.0	0.2
12	CR x KR	49.4	178.15	1.12	25.8	4.2	2.0
13	HR x LR	34.7	203.80	1.22	24.6	5.2	0.4
14	PR x DT	43.0	173.15	1.18	27.2	3.8	0
15	LR x OG	33.5	153.10	1.14	27.0	3.6	1.2
16	PR x LR	40.2	208.60	1.26	28.8	4.8	0.2
17	DT x HR	43.7	168.10	1.08	29.0	4.8	0.2
18	KR x LR	41.8	167.45	1.28	29.0	4.4	0.6
19	PR x DT (1)	43.4	152.10	0.92	27.0	5.2	0
20	OG x DT	44.2	184.90	1.34	30.2	4.4	1.0
	Mean	44.775	217.123	1.198	26.730	5.760	0.660
	CD (0.05)	4.552	32.182	0.239	3.022	1.830	0.890

a. Anthracnose

b. Bacterial leaf blight

Plate 3. Pest and disease incidence in anthurium

c. Caterpillar attack

d. Scale insect attack

Plate 3. (Continued) Pest and disease incidence in anthurium

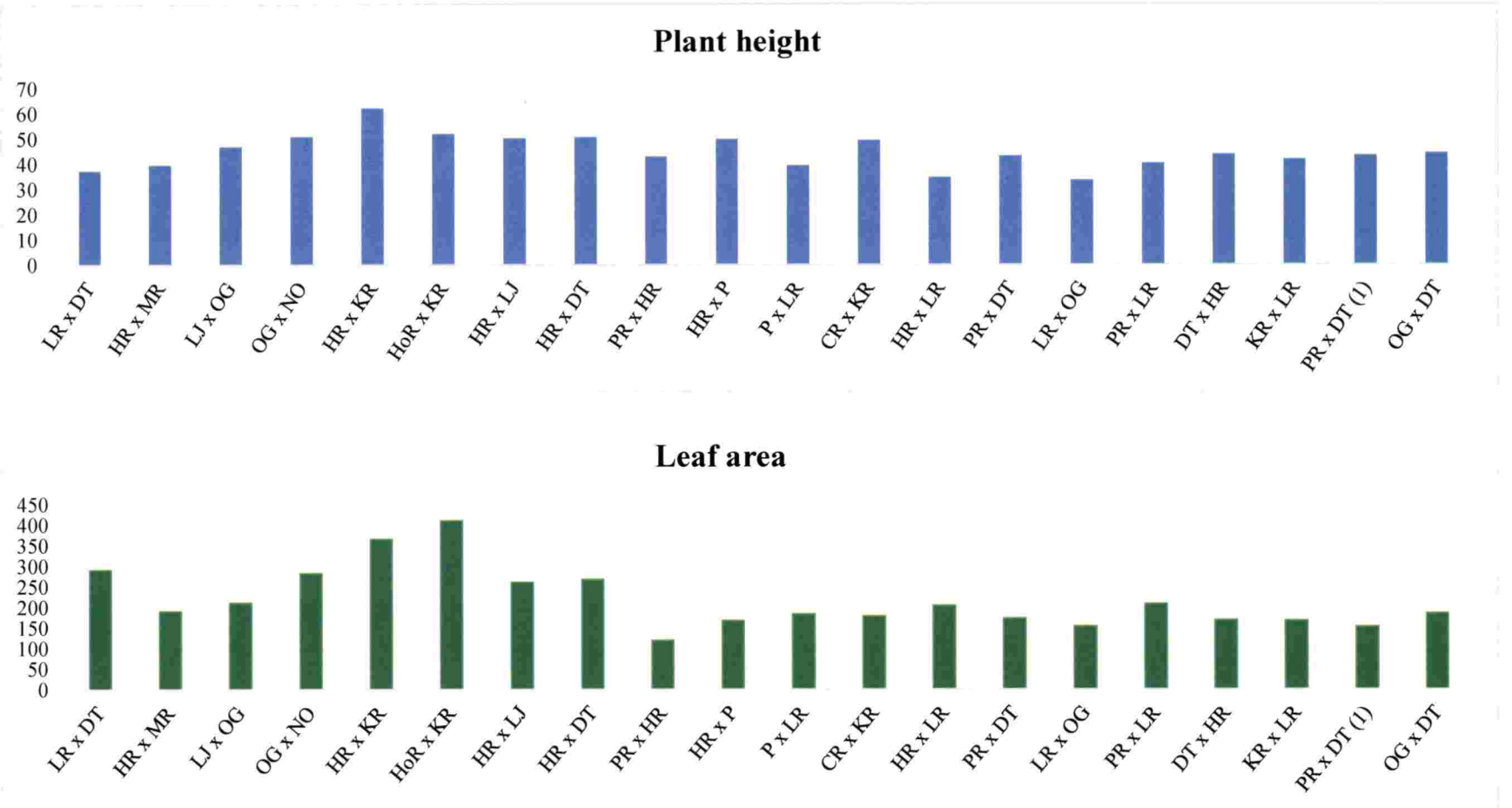


Fig. 1a. Mean performance of 20 *Anthurium andreanum* hybrids

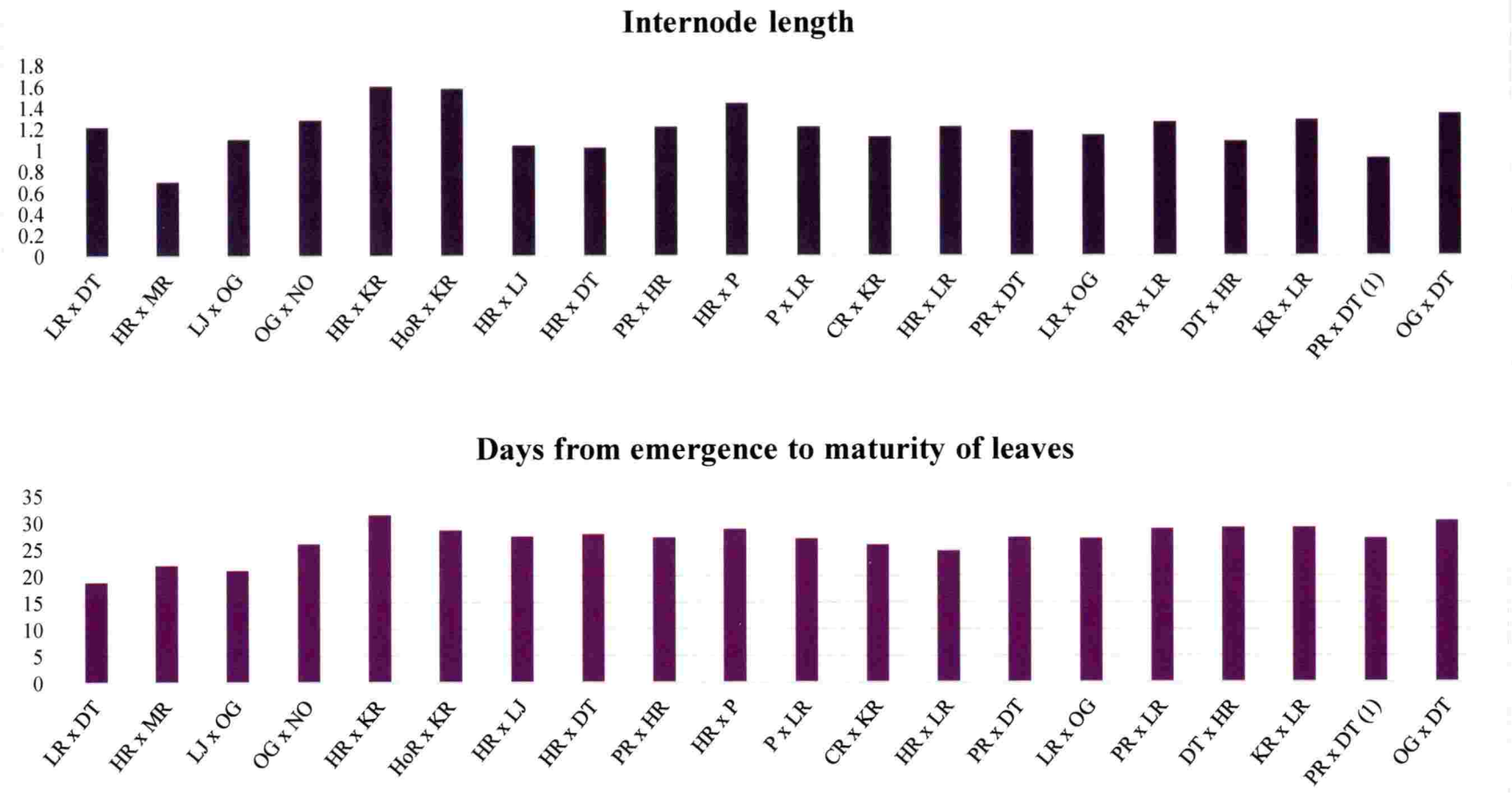


Fig. 1b. Mean performance of 20 *Anthurium andreanum* hybrids

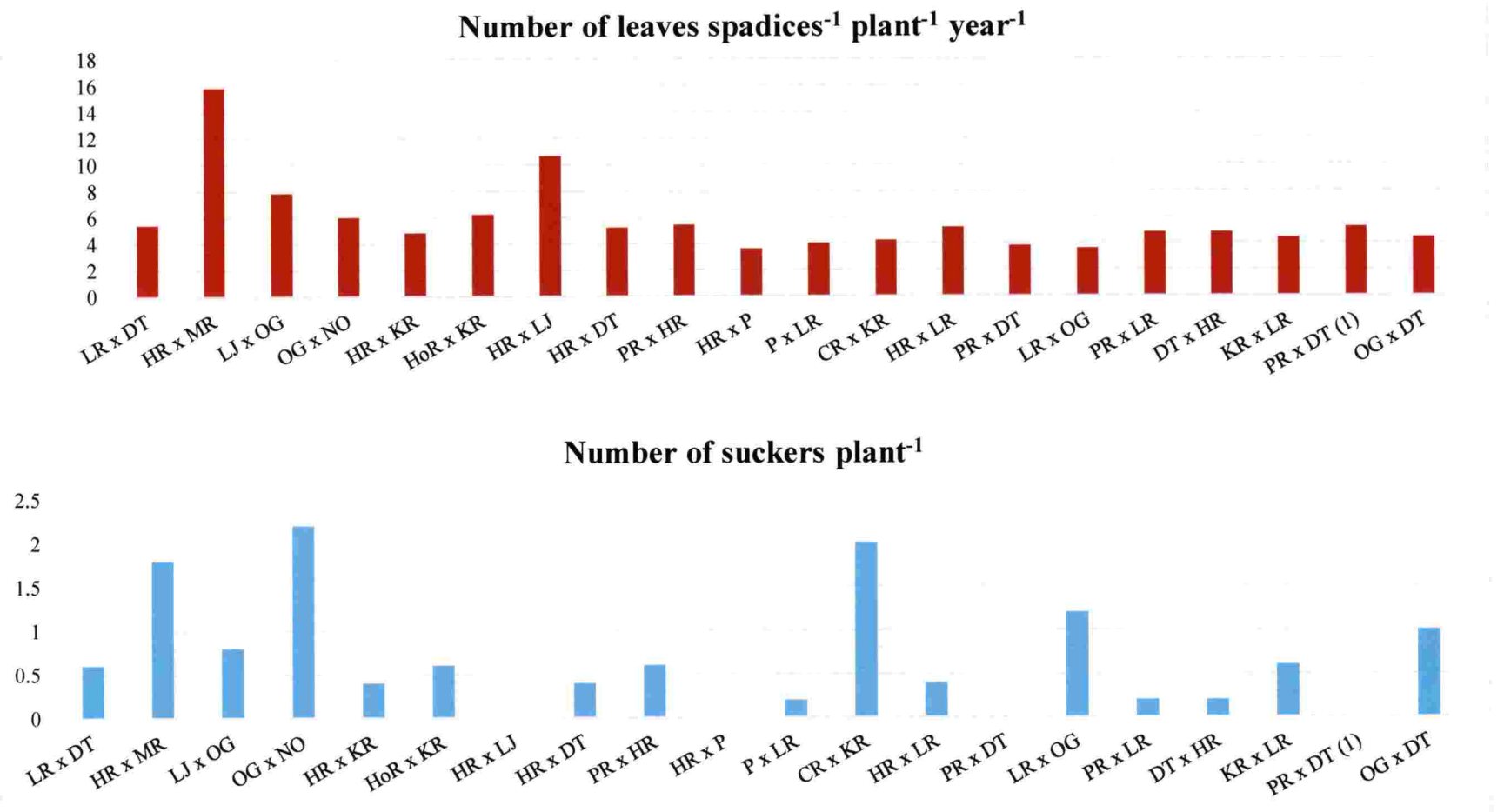


Fig. 1c. Mean performance of 20 *Anthurium andreanum* hybrids

4.1.1.2 Floral Characters

4.1.1.2.1 Days from Emergence to Maturity of Inflorescence

The hybrid HR x LJ and PR x LR showed the maximum duration from emergence to maturity of inflorescence (30.8 days) and the value was on par with HoR x KR (29.6 days), KR x LR (29.2 days), HR x MR (28.8 days) and OG x DT (28.6 days). The hybrid HR x LR (21.6 days) took the least number of days from emergence to maturity of inflorescence and was on par with HR x DT (22.6 days), PR x DT (23.2 days), LR x OG (23.8 days) and OG x NO (24 days).

4.1.1.2.2 Spathe Size

The maximum spathe size was observed for the hybrid HoR x KR (112.30 cm^2) followed by HR x LR (96.55) and HR x KR (91.60). The smallest spathe was observed for the hybrid HR x MR (27.60 cm^2) which was on par with HR x LJ (38.45 cm^2), DT x HR (38.00 cm^2), P x LR (37.45 cm^2), LG x OG (35.05 cm^2), PR x HR (32.85 cm^2) and KR x LR (30.00 cm^2).

4.1.1.2.3 Spadix Length

Mean spadix length was the maximum for the hybrid HR x KR (8.58 cm) while the minimum was for the hybrid KR x LR (3.64 cm) which was on par with OG x DT (4.30 cm), LJ x OG (4.30 cm), DT x HR (4.14 cm), HR x MR (4.04 cm), CR x KR (4.04 cm), PR x HR (3.98 cm), PR x DT (1) (3.82) and PR x LR (3.74).

4.1.1.2.4 Number of Flowers Spadix^{-1}

The maximum number of flowers spadix^{-1} was observed for the hybrid HR x KR (678.0) followed by HR x LJ (632.2) and OG x NO (503.6). The least number of flowers spadix^{-1} was recorded in the hybrid HR x MR (223.6).

Table 5b. Mean performance of floral characters in *Anthurium andreanum* hybrids.

Sl. No.	Genotypes	Days from emergence to maturity of inflorescence	Spathe size (cm^2)	Spadix length (cm)	Number of flowers $spadix^{-1}$	Life of spadix (days)
1	LR x DT	27.6	86.85	5.50	452.4	75.2
2	HR x MR	28.8	27.60	4.04	223.6	69.6
3	LJ x OG	26.6	35.05	4.30	379.6	77.6
4	OG x NO	24.0	89.40	5.38	503.6	95.0
5	HR x KR	27.2	91.60	8.58	678.0	87.2
6	HoR x KR	29.6	112.3	6.04	379.2	90.2
7	HR x LJ	30.8	38.45	6.76	632.2	85.6
8	HR x DT	22.6	90.05	5.18	273.6	77.0
9	PR x HR	27.0	32.85	3.98	283.2	69.4
10	HR x P	26.0	46.10	6.04	452.8	58.8
11	P x LR	27.2	37.45	5.52	373.8	78.0
12	CR x KR	27.4	90.75	4.04	275.6	67.8
13	HR x LR	21.6	96.55	5.26	291.6	72.4
14	PR x DT	23.2	58.15	5.60	416.2	87.0
15	LR x OG	23.8	42.10	4.96	335.8	74.8
16	PR x LR	30.8	48.15	3.74	339.4	73.0
17	DT x HR	24.6	38.00	4.14	319.0	78.0
18	KR x LR	29.2	30.00	3.64	261.6	67.8
19	PR x DT (1)	26.8	61.60	3.82	295.4	78.0
20	OG x DT	28.6	57.75	4.30	302.8	81.6
	Mean	26.670	60.538	5.041	373.470	77.200
	CD (0.05)	2.440	12.234	0.707	34.031	6.109

4.1.1.2.5 Life of Spadix

The highest mean value for life of spadix on plant was observed in the hybrid OG x NO (95.0 days) which was on par with HoR x KR (90.2 days). The shortest spadix life was recorded in the genotype HR x P (58.8 days).

4.1.1.2.6 Days to Initiation of Female Phase

The hybrid HoR x KR took the maximum days (9.4 days) for initiation of female phase on the candle after flower opening. Initiation of female phase was the fastest in the hybrids HR x KR (4.4 days), CR x KR (4.4 days), HR x LR (4.4 days) and were on par with PR x DT (5.0 days), HR x MR (5.0 days) and LJ x OG (5.0 days).

4.1.1.2.7 Duration of Female Phase

Mean duration of female phase was observed to be the maximum in the hybrid HR x P (10.4 days) which was on par with HR x LJ (10.2 days), P x LR (9.4 days) and OG x DT (9.2 days).

4.1.1.2.8 Duration of Interphase

The highest mean number of days for interphase was recorded in the hybrid PR x DT (9.4 days) followed by LJ x OG (7.8 days) and HR x LJ (7.0 days). The minimum duration of interphase was noticed in HoR x KR (4.4 days), HR x DT (4.4 days), CR x KR (4.4 days), HR x LR (4.4 days), LR x OG (4.4 days) and DT x HR (4.4 days) which were on par with the hybrids PR x LR (5.6 days), HR x KR (5.2 days), HR x P (5.2 days), LR x DT (4.8 days), HR x MR (4.8 days), PR x HR (4.8 days) and OG x DT (4.8 days).

4.1.1.2.9 Duration of Male Phase

The longest mean duration of male phase was recorded by the hybrid HR x LJ (10.8 days) which was on par with the hybrid PR x DT (10.4 days), OG x NO (9.8 days) and HR x KR (9.6 days). The least mean duration of male phase was

Table 5b. Continued

Sl. No.	Genotypes	Days to initiation of female phase	Duration of female phase	Duration of interphase	Duration of male phase
1	LR X DT	6.4	6.8	4.8	8.0
2	HR X MR	5.0	5.4	4.8	7.6
3	LJ X OG	5.0	9.0	7.8	6.6
4	OG X NO	7.2	9.8	6.8	9.8
5	HR X KR	4.4	8.6	5.2	9.6
6	HoR X KR	9.4	8.4	4.4	8.2
7	HR X LJ	6.2	10.2	7.0	10.8
8	HR X DT	5.0	6.8	4.4	7.4
9	PR X HR	5.8	8.4	4.8	8.0
10	HR X P	6.2	10.4	5.2	8.2
11	P X LR	5.8	9.4	6.6	7.4
12	CR X KR	4.4	7.0	4.4	8.2
13	HR X LR	4.4	8.2	4.4	8.6
14	PR X DT	5.0	7.6	9.4	10.4
15	LR X OG	6.4	7.2	4.4	7.6
16	PR X LR	7.4	6.8	5.6	6.2
17	DT X HR	6.0	6.6	4.4	6.6
18	KR X LR	5.8	6.8	5.8	8.4
19	PR X DT (1)	6.0	6.8	6.6	8.8
20	OG X DT	6.8	9.2	4.8	7.6
	Mean	5.930	7.970	5.580	8.200
	CD (0.05)	1.108	1.390	1.329	1.311

observed in PR x LR (6.2 days) which was on par with HR x DT (7.4 days), P x LR (7.4 days) and DT x HR (6.6 days).

4.1.1.2.10 Inclination of Candle with the Spathe

The angle between the candle and the spathe was the highest in the hybrid LR x DT (103.2°). The lowest mean angle of inclination of candle with the spathe was recorded in the hybrid P x LR (37.2°) which was on par with OD x NO (42.6°), DT x HR (39.0°) and PR x DT (1) (39.0°).

4.1.1.2.11 Anthocyanin Content

The total anthocyanin content showed wide variation among the hybrids and it ranged from 36.184 mg g^{-1} in LR x OG to 326.434 mg g^{-1} in the hybrid LR x DT. The hybrids with orange coloured spathe such as LR x OG (36.184 mg g^{-1}), OG x DT (38.770 mg g^{-1}) and OG x NO (41.712 mg g^{-1}) showed the lowest anthocyanin content. Among the red spathe genotypes the highest anthocyanin content was observed in the hybrid LR x DT (326.434 mg g^{-1}) followed by PR x LR (283.984 mg g^{-1}) and HR x LR (272.378 mg g^{-1}).

4.1.1.2.12 Vase Life

The mean vase life of the cut flower in water was the highest for the hybrid HoR x KR (24.4 days) which was on par with the genotype LJ x OG (23.4 days). The lowest mean vase life was observed for the genotype KR x LR (10 days) which was on par with the hybrid P x LR (11.4 days).

4.1.1.2.13 Number of Inflorescence $year^{-1}$

The hybrid HR x MR (6.4) produced the maximum number of inflorescence in a year compared to all other hybrids. The minimum number of inflorescence was produced by the genotype CR x KR (2.2) which was on par with the hybrids LR x OG (2.8), KR x LR (2.8), HR x KR (2.6), HR x DT (2.6), HR x P (2.6), P x LR (2.4) and OG x DT (2.4).

Table 5b. Continued

Sl. No.	Genotypes	Inclination of candle with spathe (degrees)	Anthocyanin content (mg g^{-1})	Vase life (days)	Number of inflorescence $year^{-1}$
1	LR x DT	103.2	326.434	18.4	4.0
2	HR x MR	43.6	118.240	17.2	6.4
3	LJ x OG	48.4	50.574	23.4	5.0
4	OG x NO	42.6	41.712	22.4	5.0
5	HR x KR	71.8	174.904	20.4	2.6
6	HoR x KR	71.4	233.626	24.4	4.4
7	HR x LJ	62.4	57.648	21.2	4.0
8	HR x DT	52.4	181.772	17.0	2.6
9	PR x HR	51.2	120.182	15.0	4.6
10	HR x P	47.6	78.452	10.4	2.6
11	P x LR	37.2	182.286	11.4	2.4
12	CR x KR	64.2	168.954	11.8	2.2
13	HR x LR	56.6	272.378	20.4	4.4
14	PR x DT	43.2	225.422	15.6	3.4
15	LR x OG	63.4	36.184	11.8	2.8
16	PR x LR	55.8	283.984	13.2	3.2
17	DT x HR	39.0	263.476	14.4	3.0
18	KR x LR	62.6	263.824	10.0	2.8
19	PR x DT (1)	39.0	237.170	15.8	3.6
20	OG x DT	64.6	38.770	13.6	2.4
	Mean	56.010	167.800	16.390	3.570
	CD (0.05)	5.545	9.029	1.421	0.999

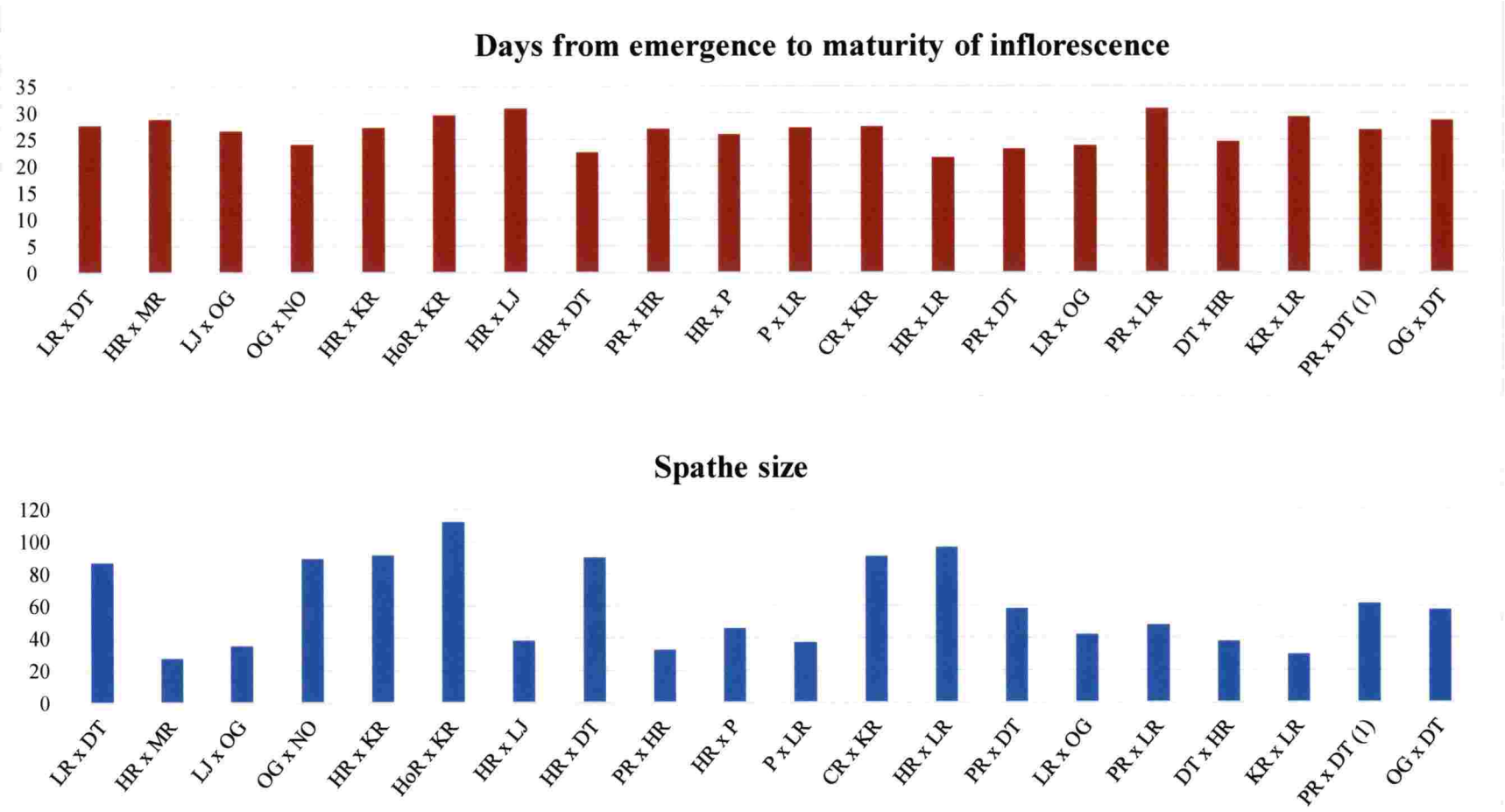


Fig. 1d. Mean performance of 20 *Anthurium andreanum* hybrids

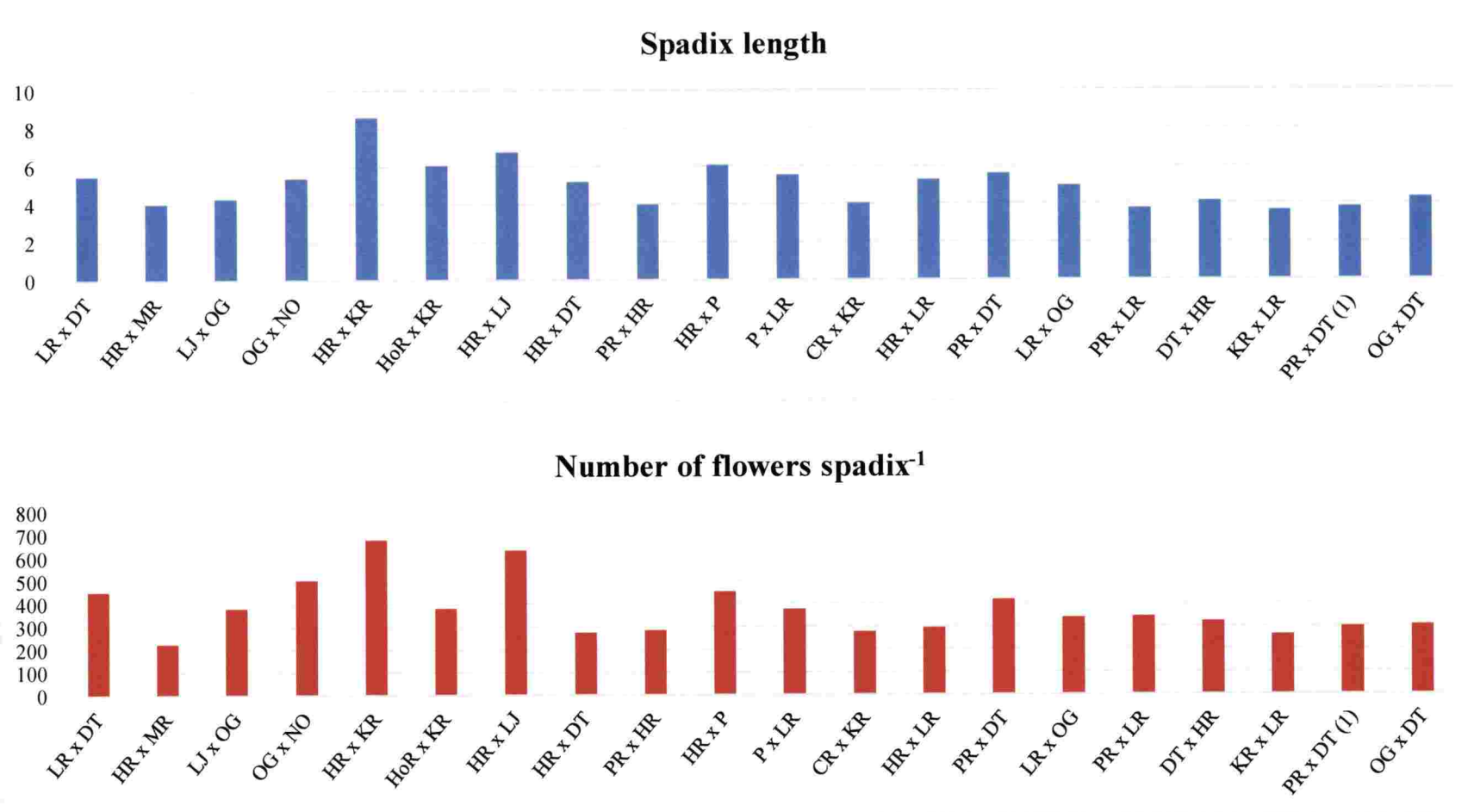


Fig. 1e. Mean performance of 20 *Anthurium andreanum* hybrids

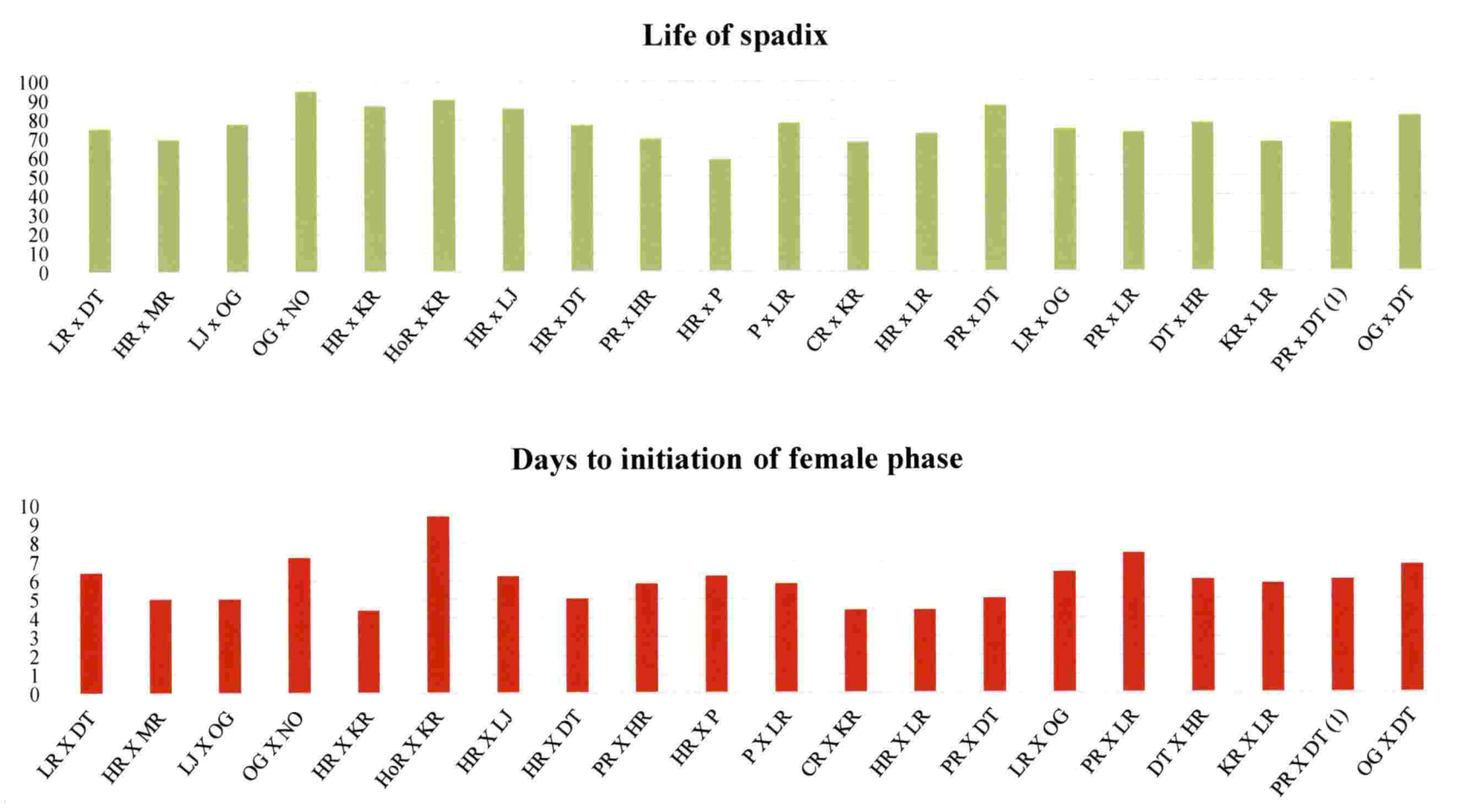


Fig. 1f. Mean performance of 20 *Anthurium andreanum* hybrids

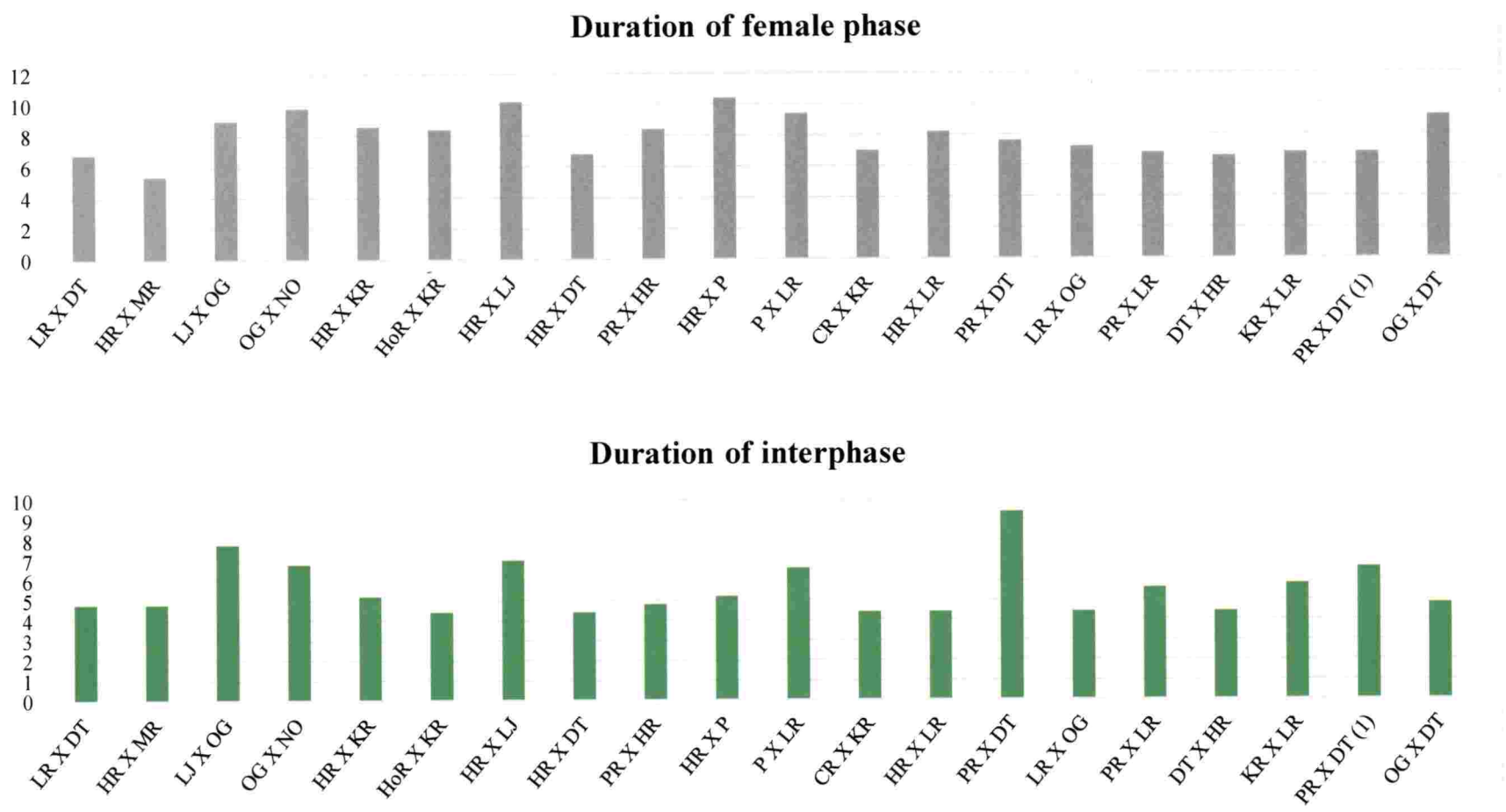


Fig. 1g. Mean performance of 20 *Anthurium andreanum* hybrids

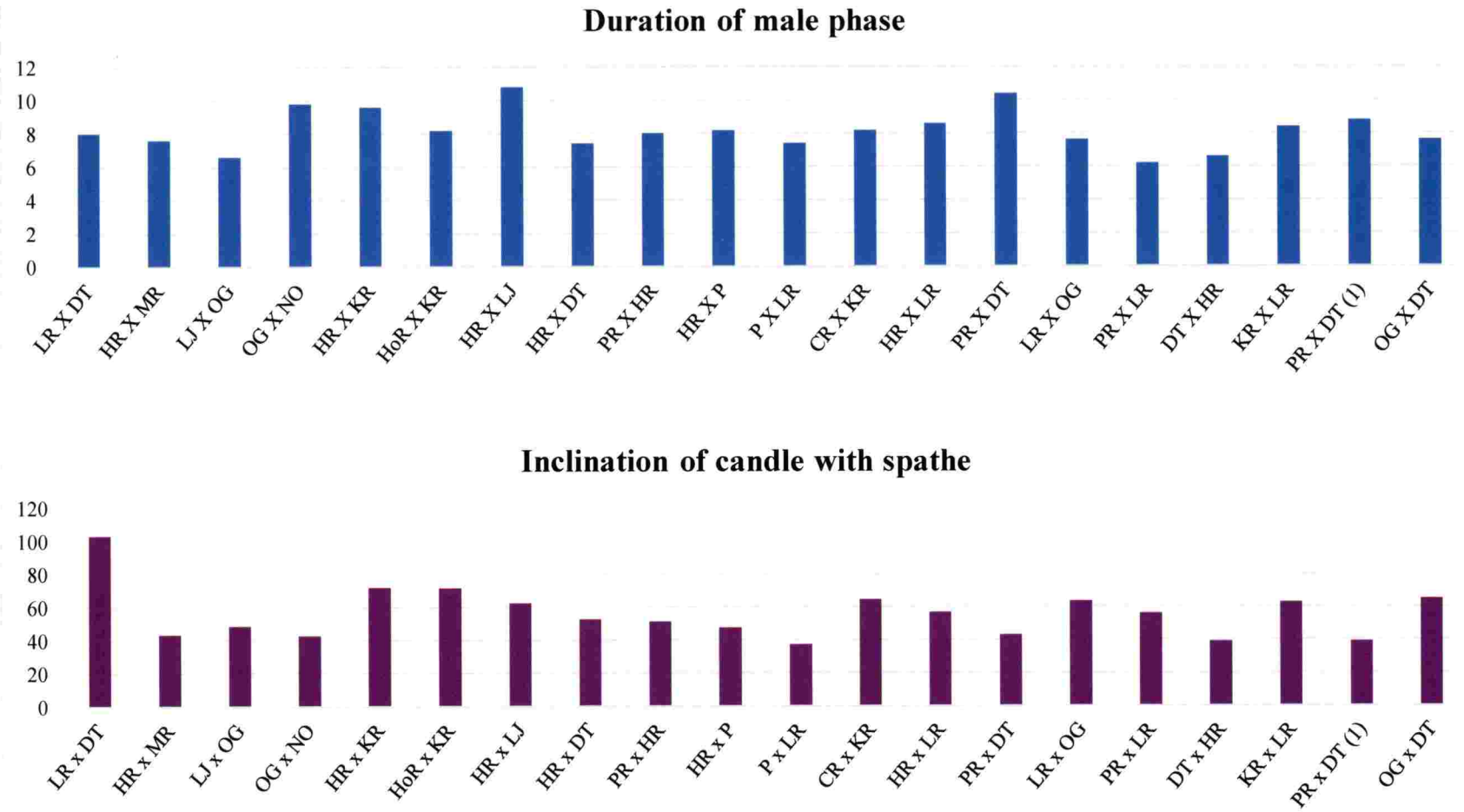


Fig. 1h. Mean performance of 20 *Anthurium andreanum* hybrids

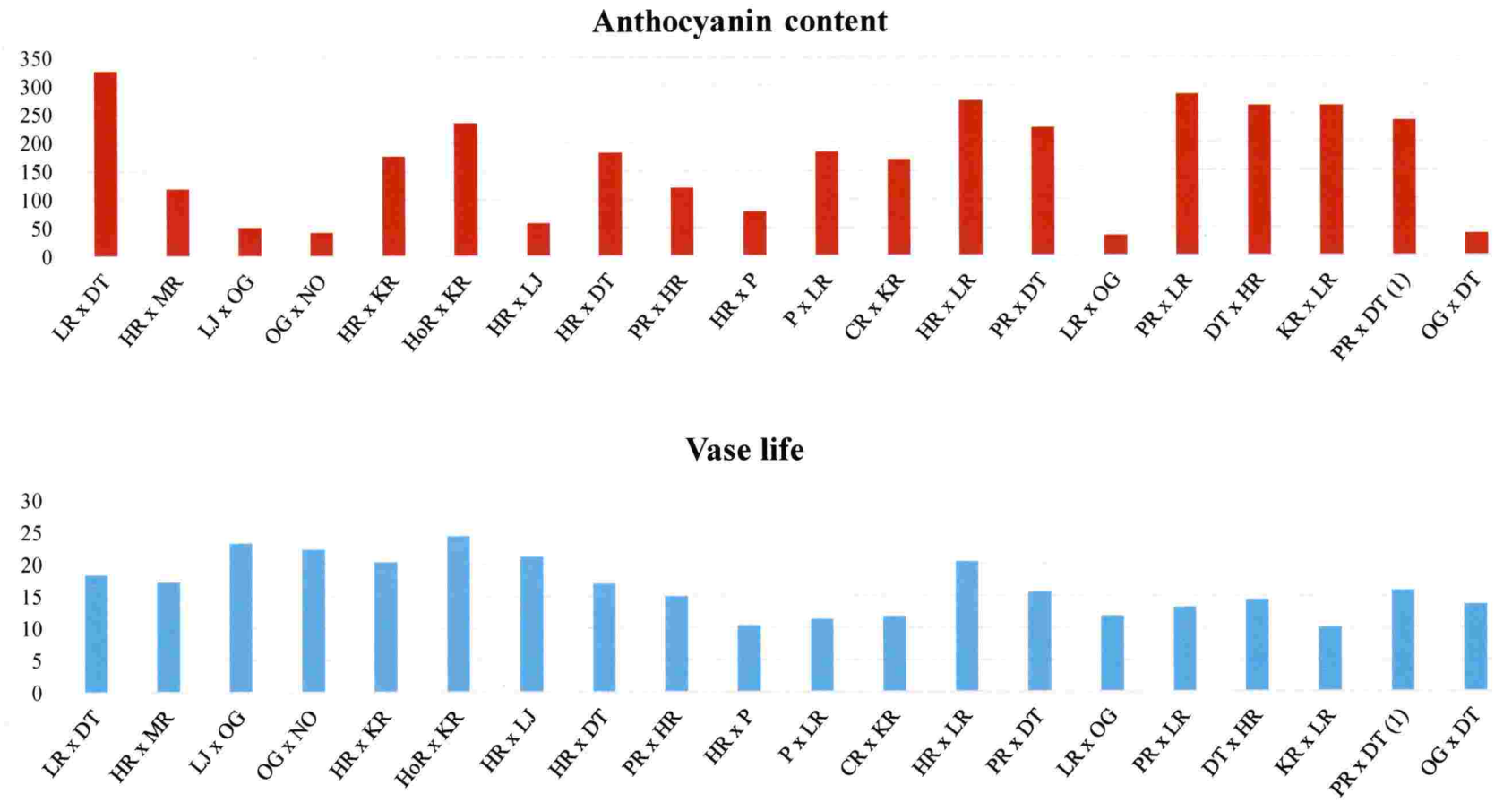


Fig. 1i. Mean performance of 20 *Anthurium andreanum* hybrids

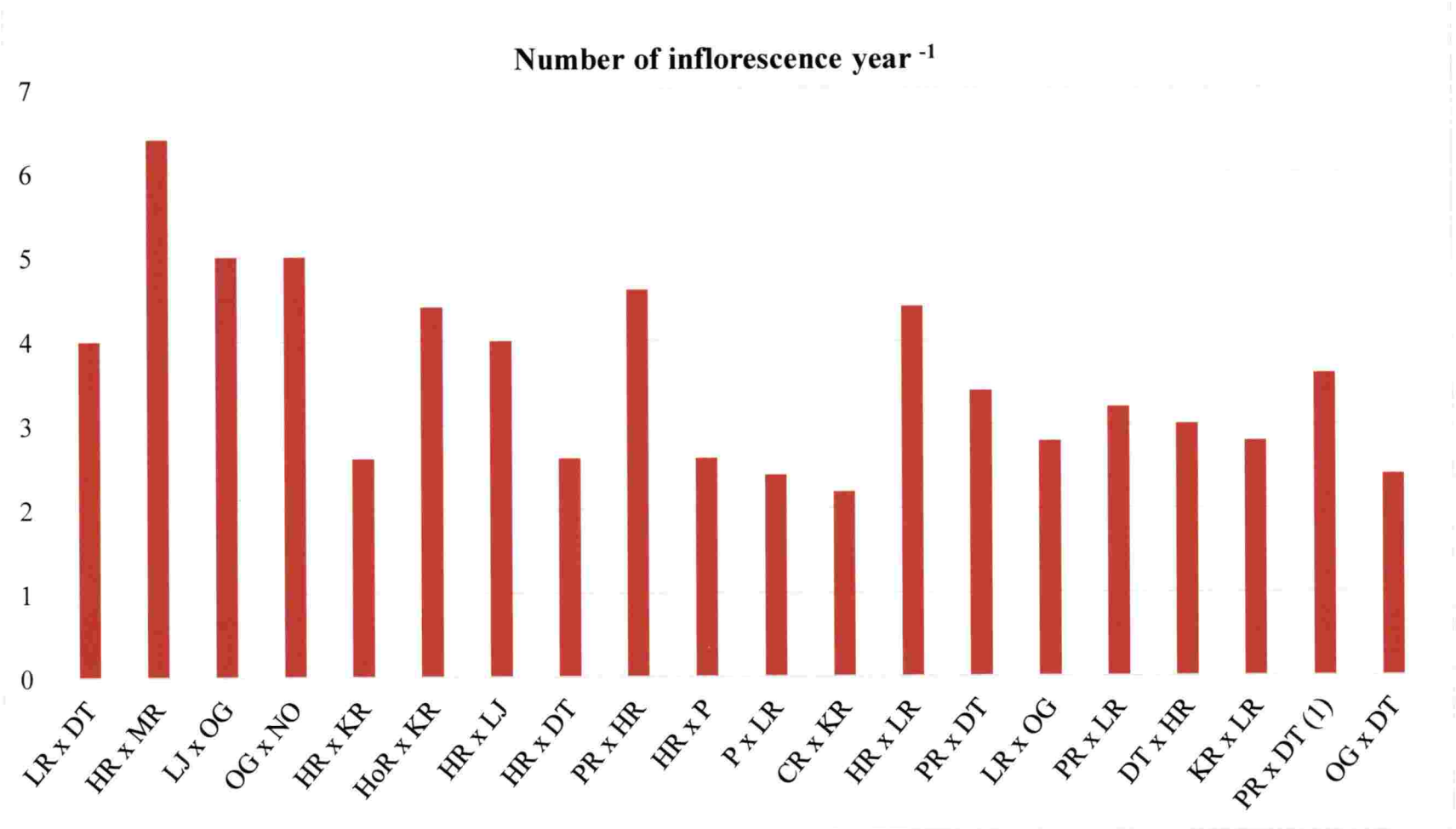


Fig. 1j. Mean performance of 20 *Anthurium andreanum* hybrids

4.1.1.3 Qualitative Characters

4.1.1.3.1 Spathe Colour

The colour of the spathe recorded wide variation and was found to occur in dark red, red, light red, orange, light orange, light pink and pinkish white.

4.1.1.3.2 Spadix Colour

The spadix or candle colour showed variations like yellowish red, whitish red, greenish red, whitish yellow, yellowish green, yellowish white, reddish white, and white.

4.1.1.3.3 Type of Inflorescence Axis

Type of Inflorescence axis showed wide variations like long curved thick, long straight thick, medium straight thin, medium curved thin, medium curved thick, short curved thick and short curved thin.

4.1.1.3.4 Pollen Emergence Pattern

Pollen emergence was found to be higher in winter months and it was very low in summer months i.e., from March to June. High pollen emergence was noticed in the hybrids HR x KR, HR x MR and low in OG x DT and OG x NO.

4.1.1.3.5 Pollen Shape

Pollen shape varied from round to oval among the twenty anthurium hybrids studied. The hybrids LJ x OG, HR x LJ and OG x DT had oval shaped pollen while all the other hybrids had round pollen.

4.1.1.3.5 Pollen Colour

Pollen colour ranged from cream to white among the hybrids under study. The hybrids LJ x OG and HoR x KR had cream coloured pollen while all the other hybrids had white coloured pollen.

Table 5c. Qualitative characters of *Anthurium andreanum* hybrids

Genotype	Colour of petiole	Colour of young leaf	Spathe colour	Spadix colour	Type of inflorescence axis	Pollen shape	Pollen colour
LR x DT	Greenish Brown	Greenish Brown	Dark Red	Yellowish Red	Medium Curved Thick	Round	White
HR x MR	Green	Brownish Green	Light Red	Whitish Red	Medium Straight Thin	Round	White
LJ x OG	Dark Green	Brownish Green	Light Pink (Rose)	White	Medium Curved Thick	Oval	Cream
OG x NO	Dark Green	Light Green	Orange	Yellowish White	Long Curved Thick	Round	White
HR x KR	Greenish Brown	Greenish Brown	Red	Whitish Red	Long Curved Thick	Round	White
HoR x KR	Greenish Brown	Light Green	Light Red	Whitish Red	Long Straight Thick	Round	Cream
HR x LJ	Green	Brownish Green	Pinkish White	White	Long Curved Thick	Oval	White
HR x DT	Brownish Green	Brown	Red	Whitish Red	Short Curved Thick	Round	White
PR x HR	Greenish Brown	Brownish Green	Dark Red	Yellowish Red	Long Straight Thin	Round	White
HR x P	Greenish Brown	Brownish Green	Light Red	Greenish Red	Medium Curved Thick	Round	White
P x LR	Greenish Brown	Brown	Dark Red	Whitish Yellow	Medium Curved Thick	Round	White
CR x KR	Greenish Brown	Brown	Red	Reddish White	Medium Straight Thick	Round	White
HR x LR	Green	Brown	Light Red	Yellowish Red	Long Straight Thick	Round	White
PR x DT	Greenish Brown	Brownish Green	Red	Whitish Red	Medium Curved Thick	Round	White
LR x OG	Green	Green	Light Orange	Yellowish Green	Short Curved Thin	Round	White
PR x LR	Greenish Brown	Brown	Dark Red	Whitish Red	Medium Straight Thin	Round	White
DT x HR	Brownish Green	Brown	Dark Red	Yellowish Red	Short Curved Thin	Round	White
KR x LR	Brownish Green	Brown	Dark Red	Yellowish White	Medium Curved Thin	Round	White
PR x DT (1)	Greenish Brown	Brownish Green	Dark Red	Yellowish White	Medium Curved Thin	Round	White
OG x DT	Green	Brown	Light Orange	Yellowish White	Medium Curved Thin	Oval	White

4.1.2 Estimation of variability components i.e., PCV and GCV

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), which are the relative measures of variation used for comparison among characters measured in different units were computed and presented in Table 6. For all the observed characters, phenotypic coefficient of variation (PCV) is greater in magnitude than genotypic coefficient of variation (GCV). This indicated that environment has significant role in expression of the characters.

The maximum phenotypic (139.474 per cent) and genotypic (89.198 per cent) coefficients of variation were observed for number of suckers plant^{-1} followed by number of leaves spadices^{-1} plant^{-1} year^{-1} (GCV 48.265 per cent and PCV 54.562 per cent), spathe size (GCV 44.121 per cent and PCV 47.068 per cent), leaf area (GCV 33.992 per cent and PCV 36.010 per cent) and number of flowers spadix^{-1} (GCV 32.168 per cent and PCV 32.984 per cent).

The minimum phenotypic (9.381 per cent) and genotypic (11.970 per cent) coefficients of variation were recorded for the character days from emergence to maturity of inflorescence followed by days from emergence to maturity of leaves (GCV 10.849 per cent and PCV 14.191 per cent), life of spadix (GCV 10.981 per cent and PCV 12.710 per cent) and duration of male phase (GCV 13.750 per cent and PCV 18.680 per cent). Low GCV and medium PCV were observed in the trait days from emergence to maturity of inflorescence.

The characters number of suckers plant^{-1}, number of inflorescence year^{-1}, duration of interphase and number of leaves spadices^{-1} plant^{-1} year^{-1} showed the maximum differences between GCV and PCV. This indicated that the expression of these characters was considerably influenced by environmental factors. A small difference between GCV and PCV was observed for the characters namely anthocyanin content, number of flowers spadix^{-1}, vase life and inclination of candle with spathe, which proves that environmental factors have negligible effect on these characters and their expression is mainly due to genetic factors.

Table 6. Phenotypic coefficient of variation and genotypic coefficient of variation of 19 characters in *Anthurium andreanum* hybrids

Sl. No.	Characters	GCV (per cent)	PCV (per cent)
1	Plant height (cm)	14.803	16.946
2	Leaf area (cm^2)	33.992	36.010
3	Internode length (cm)	15.809	22.476
4	Days from emergence to maturity of leaves	10.849	14.191
5	Number of leaves spadices^{-1} plant^{-1} year^{-1}	48.265	54.562
6	Number of suckers plant^{-1}	89.198	139.474
7	Days from emergence to maturity of inflorescence	9.381	11.970
8	Spathe size (cm^2)	44.121	47.068
9	Spadix length (cm)	23.806	26.366
10	Number of flowers spadix^{-1}	32.168	32.984
11	Life of spadix (days)	10.981	12.710
12	Days to initiation of female phase	19.237	24.348
13	Duration of female phase	16.446	20.902
14	Duration of interphase	23.187	30.196
15	Duration of male phase	13.750	18.680
16	Inclination of candle with spathe (degrees)	27.542	28.546
17	Anthocyanin content (mg /g)	56.230	56.395
18	Vase life	27.049	27.927
19	Number of inflorescence year^{-1}	29.832	37.421

PCV -Phenotypic coefficient of variation

GCV -Genotypic coefficient of variation

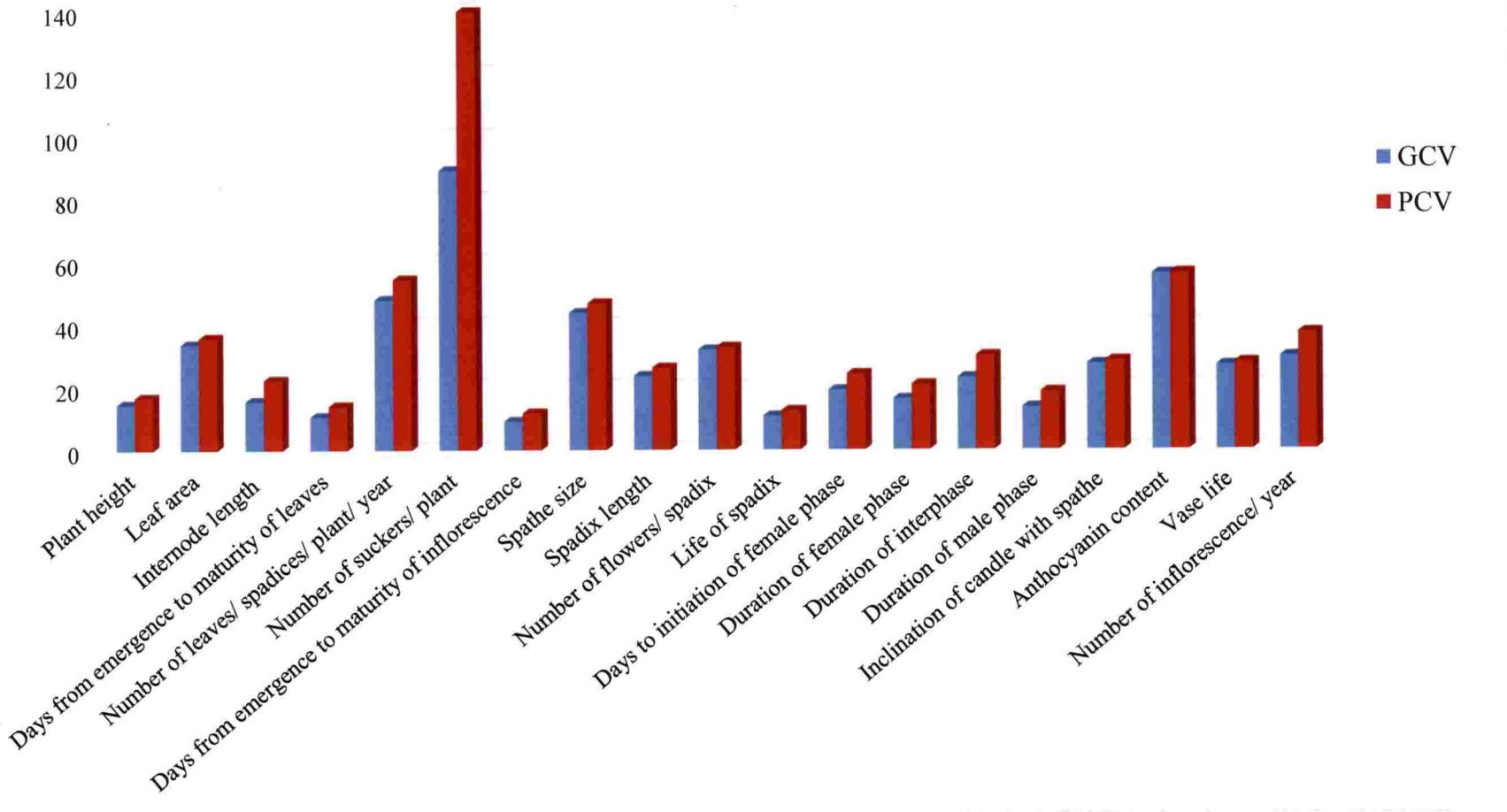


Fig. 2. PCV and GCV for 19 characters in *Anthurium andreanum* hybrids

4.1.3 Estimation of Heritability and Genetic Advance

Heritability estimates of a character provides the measure of effectiveness of selection for that particular character. The heritability and genetic advance estimates of the various characters studied are depicted in Table 7. High heritability (above 60 per cent) was observed for the characters such as anthocyanin content (99.417 per cent), number of flowers spadix^{-1} (95.114 per cent), vase life (93.810 per cent), inclination of candle with spathe (93.086 per cent), leaf area (89.103 per cent), spathe size (87.870 per cent), spadix length (81.524 per cent), number of leaves spadices^{-1} plant^{-1} year^{-1} (78.251 per cent), plant height (76.304 per cent), life of spadix (74.651 per cent), number of inflorescence year^{-1} (63.551 per cent), days to initiation of female phase (62.421 per cent), duration of female phase (61.910 per cent) and days from emergence to maturity of inflorescence (61.416 per cent). The traits internode length, days from emergence to maturity of leaves, number of suckers plant^{-1}, duration of interphase and male phase exhibited medium heritability (30-60 per cent).

Genetic advance as percentage of mean is independent of the unit of measurement and hence is used for comparison of characters. High genetic advance (>20 per cent) was exhibited for the traits number of suckers plant^{-1} (117.514 per cent), anthocyanin content (115.496 per cent), number of leaves spadices^{-1} plant^{-1} year^{-1} (87.951 per cent), spathe size (85.199 per cent), leaf area (66.098 per cent), number of flowers spadix^{-1} (64.627 per cent), inclination of candle with spathe (54.739 per cent), vase life (53.969 per cent), number of inflorescence year^{-1} (48.990 per cent), spadix length (44.279 per cent), duration of interphase (36.667 per cent), days to initiation of female phase (31.309 per cent), duration of female phase (26.658 per cent), plant height (26.636 per cent) internode length (22.905 per cent) and duration of male phase (20.850 per cent). Low genetic advance was observed for life of spadix (19.545 per cent), days from emergence to maturity of leaves (17.086 per cent) and days from emergence to maturity of inflorescence (15.144 per cent).

Table 7. Heritability and genetic advance of 19 characters in *Anthurium andreanum* hybrids

Sl. No.	Characters	Heritability (per cent)	Genetic advance (percentage of mean)
1	Plant height (cm)	76.304	26.636
2	Leaf area (cm^2)	89.103	66.098
3	Internode length (cm)	49.470	22.905
4	Days from emergence to maturity of leaves	58.450	17.086
5	Number of leaves spadices^{-1} plant^{-1} year^{-1}	78.251	87.951
6	Number of suckers plant^{-1}	40.901	117.514
7	Days from emergence to maturity of inflorescence	61.416	15.144
8	Spathe size (cm^2)	87.870	85.199
9	Spadix length (cm)	81.524	44.279
10	Number of flowers spadix^{-1}	95.114	64.627
11	Life of spadix (days)	74.651	19.545
12	Days to initiation of female phase	62.421	31.309
13	Duration of female phase	61.910	26.658
14	Duration of interphase	58.964	36.677
15	Duration of male phase	54.183	20.850
16	Inclination of candle with spathe (degrees)	93.086	54.739
17	Anthocyanin content (mg /g)	99.417	115.496
18	Vase life	93.810	53.969
19	Number of inflorescence year^{-1}	63.551	48.990

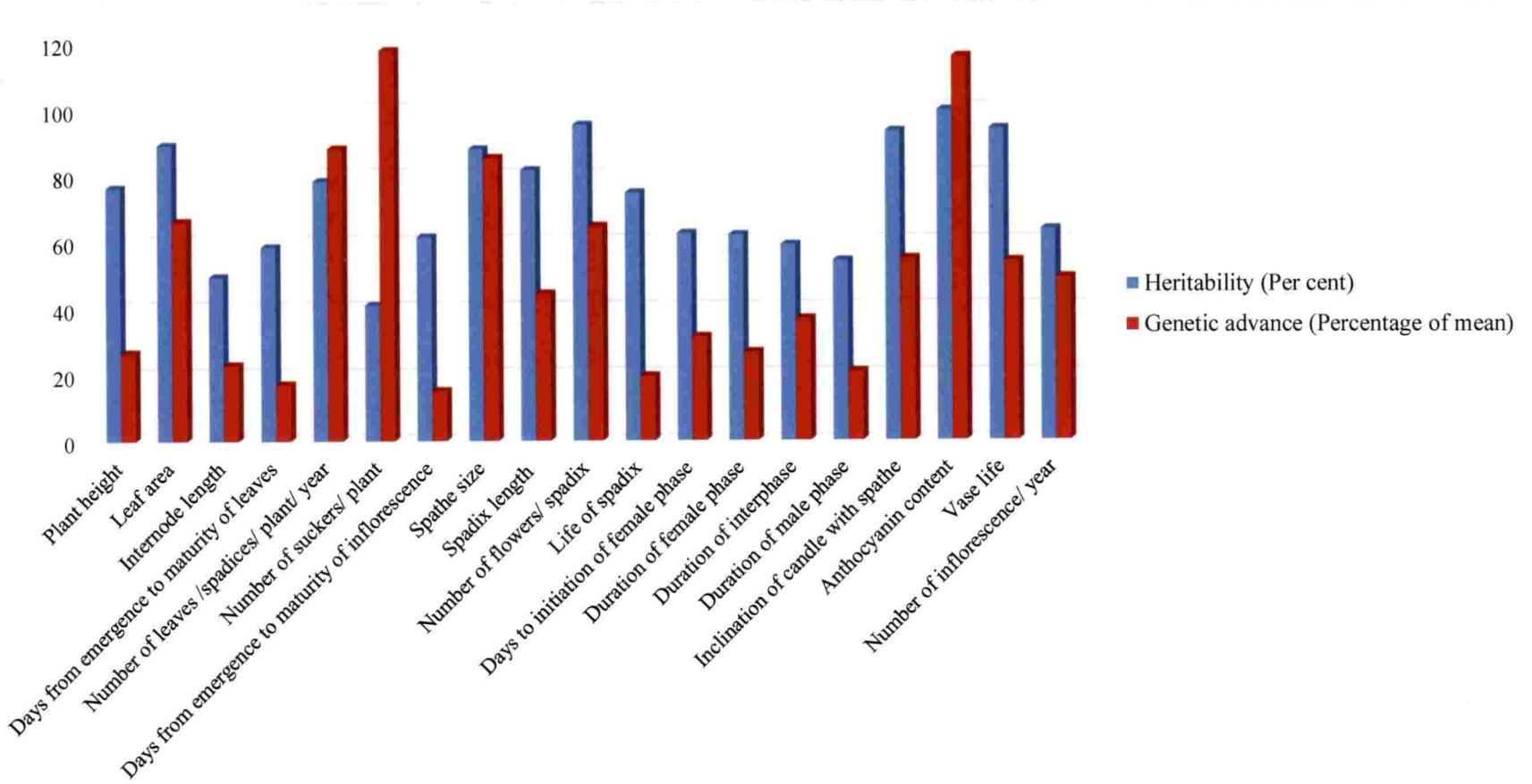


Fig. 3. Heritability and genetic advance as percentage mean for 19 characters in *Anthurium andreanum* hybrids

High heritability coupled with high genetic advance was observed for the characters namely plant height, leaf area, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size, spadix length, number of flowers spadix^{-1}, days to initiation of female phase, duration of female phase, inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1}.

4.1.4 Correlation Analysis

Correlation analysis helps in identification of correlated response among the characters and thereby equip the plant breeders in selection of one or more attributes at a time. The results of correlation analysis are represented in Table 8.

4.1.4.1 *Phenotypic Correlation*

On studying the phenotypic correlation (Table 8a), the character plant height showed a positive and significant correlation with leaf area (0.583), internode length (0.434), days from emergence to maturity of leaves (0.472), spathe size (0.382), spadix length (0.540), number of flowers spadix^{-1} (0.538), life of spadix (0.350), duration of female phase (0.318), duration of male phase (0.273), and vase life (0.341).

Leaf area recorded positive and significant correlation with plant height (0.583), internode length (0.453), spathe size (0.670), spadix length (0.643), number of flowers spadix^{-1} (0.553), life of spadix (0.564), days to initiation of female phase (0.304), inclination of candle with spathe (0.467) and vase life (0.660).

Internode length of the hybrids found to have significant positive correlation with plant height (0.434), leaf area (0.453), days from emergence to maturity of leaves (0.474), spathe size (0.419), spadix length (0.473), number of flowers spadix^{-1} (0.373), life of spadix (0.242), days to initiation of female phase (0.334), duration of female phase (0.373) and inclination of candle with spathe

(0.325). A significant negative correlation exists between internode length and number of leaves spadices^{-1} plant^{-1} year^{-1} (0.364).

Days from emergence to maturity of leaves was positively correlated with plant height (0.472), internode length (0.474) and spadix length (0.280). Days from emergence to maturity of leaves had a significant negative correlation with with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.331), vase life (0.244) and number of inflorescence year^{-1} (0.372).

Number of leaves spadices^{-1} plant^{-1} year^{-1} was positively correlated with days from emergence to maturity of inflorescence (0.332), vase life (0.393) and number of inflorescence year^{-1} (0.672) while it was negatively correlated with internode length (0.364), days from emergence to maturity of leaves (0.331), spathe size (0.219) and anthocyanin content (0.223).

Number of suckers plant^{-1} was found to have positive correlation with number of inflorescence year^{-1} (0.254) and had negative correlation with anthocyanin content (0.295).

Days from emergence to maturity of inflorescence showed positive and significant correlation with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.332), days to initiation of female phase (0.298) and inclination of candle with spathe (0.213).

Spathe size registered a positive and significant correlation with plant height (0.382), leaf area (0.670), internode length (0.419), spadix length (0.434), number of flowers spadix^{-1} (0.224), life of spadix (0.415), inclination of candle with spathe (0.412), anthocyanin content (0.267), vase life (0.455) and was negatively correlated with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.219) and duration of interphase (0.237).

Spadix length had a significant and positive correlation with plant height (0.540), leaf area (0.643), internode length (0.473), days from emergence to maturity of leaves (0.280), spathe size (0.434), number of flowers spadix^{-1}

(0.831), life of spadix (0.483), duration of female phase (0.425), duration of male phase (0.395), inclination of candle with spathe (0.271) and vase life (0.392).

Number of flowers spadix^{-1} was found to have positive correlation with plant height (0.538), leaf area (0.553), internode length (0.373), spathe size (0.224), spadix length (0.831), life of spadix (0.506), duration of female phase (0.504), duration of interphase (0.259), duration of male phase (0.475), inclination of candle with spathe (0.255) and vase life (0.421) while anthocyanin content (0.203) displayed a negative correlation.

Life of spadix had significant positive correlation with plant height (0.350), leaf area (0.564), internode length (0.242), spathe size (0.415), spadix length (0.483), number of flowers spadix^{-1} (0.506), days to initiation of female phase (0.309), duration of female phase (0.242), duration of interphase (0.229), duration of male phase (0.331) and vase life (0.599).

Days to initiation of female phase had a positive correlation with leaf area (0.304), internode length (0.334), days from emergence to maturity of inflorescence (0.298), life of spadix (0.309) and duration of female phase (0.209).

Duration of female phase showed a significant positive correlation with plant height (0.318), internode length (0.373), spadix length (0.425), number of flowers spadix^{-1} (0.504), life of spadix (0.242), days to initiation of female phase (0.209), duration of interphase (0.244), duration of male phase (0.316) and vase life (0.202) and exhibited a negative correlation with anthocyanin content (0.457).

Duration of interphase showed a positive correlation with number of flowers spadix^{-1} (0.259), life of spadix (0.229), duration of female phase (0.244), duration of male phase (0.350) and negative correlation with spathe size (0.237) and inclination of candle with spathe (0.281).

Duration of male phase was positively correlated with plant height (0.273), spadix length (0.395), number of flowers spadix^{-1} (0.475), life of spadix (0.331),

duration of female phase (0.316), duration of interphase (0.350) and vase life (0.245).

Inclination of candle with spathe showed a significant positive correlation with leaf area (0.467), internode length (0.325), days from emergence to maturity of inflorescence (0.213), spathe size (0.412), spadix length (0.271), number of flowers spadix^{-1} (0.255) and vase life (0.238) while the trait was negatively correlated with duration of interphase (0.281).

Anthocyanin content was positively correlated with spathe size (0.267) and inclination of candle with spathe (0.238). Negative correlation was observed for anthocyanin content with plant height (0.217), number of leaves spadices^{-1} plant^{-1} year^{-1} (0.223), number of suckers plant^{-1} (0.295), number of flowers spadix^{-1} (0.203) and duration of female phase (0.457).

Vase life was found to have positive and significant correlation with plant height (0.341), leaf area (0.660), number of leaves spadices^{-1} plant^{-1} year^{-1} (0.393), spathe size (0.455), spadix length (0.392), number of flowers spadix^{-1} (0.421), life of spadix (0.599), duration of female phase (0.202), duration of male phase (0.245) and number of inflorescence year^{-1} (0.542). Days from emergence to maturity of leaves (0.244) had a negative correlation with vase life.

Number of inflorescence year^{-1} registered a positive and significant correlation with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.672), number of suckers plant^{-1} (0.254) and vase life (0.542) and was negatively correlated with days from emergence to maturity of leaves (0.372).

4.1.4.2 *Genotypic Correlation*

On studying the genotypic correlation (Table 8b), the character plant height showed a positive and significant correlation with leaf area (0.595), internode length (0.428), days from emergence to maturity of leaves (0.409), spathe size (0.378), spadix length (0.590), number of flowers spadix^{-1} (0.599), life

of spadix (0.413), duration of female phase (0.427), duration of male phase (0.407), and vase life (0.391). Negative correlation was observed between plant height and anthocyanin content (0.242).

Leaf area recorded positive and significant correlation with plant height (0.595), internode length (0.518), spathe size (0.702), spadix length (0.685), number of flowers spadix^{-1} (0.572), life of spadix (0.632), days to initiation of female phase (0.374), duration of female phase (0.200), duration of male phase (0.286) and inclination of candle with spathe (0.512).

Internode length of the hybrids was found to have significant positive correlation with plant height (0.428), leaf area (0.518), days from emergence to maturity of leaves (0.493), spathe size (0.439), spadix length (0.557), number of flowers spadix^{-1} (0.498), life of spadix (0.233), days to initiation of female phase (0.402), duration of female phase (0.582) and inclination of candle with spathe (0.417). A significant negative correlation exists between internode length and number of inflorescence year^{-1} (0.453).

Days from emergence to maturity of leaves was positively correlated with plant height (0.409), internode length (0.493), spadix length (0.208), days to initiation of female phase (0.224) and duration of female phase (0.269). A significant negative correlation exists between days from emergence to maturity of leaves with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.478), number of suckers plant^{-1} (0.418), inclination of candle with spathe (0.240), vase life (0.340) and number of inflorescence year^{-1} (0.705).

Number of leaves spadices^{-1} plant^{-1} year^{-1} was positively correlated with number of sucker plant $^{-1}$ (0.348), days from emergence to maturity of inflorescence (0.356), vase life (0.431) and number of inflorescence year^{-1} (0.793) while it was negatively correlated with internode length (0.662), days from emergence to maturity of leaves (0.478), spathe size (0.269) and anthocyanin content (0.249).

Number of suckers plant^{-1} was found to have positive correlation with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.348) and number of inflorescence year^{-1} (0.358) and had negative correlation with internode length (0.267), days from emergence to maturity of leaves (0.418), spadix length (0.297), number of flowers spadix^{-1} (0.243), duration of interphase (0.278) and anthocyanin content (0.460).

Days from emergence to maturity of inflorescence showed positive and significant correlation with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.356), days to initiation of female phase (0.454) and inclination of candle with spathe (0.329) while spathe size registered a negative correlation.

Spathe size registered a positive and significant correlation with plant height (0.378), leaf area (0.702), internode length (0.439), spadix length (0.412), number of flowers spadix^{-1} (0.204), life of spadix (0.404), duration of male phase (0.299), inclination of candle with spathe (0.443), anthocyanin content (0.291) and vase life (0.481) and was negatively correlated with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.269), duration of interphase (0.288) and days from emergence to maturity of inflorescence (0.293).

Spadix length had a significant and positive correlation with plant height (0.590), leaf area (0.685), internode length (0.557), days from emergence to maturity of leaves (0.208), spathe size (0.412), number of flowers spadix^{-1} (0.868), life of spadix (0.448), duration of female phase (0.548), duration of male phase (0.640), inclination of candle with spathe (0.311) and vase life (0.428).

Number of flowers spadix^{-1} was found to have positive correlation with plant height (0.599), leaf area (0.572), internode length (0.498), spathe size (0.204), spadix length (0.868), life of spadix (0.526), duration of female phase (0.638), duration of interphase (0.364), duration of male phase (0.651), inclination of candle with spathe (0.277), vase life (0.432) while number of suckers plant^{-1} (0.243) and anthocyanin content (0.208) displayed a negative correlation.

Life of spadix had significant positive correlation with plant height (0.413), leaf area (0.632), internode length (0.233), spathe size (0.404), spadix length (0.448), number of flowers spadix^{-1} (0.526), days to initiation of female phase (0.343), duration of female phase (0.292), duration of interphase (0.444), duration of male phase (0.516) and vase life (0.674).

Days to initiation of female phase had a positive correlation with leaf area (0.374), internode length (0.402), days from emergence to maturity of leaves (0.224), days from emergence to maturity of inflorescence (0.454) and life of spadix (0.343).

Duration of female phase showed a significant positive correlation with plant height (0.427), leaf area (0.200), internode length (0.582), days from emergence to maturity of leaves (0.269), spadix length (0.548), number of flowers spadix^{-1} (0.638), life of spadix (0.292), duration of interphase (0.325), duration of male phase (0.399) and vase life (0.249) and expressed a negative correlation with anthocyanin content (0.584).

Duration of interphase showed a positive correlation with number of flowers spadix^{-1} (0.364), life of spadix (0.444), duration of female phase (0.325), duration of male phase (0.480) and exhibited negative correlation with number of sucker plant^{-1} (0.278), spathe size (0.288) and inclination of candle with spathe (0.413).

Duration of male phase was positively correlated with plant height (0.407), leaf area (0.286), spathe size (0.299), spadix length (0.640), number of flowers spadix^{-1} (0.651), life of spadix (0.516), duration of female phase (0.399), duration of interphase (0.480) and vase life (0.330).

Inclination of candle with spathe showed a significant positive correlation with leaf area (0.512), internode length (0.417), days from emergence to maturity of inflorescence (0.329), spathe size (0.443), spadix length (0.311), number of flowers spadix^{-1} (0.277) and anthocyanin content (0.246) while the trait was

negatively correlated with days from emergence to maturity of leaves (0.240), duration of interphase (0.413).

Anthocyanin content was positively correlated with spathe size (0.291) and inclination of candle with spathe (0.246). Negative correlation was observed for the trait with plant height (0.242), number of leaves spadices^{-1} plant^{-1} year^{-1} (0.249), number of suckers plant^{-1} (0.460), number of flowers spadix^{-1} (0.208) and duration of female phase (0.584).

Vase life was found to have positive and significant correlation with plant height (0.391), number of leaves spadices^{-1} plant^{-1} year^{-1} (0.431), spathe size (0.481), spadix length (0.428), number of flowers spadix^{-1} (0.432), life of spadix (0.674), duration of female phase (0.249), duration of male phase (0.330) and number of inflorescence year^{-1} (0.660). Days from emergence to maturity of leaves (0.340) had a negative correlation with vase life.

Number of inflorescence year^{-1} registered a positive and significant correlation with number of leaves spadices^{-1} plant^{-1} year^{-1} (0.793), number of suckers plant^{-1} (0.358) and vase life (0.660) and was negatively correlated with internode length (0.453) and days from emergence to maturity of leaves (0.705).

4.1.5 Path Analysis

Analysis of direct and indirect effects of characters toward the flower yield was worked out using path analysis. For path analysis, the character, number of inflorescence year^{-1} was taken as the dependent character. The independent characters included plant height, leaf area, internode length, days from emergence to maturity of leaves, number of leaves spadices^{-1} plant^{-1} year^{-1}, number of suckers plant^{-1}, days from emergence to maturity of inflorescence, spathe size, spadix length, number of flowers spadix^{-1}, life of spadix, days to initiation of female phase, duration of female phase, duration of interphase, duration of male phase, inclination of candle with spathe, anthocyanin content and vase life. The analysis

revealed the direct and indirect effects of various characters on flower yield as presented in the Table 9.

The highest positive direct effect was observed for the character plant height (1.344) followed by anthocyanin content (1.210), number of leaves spadices^{-1} plant^{-1} year^{-1} (1.178), life of spadix (1.100), duration of male phase (1.028), internode length (0.728), inclination of candle with spathe (0.581), duration of female phase (0.510), number of suckers plant^{-1} (0.416), vase life (0.098) and leaf area (0.077). Days from emergence to maturity of leaves (-0.865), days from emergence to maturity of inflorescence (-1.793), spathe size (-2.022), spadix length (-0.600), number of flowers spadix^{-1}(-0.728), life of spadix (-0.107) and duration of interphase (-0.252) showed negative direct effect towards flower yield.

The correlation between leaf area and number of inflorescence year^{-1} was 0.139 and its direct effect was found to be positive and low (0.077). Indirect effect via., days to initiation of female phase (0.412), duration of male phase (0.294), inclination of candle with the spathe (0.298) and number of leaves spadices^{-1} plant^{-1} year^{-1} (0.162) and anthocyanin content (0.162) contributed towards the above correlation.

Number of leaves spadices^{-1} plant^{-1} year^{-1} showed a significant positive correlation of 0.793 with number of inflorescence year^{-1} and had high direct effect (1.178). The characters namely spathe size (0.545) and number of suckers plant^{-1} (0.145) mainly contributed to the correlation, indirectly.

Number of suckers plant^{-1} registered a significant positive correlation of 0.358 with number of inflorescence year^{-1} and the direct effect (0.416) was found to be high and positive. Indirect effect due to the characters via number of leaves spadices^{-1} plant^{-1} year^{-1} (0.410), days from emergence to maturity of leaves (0.362), spadix length (0.178) and number of flowers spadix^{-1} (0.177).

Correlation between days from emergence to maturity of inflorescence was 0.058 and number of inflorescence year^{-1} was found to be negative and high (-1.793). The indirect effect on number of inflorescence year^{-1} was mainly due to spathe size (0.593), days to initiation of female phase (0.499), number of leaves spadices^{-1} plant^{-1} year^{-1} (0.420), plant height (0.229) and inclination of candle with spathe (0.191).

The correlation between life of spadix and number of inflorescence year^{-1} was 0.165 and its direct effect was found to be negative and low (-0.107). Indirect effect via plant height (0.555), days to initiation of female phase (0.377) and duration of male phase (0.531) contributed towards the correlation.

Days to initiation of female phase and number of inflorescence year^{-1} showed a positive correlation of 0.092 and had positive and high direct effect of 1.100. The traits such as internode length (0.292) and inclination of candle with spathe (0.112) mainly contributed towards the correlation.

The correlation between duration of interphase and number of inflorescence year^{-1} was 0.163 and the direct effect was found to be negative (-0.252). Spathe size (0.581), duration of male phase (0.494) and days from emergence to maturity of leaves (0.102) mainly contributed for the correlation.

The correlation between duration of male phase and number of inflorescence year^{-1} was 0.112 and had positive and high direct effect of 1.028. Plant height (0.547), duration of female phase (0.204), internode length (0.134), days from emergence to maturity of inflorescence (0.133) and number of leaves spadices^{-1} plant^{-1} year^{-1} (0.110) contributed towards the correlation.

A positive correlation of 0.660 was registered between vase life and number of inflorescence year^{-1} and had a positive direct effect of 0.098. The traits such as plant height (0.525), number of leaves spadices^{-1} plant^{-1} year^{-1} (0.507), duration of male phase (0.340) and days from emergence to maturity of leaves (0.294) also contributed highly towards the correlation.

Table 8a. Phenotypic correlation coefficients among 19 characters in *Anthurium andreanum* hybrids

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}
X_1	1																		
X_2	0.583**	1																	
X_3	0.434**	0.453**	1																
X_4	0.472**	0.136^{NS}	0.474**	1															
X_5	-0.008^{NS}	0.103^{NS}	-0.364**	-0.331**	1														
X_6	0.111^{NS}	0.063^{NS}	-0.030^{NS}	-0.117^{NS}	0.178^{NS}	1													
X_7	0.135^{NS}	0.168^{NS}	0.100^{NS}	0.131^{NS}	0.332**	-0.082^{NS}	1												
X_8	0.382**	0.670**	0.419**	0.082^{NS}	-0.219*	0.173^{NS}	-0.187^{NS}	1											
X_9	0.540**	0.643**	0.473**	0.280**	-0.006^{NS}	-0.148^{NS}	-0.024^{NS}	0.434**	1										
X_{10}	0.538**	0.553**	0.373**	0.170^{NS}	0.005^{NS}	-0.157^{NS}	0.121^{NS}	0.224*	0.831**	1									
X_{11}	0.350**	0.564**	0.242*	0.185^{NS}	0.045^{NS}	0.016^{NS}	0.009^{NS}	0.415**	0.483**	0.506**	1								
X_{12}	0.013^{NS}	0.304**	0.334**	0.150^{NS}	-0.037^{NS}	0.006^{NS}	0.298**	0.121^{NS}	0.031^{NS}	0.056^{NS}	0.309**	1							
X_{13}	0.318**	0.159^{NS}	0.373**	0.169^{NS}	-0.104^{NS}	-0.126^{NS}	0.075^{NS}	0.057^{NS}	0.425**	0.504**	0.242*	0.209*	1						
X_{14}	0.084^{NS}	-0.081^{NS}	-0.066^{NS}	-0.068^{NS}	0.007^{NS}	-0.155^{NS}	-0.002^{NS}	-0.237*	0.038^{NS}	0.259**	0.229*	-0.093^{NS}	0.244*	1					
X_{15}	0.273**	0.189^{NS}	0.059^{NS}	0.064^{NS}	0.017^{NS}	-0.080^{NS}	-0.089^{NS}	0.171^{NS}	0.395**	0.475**	0.331**	-0.049^{NS}	0.316**	0.350**	1				
X_{16}	0.050^{NS}	0.467**	0.325**	-0.140^{NS}	-0.102^{NS}	0.072^{NS}	0.213*	0.412**	0.271**	0.255*	0.045^{NS}	0.153^{NS}	-0.071^{NS}	-0.281**	0.086^{NS}	1			
X_{17}	-0.217*	0.124^{NS}	0.047^{NS}	-0.056^{NS}	-0.223*	-0.295**	0.010^{NS}	0.267**	-0.114^{NS}	-0.203*	-0.096^{NS}	0.000^{NS}	-0.457**	-0.100^{NS}	-0.111^{NS}	0.238*	1		
X_{18}	0.341**	0.660**	0.107^{NS}	-0.244*	0.393**	0.053^{NS}	-0.004^{NS}	0.455**	0.392**	0.421**	0.599**	0.157^{NS}	0.202*	0.125^{NS}	0.245*	0.172^{NS}	-0.075^{NS}	1	
X_{19}	-0.091^{NS}	0.124^{NS}	-0.190^{NS}	-0.372**	0.672**	0.254*	0.076^{NS}	-0.028^{NS}	-0.075^{NS}	-0.033^{NS}	0.142^{NS}	0.085^{NS}	-0.011^{NS}	0.095^{NS}	0.009^{NS}	-0.079^{NS}	-0.109^{NS}	0.542**	1

*Significant at 5 percent level **Significant at 1 per cent level

X_1 Plant height (cm)
X_2 Leaf area (cm^2)
X_3 Internode length (cm)
X_4 Days from emergence to maturity of leaves
X_5 Number of leaves spadices^{-1} plant^{-1} year^{-1}
X_6 Number of suckers plant^{-1}
X_7 Days from emergence to maturity of inflorescence
X_8 Spathe size (cm^2)
X_9 Spadix length (cm)
X_{10} Number of flowers spadix^{-1}
X_{11} Life of spadix (days)
X_{12} Days to initiation of female phase
X_{13} Duration of female phase
X_{14} Duration of interphase
X_{15} Duration of male phase
X_{16} Inclination of candle with spathe (degrees)
X_{17} Anthocyanin content (mg /g)
X_{18} Vase life
X_{19} Number of inflorescence year^{-1}

Table 8b. Genotypic correlation coefficients among 19 characters in *Anthurium andreanum* hybrids

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}
X_1	1																		
X_2	0.595**	1																	
X_3	0.428**	0.518**	1																
X_4	0.409**	0.005^{NS}	0.493**	1															
X_5	0.015^{NS}	0.137^{NS}	-0.662**	-0.478**	1														
X_6	-0.037^{NS}	0.023^{NS}	-0.267**	-0.418**	0.348**	1													
X_7	0.170^{NS}	0.187^{NS}	0.096^{NS}	0.074^{NS}	0.356**	-0.006^{NS}	1												
X_8	0.378**	0.702**	0.439**	-0.016^{NS}	-0.269**	0.172^{NS}	-0.293**	1											
X_9	0.590**	0.685**	0.557**	0.208*	-0.052^{NS}	-0.297**	-0.083^{NS}	0.412**	1										
X_{10}	0.599**	0.572**	0.498**	0.157^{NS}	-0.009^{NS}	-0.243*	0.140^{NS}	0.204*	0.868**	1									
X_{11}	0.413**	0.632**	0.233*	0.148^{NS}	0.042^{NS}	0.075^{NS}	-0.066^{NS}	0.404**	0.448**	0.526**	1								
X_{12}	0.004^{NS}	0.374**	0.402**	0.224*	-0.092^{NS}	-0.038^{NS}	0.454**	0.129^{NS}	-0.053^{NS}	0.076^{NS}	0.343**	1							
X_{13}	0.427**	0.200*	0.582**	0.269**	-0.196^{NS}	-0.163^{NS}	0.014^{NS}	0.035^{NS}	0.548**	0.638**	0.292**	0.192^{NS}	1						
X_{14}	0.079^{NS}	-0.124^{NS}	-0.136^{NS}	-0.118^{NS}	0.049^{NS}	-0.278**	-0.026^{NS}	-0.288**	0.118^{NS}	0.364**	0.444**	-0.118^{NS}	0.325**	1					
X_{15}	0.407**	0.286**	0.184^{NS}	0.179^{NS}	0.093^{NS}	-0.033^{NS}	-0.074^{NS}	0.299**	0.640**	0.651**	0.516**	-0.137^{NS}	0.399**	0.480**	1				
X_{16}	0.036^{NS}	0.512**	0.417**	-0.240*	-0.095^{NS}	0.061^{NS}	0.329**	0.443**	0.311**	0.277**	0.024^{NS}	0.192^{NS}	-0.082^{NS}	-0.413**	0.074^{NS}	1			
X_{17}	-0.242*	0.134^{NS}	0.075^{NS}	-0.066^{NS}	-0.249*	-0.460**	0.012^{NS}	0.291**	-0.118^{NS}	-0.208*	-0.109^{NS}	0.003^{NS}	-0.584**	-0.147^{NS}	-0.151^{NS}	0.246*	1		
X_{18}	0.391**	0.723**	0.091^{NS}	-0.340**	0.431**	0.119^{NS}	-0.043^{NS}	0.481**	0.428**	0.432**	0.674**	0.156^{NS}	0.249*	0.183^{NS}	0.330**	0.181^{NS}	-0.078^{NS}	1	
X_{19}	-0.167^{NS}	0.139^{NS}	-0.453**	-0.705**	0.793**	0.358**	0.058^{NS}	-0.089^{NS}	-0.149^{NS}	-0.066^{NS}	0.165^{NS}	0.092^{NS}	-0.119^{NS}	0.163^{NS}	0.112^{NS}	-0.119^{NS}	-0.132^{NS}	0.660**	1

*Significant at 5 percent level **Significant at 1 per cent level

X_1 Plant height (cm)
X_2 Leaf area (cm^2)
X_3 Internode length (cm)
X_4 Days from emergence to maturity of leaves
X_5 Number of leaves spadices^{-1} plant^{-1} year^{-1}
X_6 Number of suckers plant^{-1}
X_7 Days from emergence to maturity of inflorescence
X_8 Spathe size (cm^2)
X_9 Spadix length (cm)
X_{10} Number of flowers spadix^{-1}
X_{11} Life of spadix (days)
X_{12} Days to initiation of female phase
X_{13} Duration of female phase
X_{14} Duration of interphase
X_{15} Duration of male phase
X_{16} Inclination of candle with spathe (degrees)
X_{17} Anthocyanin content (mg /g)
X_{18} Vase life
X_{19} Number of inflorescence year^{-1}

Table 9. Direct and indirect effects of component characters on number of inflorescence year^{-1} in *Anthurium andreanum* hybrids

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	Genotypic correlation with number of inflorescence year^{-1}
X_1	**1.344**	0.046	0.311	-0.354	0.017	-0.015	-0.305	-0.764	-0.354	-0.436	-0.044	0.005	0.217	-0.02	0.418	0.021	-0.292	0.038	-0.167
X_2	0.799	**0.077**	0.377	-0.005	0.162	0.01	-0.335	-1.419	-0.411	-0.416	-0.067	0.412	0.102	0.031	0.294	0.298	0.162	0.071	0.139
X_3	0.575	0.04	**0.728**	-0.427	-0.779	-0.111	-0.172	-0.888	-0.335	-0.362	-0.025	0.442	0.297	0.034	0.189	0.242	0.09	0.009	-0.453
X_4	0.55	0	0.359	**-0.865**	-0.564	-0.174	-0.133	0.032	-0.125	-0.114	-0.016	0.246	0.137	0.03	0.184	-0.14	-0.08	-0.033	-0.705
X_5	0.02	0.011	-0.481	0.414	**1.178**	0.145	-0.639	0.545	0.031	0.006	-0.005	-0.101	-0.1	-0.012	0.096	-0.055	-0.301	0.042	0.793
X_6	-0.05	0.002	-0.195	0.362	0.41	**0.416**	0.01	-0.347	0.178	0.177	-0.008	-0.041	-0.083	0.07	-0.034	0.036	-0.556	0.012	0.358
X_7	0.229	0.014	0.07	-0.064	0.42	-0.002	**-1.793**	0.593	0.05	-0.102	0.007	0.499	0.007	0.006	-0.076	0.191	0.015	-0.004	0.058
X_8	0.508	0.054	0.32	0.014	-0.317	0.072	0.526	**-2.022**	-0.247	-0.148	-0.043	0.142	0.018	0.072	0.307	0.258	0.353	0.047	-0.089
X_9	0.793	0.053	0.406	-0.18	-0.062	-0.123	0.149	-0.833	**-0.6**	-0.632	-0.048	-0.059	0.279	-0.03	0.658	0.181	-0.143	0.042	-0.149
X_{10}	0.805	0.044	0.362	-0.136	-0.01	-0.101	-0.252	-0.412	-0.521	**-0.728**	-0.056	0.084	0.325	-0.092	0.669	0.161	-0.251	0.042	-0.066
X_{11}	0.555	0.049	0.17	-0.128	0.05	0.031	0.119	-0.816	-0.269	-0.382	**-0.107**	0.377	0.149	-0.112	0.531	0.014	-0.132	0.066	0.165
X_{12}	0.006	0.029	0.292	-0.194	-0.108	-0.016	-0.814	-0.261	0.032	-0.056	-0.037	**1.1**	0.098	0.03	-0.14	0.112	0.004	0.015	0.092
X_{13}	0.573	0.015	0.423	-0.233	-0.231	-0.068	-0.024	-0.071	-0.329	-0.464	-0.031	0.212	**0.51**	-0.082	0.411	-0.048	-0.707	0.024	-0.119
X_{14}	0.106	-0.01	-0.099	0.102	0.058	-0.116	0.046	0.581	-0.071	-0.265	-0.047	-0.13	0.166	**-0.252**	0.494	-0.24	-0.178	0.018	0.163
X_{15}	0.547	0.022	0.134	-0.155	0.11	-0.014	0.133	-0.604	-0.384	-0.474	-0.055	-0.15	0.204	-0.121	**1.028**	0.043	-0.183	0.032	0.112
X_{16}	0.048	0.039	0.303	0.208	-0.112	0.025	-0.589	-0.897	-0.187	-0.201	-0.003	0.211	-0.042	0.104	0.076	**0.581**	0.298	0.018	-0.119
X_{17}	-0.325	0.01	0.054	0.057	-0.293	-0.191	-0.022	-0.589	0.071	0.151	0.012	0.003	-0.298	0.037	-0.155	0.143	**1.21**	-0.008	-0.132
X_{18}	0.525	0.055	0.066	0.294	0.507	0.049	0.078	-0.972	-0.257	-0.314	-0.072	0.171	0.127	-0.046	0.34	0.105	-0.094	**0.098**	0.660

Residual effect: -0.2965

X_1 Plant height (cm)
X_2 Leaf area (cm^2)
X_3 Internode length (cm)
X_4 Days from emergence to maturity of leaves
X_5 Number of leaves spadices^{-1} plant^{-1} year^{-1}
X_6 Number of suckers plant^{-1}
X_7 Days from emergence to maturity of inflorescence
X_8 Spathe size (cm^2)
X_9 Spadix length (cm)
X_{10} Number of flowers spadix^{-1}
X_{11} Life of spadix (days)
X_{12} Days to initiation of female phase
X_{13} Duration of female phase
X_{14} Duration of interphase
X_{15} Duration of male phase
X_{16} Inclination of candle with spathe (degrees)
X_{17} Anthocyanin content (mg /g)
X_{18} Vase life

4.2 *IN VITRO* MASS MULTIPLICATION OF SELECTED *ANTHURIUM ANDREANUM* LINDEN HYBRIDS

Six superior hybrids were selected from experiment I, having high flower yield attributing characters and other commercial qualitative traits. The effect of various treatments at different stages of *in vitro* mass multiplication of selected *Anthurium andreanum* Linden hybrids are presented in this chapter. The hybrids used for the study were HR x MR, LJ x OG, OG x NO, HoR x KR, PR x HR and HR x LR.

4.2.1 Surface Sterilization

4.2.1.1 Percentage of survival at culture initiation stage

Five treatments were compared to assess the effect of surface sterilization treatments on microbial contamination and percentage survival of explant (Table 10) on half strength MS medium. The effects of surface sterilization treatments were found to vary among the hybrids. The treatment TS_5 (double sterilization with 5.0 per cent sodium hypochlorite for 10 minutes + 0.1 per cent mercuric chloride for five minutes) was found to be the best treatment for surface sterilization, which was capable of producing 87.5 per cent explant survival in the hybrids HR x MR, LJ x OG and OG x NO. In the same treatment, percentage survival of explants in the hybrid PR x HR and HR x LR was observed to be 62.5 per cent while 50.0 per cent survival was observed in the hybrid HoR x KR.

The second treatment, TS_2 (0.1 per cent mercuric chloride for seven minutes) was found to be the best to giving 62.5 per cent explant survival for the hybrids HR x MR, LJ x OG, OG x NO and HR x LR. The hybrid HR x MR showed same explant survival percentage in the treatments TS_5 and TS_2. The treatments TS_1 (0.1 per cent mercuric chloride for 5 minutes), TS_3 (5.0 per cent sodium hypochlorite for 12 minutes) and TS_4 (5.0 per cent sodium hypochlorite for 15 minutes) were found to be less effective in producing contamination free cultures.

Table 10. Effect of surface sterilization treatments on survival of explants in *Anthurium andreanum* hybrids (Percentage survival at culture initiation stage).

Sl. No.	Treatments	HR x MR	LJ x OG	OG x NO	HoR x KR	PR x HR	HR x LR
1	TS_1	12.5	25.0	25.0	0	12.5	12.5
2	TS_2	62.5	62.5	62.5	37.5	50.0	62.5
3	TS_3	50.0	62.5	50.0	25.0	37.5	37.5
4	TS_4	50.0	37.5	62.5	37.5	37.5	50.0
5	TS_5	87.5	87.5	87.5	50.0	62.5	62.5

Plate 4. Stage of leaf explant used for inoculation

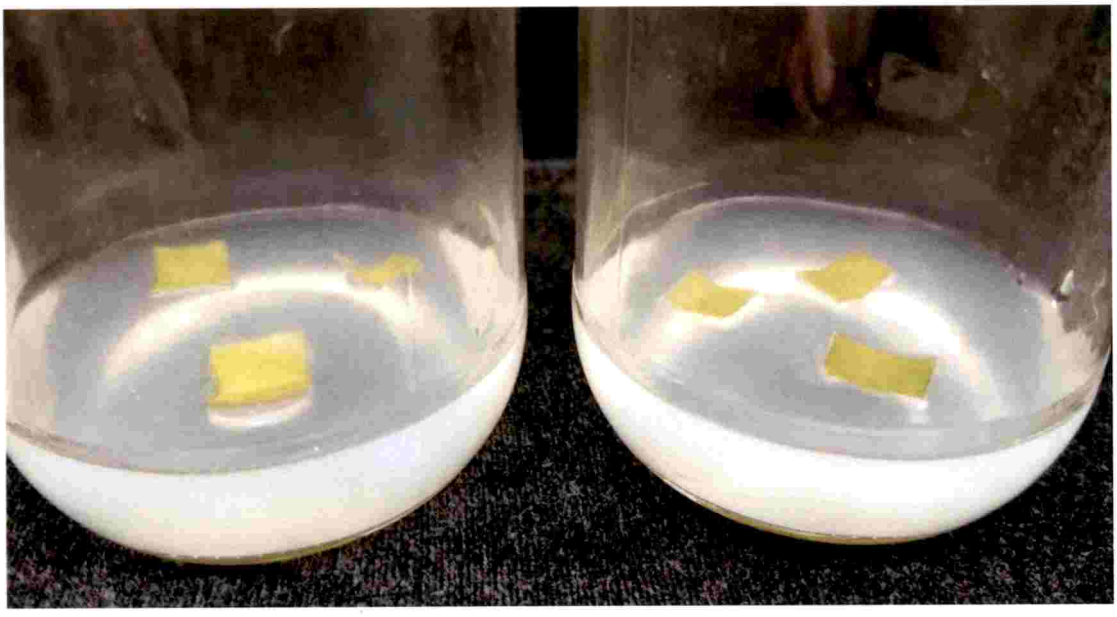


Plate 5. Inoculation of leaf explants on culture medium

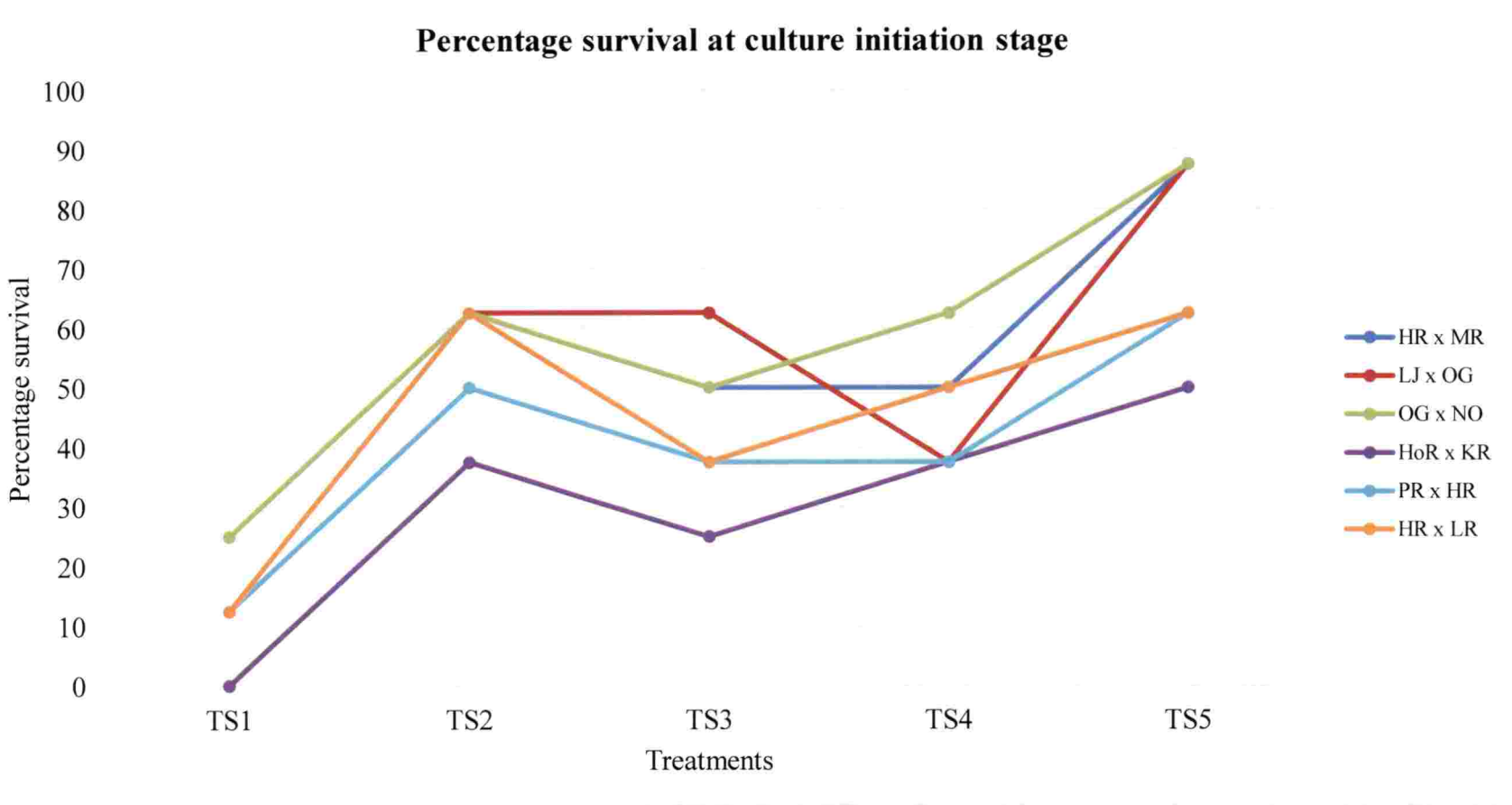


Fig. 4. Effect of surface sterilization treatments on survival of explants in *Anthurium andreanum* hybrids (Percentage survival at culture initiation stage)

4.2.2 Effect of media and hormones

4.2.2.1 Callus induction percentage

The observed results for the five callus induction treatments were converted into percentage response (Table 11). Among the five treatments tried only three were found to be responsive and the callus induction percentage ranged from 25.0 per cent to 50.0 per cent among various treatments. The highest callus induction percentage of 50.0 per cent was observed for the genotypes HR x MR and OG x NO with the treatment TC_4 (½ MS + 1.0 mg L^{-1} BA + 0.5 mg L^{-1} 2,4-D). The lowest callus induction percentage of 25.0 per cent was observed in the medium TC_5 (½ MS + 1.0 mg L^{-1} BA + 1.0 mg L^{-1} 2,4-D) for hybrids LJ x OG, HoR x KR, PR x HR and HR x LR. The hybrids HoR x KR and HR x LR showed best callus induction percentage in the medium TC_2 (½ MS + 1.0 mg L^{-1} BA). In comparison with other treatments, TC_4 (½ MS + 1.0 mg L^{-1} BA + 0.5 mg L^{-1} 2,4-D) was found to be the best callusing medium for all the genotypes studied.

4.2.2.2 Days to callus induction

On assessment of the callusing observations made (Table 12), the number of days taken for initiation of callusing varied from 56.00 to 89.33 days among the hybrids. The hybrids HR x MR (60.00 days), LJ x OG (78.67 days), OG x NO (56.00 days) and PR x HR (76.00 days) showed the fastest callus induction with the treatment TC_4 (½ MS + 1.0 mg L^{-1} BA + 0.5 mg L^{-1} 2,4-D) while the hybrid HoR x KR (78.00 days) showed the fastest callusing response with the treatment TC_2 (½ MS + 1.0 mg L^{-1} BA). HR x LR (75.33 days) had the fastest callus induction with the treatment TC_5 (½ MS + 1.0 mg L^{-1} BA + 1.0 mg L^{-1} 2,4-D). The maximum time taken for callusing response was observed with the treatment TC_2 (½ MS + 1.0 mg L^{-1} BA) for all hybrids except HoR x KR. The treatment TC_5 (½ MS + 1.0 mg L^{-1} BA + 1.0 mg L^{-1} 2,4-D) depicted quicker callusing response compared to treatment TC_2 (½ MS + 1.0 mg L^{-1} BA).

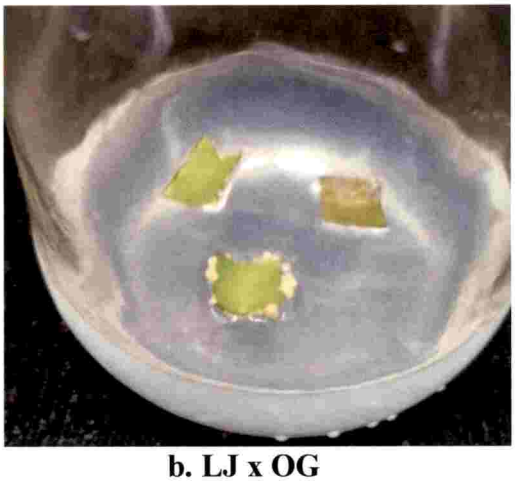
Table 11. Effect of media and hormones on callus induction of *Anthurium andreanum* hybrids (Callus induction percentage)

Sl. No.	Treatments	HR x MR	LJ x OG	OG x NO	HoR x KR	PR x HR	HR x LR
1	TC_1	0	0	0	0	0	0
2	TC_2	37.5	37.5	37.5	25.0	37.5	25.0
3	TC_3	0	0	0	0	0	0
4	TC_4	50.0	37.5	50.0	37.5	37.5	37.5
5	TC_5	37.5	25.0	37.5	25.0	25.0	25.0

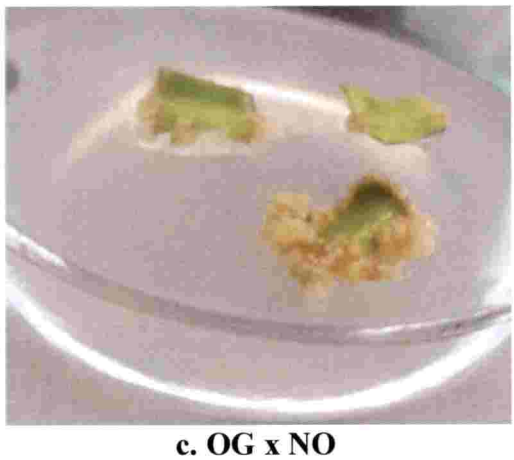
Table 12. Effect of media and hormones on callus induction of *Anthurium andreanum* hybrids (Days to callus induction)

Sl. No.	Treatments	HR x MR	LJ x OG	OG x NO	HoR x KR	PR x HR	HR x LR
1	TC_1	0	0	0	0	0	0
2	TC_2	70.67	88.67	68.67	78.00	89.33	86.00
3	TC_3	0	0	0	0	0	0
4	TC_4	60.00	78.67	56.00	79.67	76.00	75.33
5	TC_5	69.00	80.50	64.33	76.00	81.50	74.50

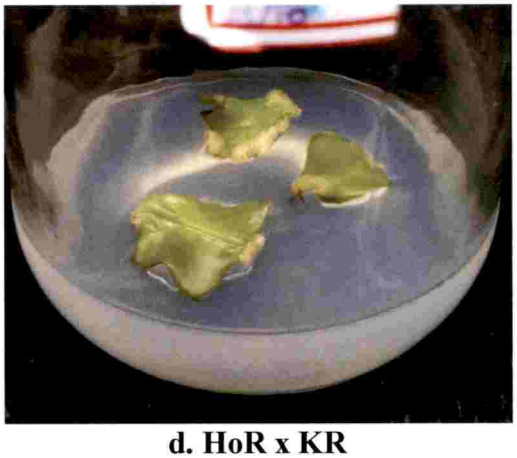
a. HR x MR



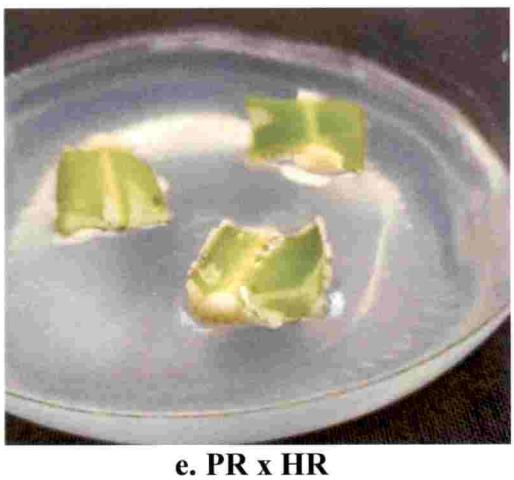
b. LJ x OG



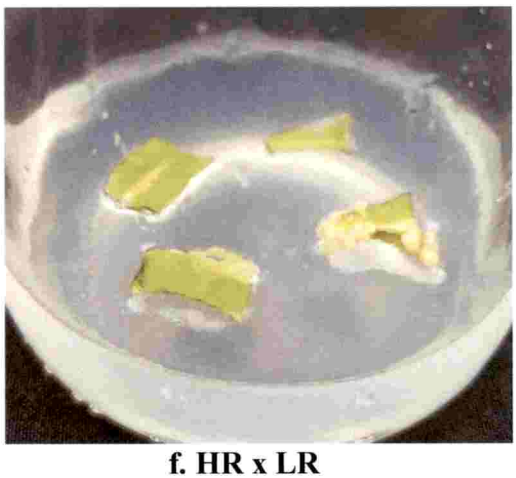
c. OG x NO



d. HoR x KR



e. PR x HR



f. HR x LR

Plate 6. Callus initiation from leaf explants of *Anthurium andreanum* Linden hybrids (12 weeks after culture)

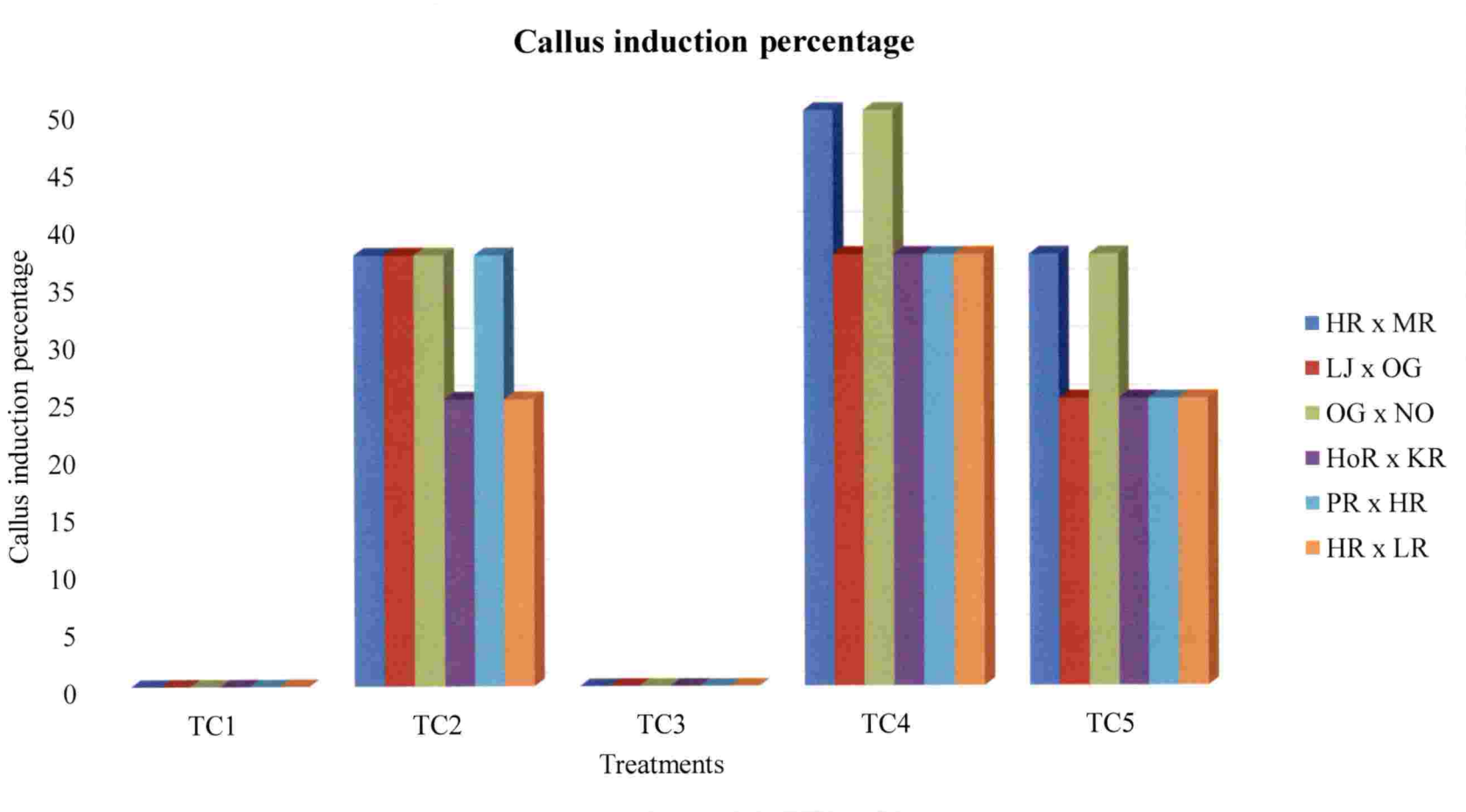


Fig. 5. Effect of media and hormones on callus induction of *Anthurium andreanum* hybrids (Callus induction percentage)

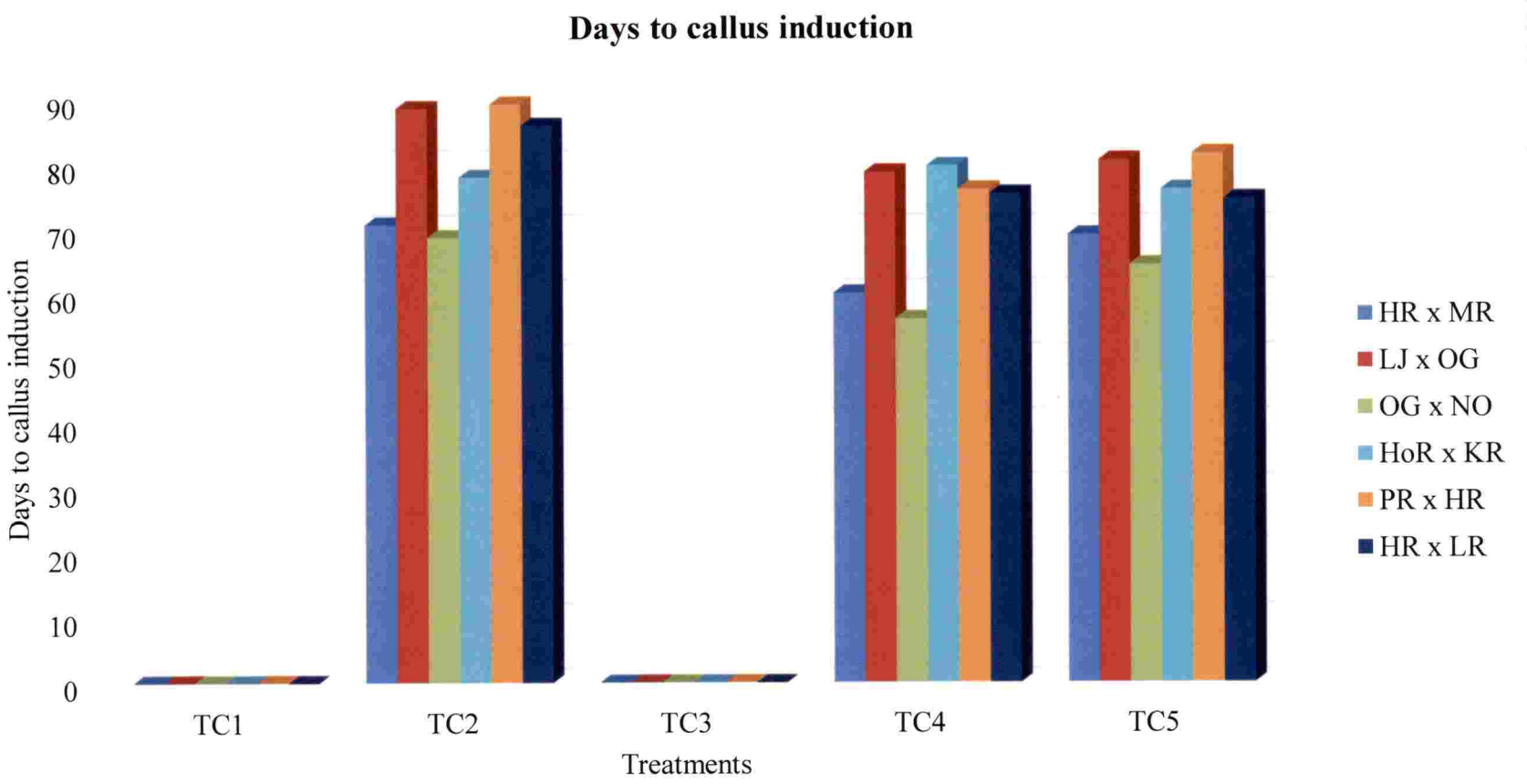


Fig. 6. Effect of media and hormones on callus induction of *Anthurium andreanum* hybrids (Days to callus induction)

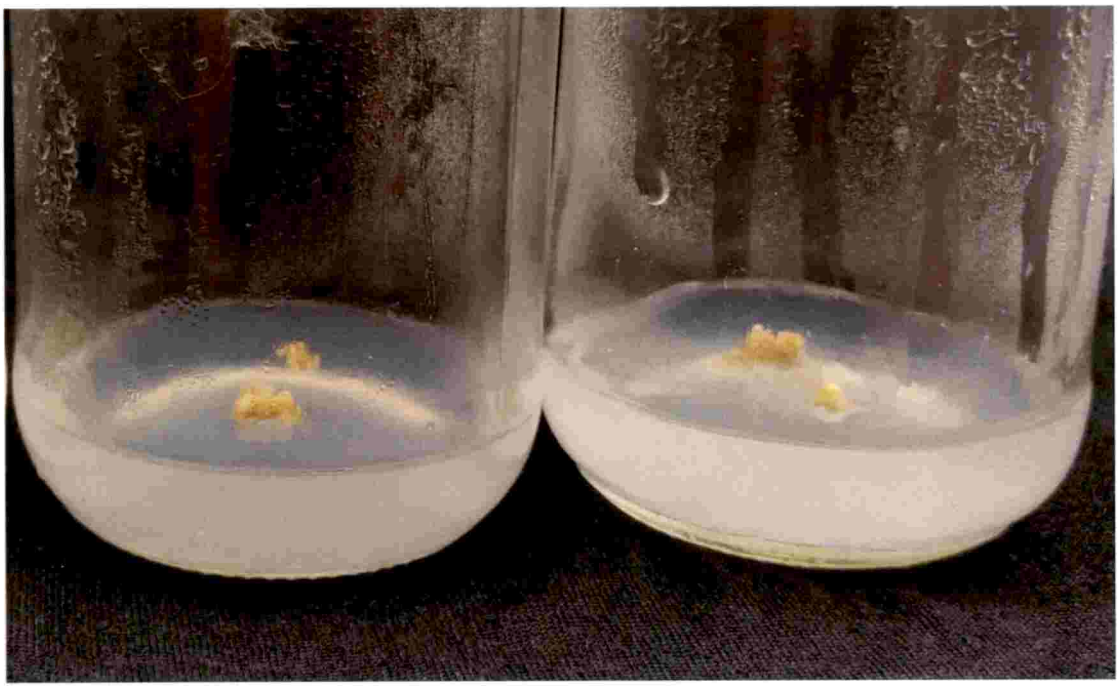


Plate 7. Subcultured callus for callus multiplication

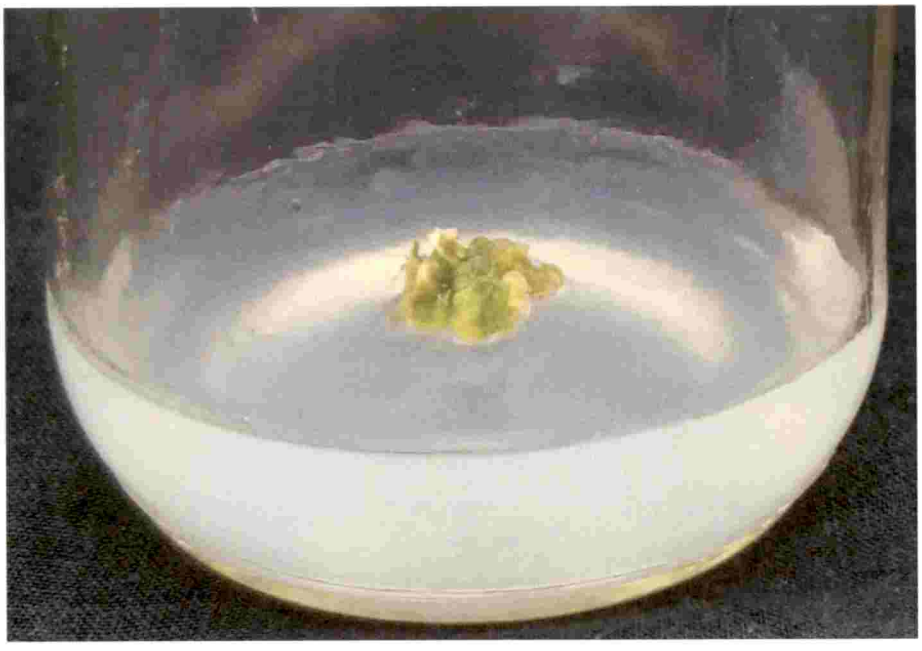


Plate 8. Initial greening of callus in shoot regeneration medium

4.2.2.3 Shoot initiation percentage

After attaining callus multiplication in two months after callus induction, the multiplied callus was subjected to shoot regeneration treatments (Table 13) and observations on shoot initiation for the five treatments were converted into percentage response. All the hybrid genotypes showed shooting response.

Shoot initiation response of the six hybrid genotypes showed variations and shoot initiation was recorded in all the treatments studied. The lowest shoot initiation percentage of 25.0 per cent was recorded for the treatment TR_1 (½ MS + 0.1 mg L^{-1} BA) and TR_5 (½ MS + 1.0 mg L^{-1} BA) in the hybrids HR x MR, OG x NO, HoR x KR and HR x LR. The treatment TR_3 (½ MS + 0.5 mg L^{-1} BA) was found to show the highest shoot initiation percentage (50.0 to 87.5 per cent) for all the hybrids under study. The highest callus induction percentage of 87.5 per cent was shown by the genotypes OG x NO and HoR x KR for the treatment TR_3.

4.2.2.4 Days to regeneration

The average number of days required for regeneration for six hybrids was analyzed (Table 14). The average number of days to regeneration ranged from 62.20 days to 82.33 days among the hybrids. All the genotypes showed the least number of days for regeneration, in the treatment TR_3 (½ MS + 0.5 mg L^{-1} BA).

For the treatment TR_3, the least number of days to regeneration was observed in the hybrid HR x MR (62.20 days) and the highest in LJ x OG (77.25 days). Next to TR_3, the treatment TR_4 (½ MS + 0.8 mg L^{-1} BA) showed had faster shoot regeneration followed by the treatment TR_2 (½ MS + 0.3 mg L^{-1} BA). More number of days for regeneration and low shooting response was observed in treatment TR_1 (½ MS + 0.1 mg L^{-1} BA) followed by TR_5 (½ MS + 1.0 mg L^{-1} BA).

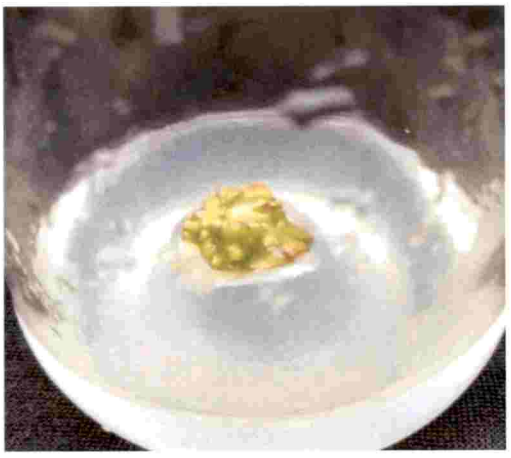
Table 13. Effect of media and hormones on shoot initiation in *Anthurium andreanum* hybrids (Shoot initiation percentage)

Sl. No.	Treatments	HR x MR	LJ x OG	OG x NO	HoR x KR	PR x HR	HR x LR
1	TR_1	0	0	25.0	0	0	0
2	TR_2	37.5	37.5	50.0	50.0	50.0	37.5
3	TR_3	62.5	50.0	87.5	87.5	62.5	50.0
4	TR_4	37.5	25.0	25.0	37.5	25.0	37.5
5	TR_5	25.0	0	25.0	25.0	0	25.0

Table 14. Effect of media and hormones on shoot initiation in *Anthurium andreanum* hybrids (Days to regeneration)

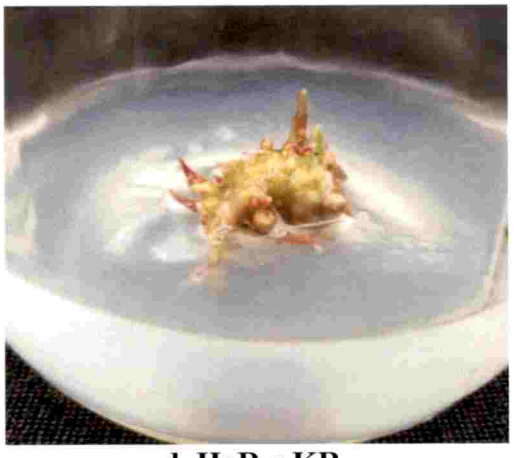
Sl. No.	Treatments	HR x MR	LJ x OG	OG x NO	HoR x KR	PR x HR	HR x LR
1	TR_1	0	0	76	0	0	0
2	TR_2	71.33	82.33	67.00	69.50	70.50	71.33
3	TR_3	62.20	77.25	62.29	62.86	62.80	66.00
4	TR_4	70.00	80.00	66.50	67.33	71.00	70.00
5	TR_5	74.00	0	72.00	76.50	0	74.50

a.HR x MR

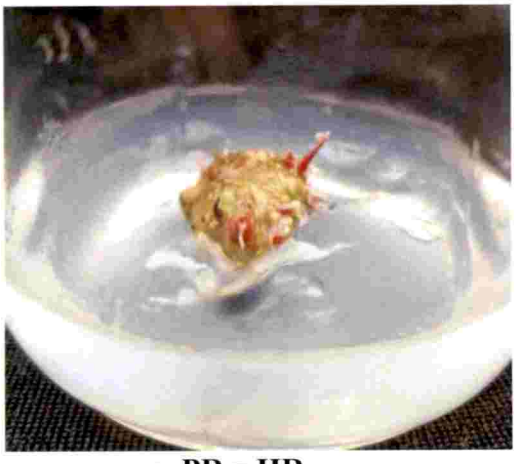


b. LJ x OG

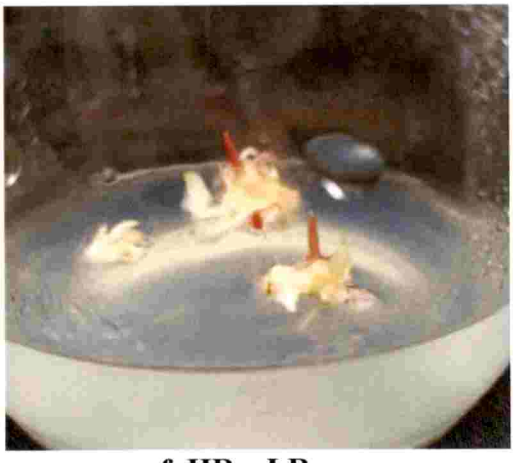
c. OG x NO



d. HoR x KR



e. PR x HR



f. HR x LR

Plate 9. Shoot regeneration response of *Anthurium andreanum* Linden hybrids (10 weeks after culture in regeneration medium)

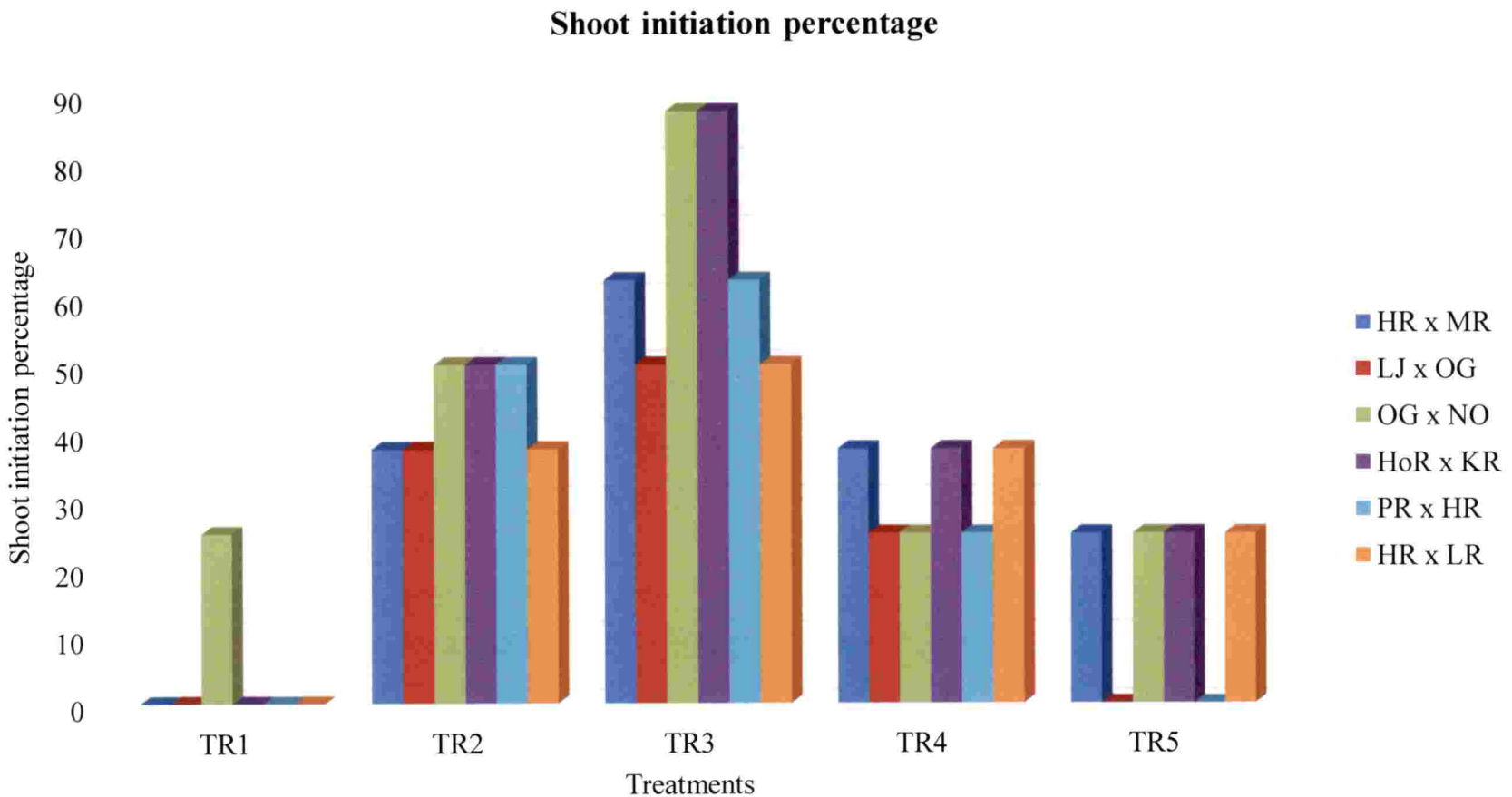


Fig. 7. Effect of media and hormones on shoot initiation in *Anthurium andreanum* hybrids (Shoot initiation percentage)

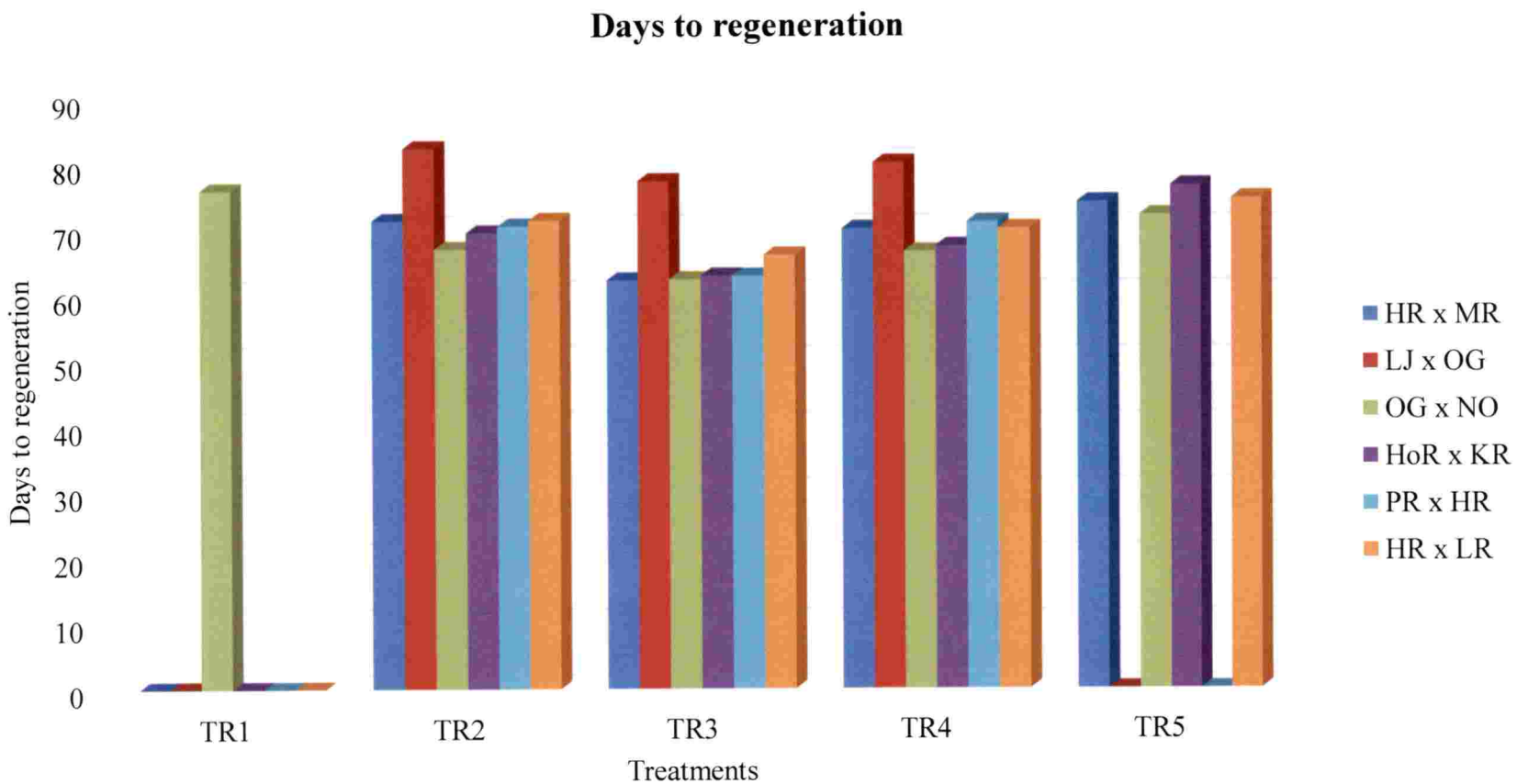


Fig. 8. Effect of media and hormones on shoot initiation in *Anthurium andreanum* hybrids (Days to regeneration)

4.2.2.5 Root initiation percentage

The observations on root initiation for the five treatments were converted into percentage response and documented (Table 15). The rooting response was observed on the same media in which shooting response occurred from the callus and hence a separate rooting phase was not necessary. In all the treatments, the shooting response was followed by rooting response.

The highest root initiation percentage of 87.5 per cent was observed for the genotype OG x NO and HoR x KR followed by 62.5 per cent for the hybrid HR x MR and PR x HR for the treatment TR_3 (½ MS + 0.5 mg L^{-1} BA). The lowest shoot initiation percentage of 12.5 per cent was recorded in the hybrid OG x NO for the treatment TR_1 (½ MS + 0.1 mg L^{-1} BA) followed by 25 per cent for the treatment TR_5 (½ MS + 1.0 mg L^{-1} BA) in the hybrids HR x MR, OG x NO, HoR x KR and HR x LR. On comparing the treatments TR_3 (½ MS + 0.5 mg L^{-1} BA) was found to show the highest root initiation percentage followed by TR_2 (½ MS + 0.3 mg L^{-1} BA) and TR_4 (½ MS + 0.8 mg L^{-1} BA).

4.2.2.6 Days to root generation

The average number of days required for root generation for six hybrids was studied (Table 16). Average number of days to root generation followed similar pattern like shoot induction and ranged from 63.20 days to 93.00 days among the hybrids. The least number of days for root generation was observed for the treatment TR_3 (½ MS + 0.5 mg L^{-1} BA) in all the genotypes except LJ x OG. LJ x OG showed the least number of days to rooting (84.00 days) for the treatment TR_2 (½ MS + 0.3 mg L^{-1} BA). The hybrid HR x MR (63.20 days) showed the fastest root generation in the treatment TR_3 while the hybrid LJ x OG (93.00 days) was the slowest in root generation response for the treatment TR_4 (½ MS + 0.8 mg L^{-1} BA). The highest number of days for root generation was observed in the treatment TR_5 (½ MS + 1.0 mg L^{-1} BA) for all hybrids except LJ x OG and PR x HR.

Table 15. Effect of media and hormones on root initiation in *Anthurium andreanum* hybrids (Root initiation percentage)

Sl. No.	Treatments	HR x MR	LJ x OG	OG x NO	HoR x KR	PR x HR	HR x LR
1	TR_1	0	0	12.5	0	0	0
2	TR_2	37.5	37.5	50.0	50.0	50.0	37.5
3	TR_3	62.5	50.0	87.5	87.5	62.5	50.0
4	TR_4	37.5	25.0	25.0	37.5	25.0	37.5
5	TR_5	25.0	0	25.0	25.0	0	25.0

Table 16. Effect of media and hormones on root initiation in *Anthurium andreanum* hybrids (Days to root generation)

Sl. No.	Treatments	HR x MR	LJ x OG	OG x NO	HoR x KR	PR x HR	HR x LR
1	TR_1	0	0	82.00	0	0	0
2	TR_2	69.33	84.00	70.25	70.75	73.50	78.00
3	TR_3	63.20	87.00	65.86	68.00	67.60	73.00
4	TR_4	75.00	93.00	76.00	71.33	79.00	78.33
5	TR_5	82.00	0	82.50	85.50	0	82.50

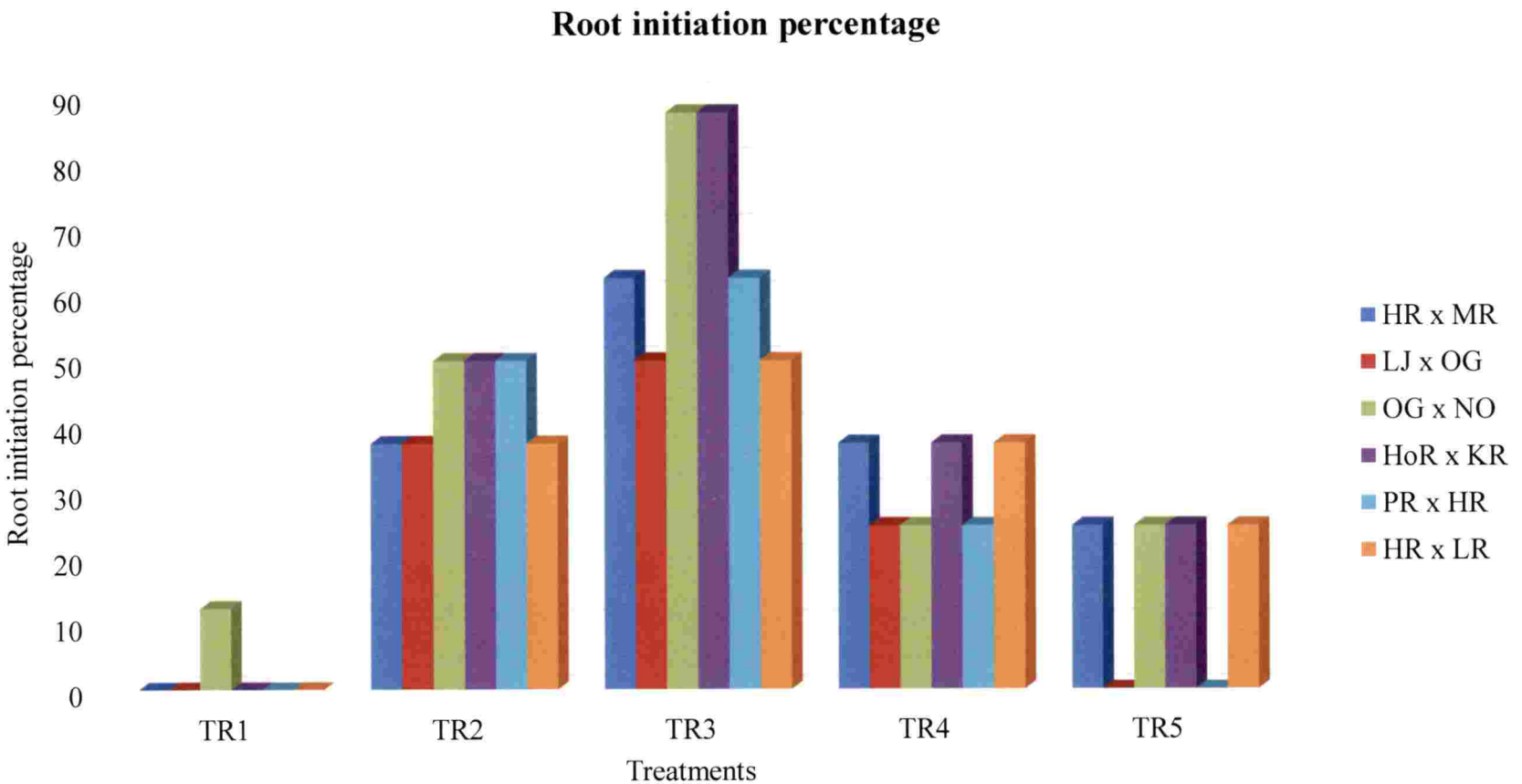


Fig. 9. Effect of media and hormones on root initiation in *Anthurium andreanum* hybrids (Root initiation percentage)

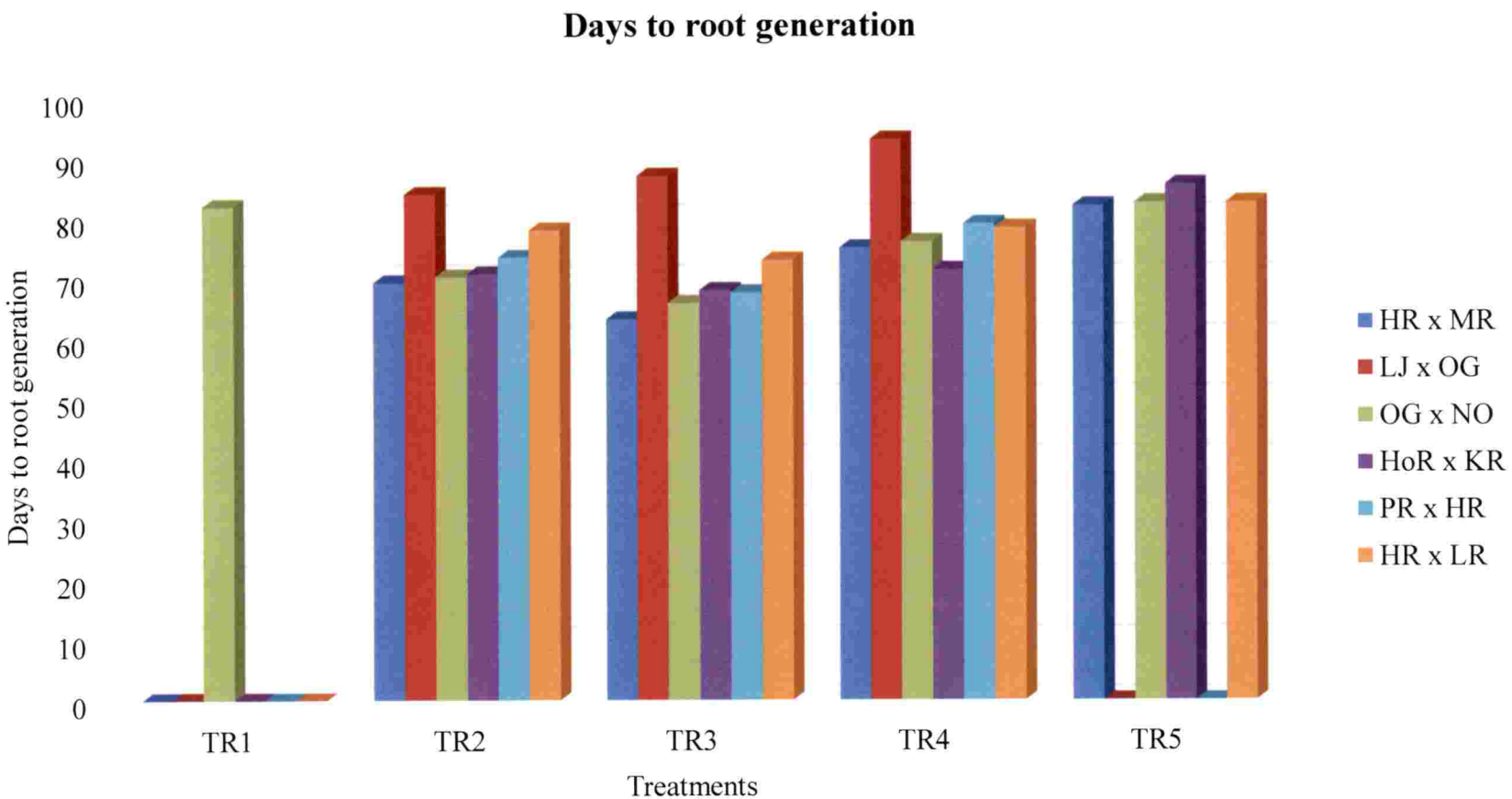


Fig. 10. Effect of media and hormones on root initiation in *Anthurium andreanum* hybrids (Days to root generation)

4.2.2.7 Days to emergence of first leaf

Number of days required for emergence of leaf was calculated from the callus subculturing stage on shooting medium to regeneration and developing a first distinguishable leaf on a shoot (Table 17). All the cultures that initiated shoot showed emergence of first leaf and the days required for emergence of the first leaf was directly correlated with the days required for regeneration. Average number of days to emergence of first leaf varied from 81.67 days in the hybrid HR x LR to 102.00 days in the hybrid LJ x OG for the treatment TR_2 (½ MS + 0.3 mg L^{-1} BA). Emergence of first leaf was the fastest for the treatment TR_3 (½ MS + 0.5 mg L^{-1} BA) in all the hybrids except HR x LR. Next to TR_3, the least number of days to emergence of first leaf was observed in the treatment TR_2 followed by TR_4 (½ MS + 0.8 mg L^{-1} BA).

4.2.3 Hardening and Acclimatization

The *in vitro* developed plantlets, after attaining 2.5 to 3.0 cm length, three to four leaves and more than two roots were transferred to a hardening medium containing sterilized fine river sand and charcoal in the ratio 3:1. The plantlets were kept in mist chamber for hardening and acclimatization.

Table 17. Effect of media and hormones on days to emergence of first leaf in *Anthurium andreanum* hybrids

Sl. No.	Treatments	HR x MR	LJ x OG	OG x NO	HoR x KR	PR x HR	HR x LR
1	TR_1	0	0	96.00	0	0	0
2	TR_2	89.67	102.00	87.50	89.75	90.25	81.67
3	TR_3	82.20	97.50	83.29	82.57	83.20	84.50
4	TR_4	91.33	101.50	87.00	89.00	91.50	85.00
5	TR_5	94.50	0	93.00	98.00	0	97.00

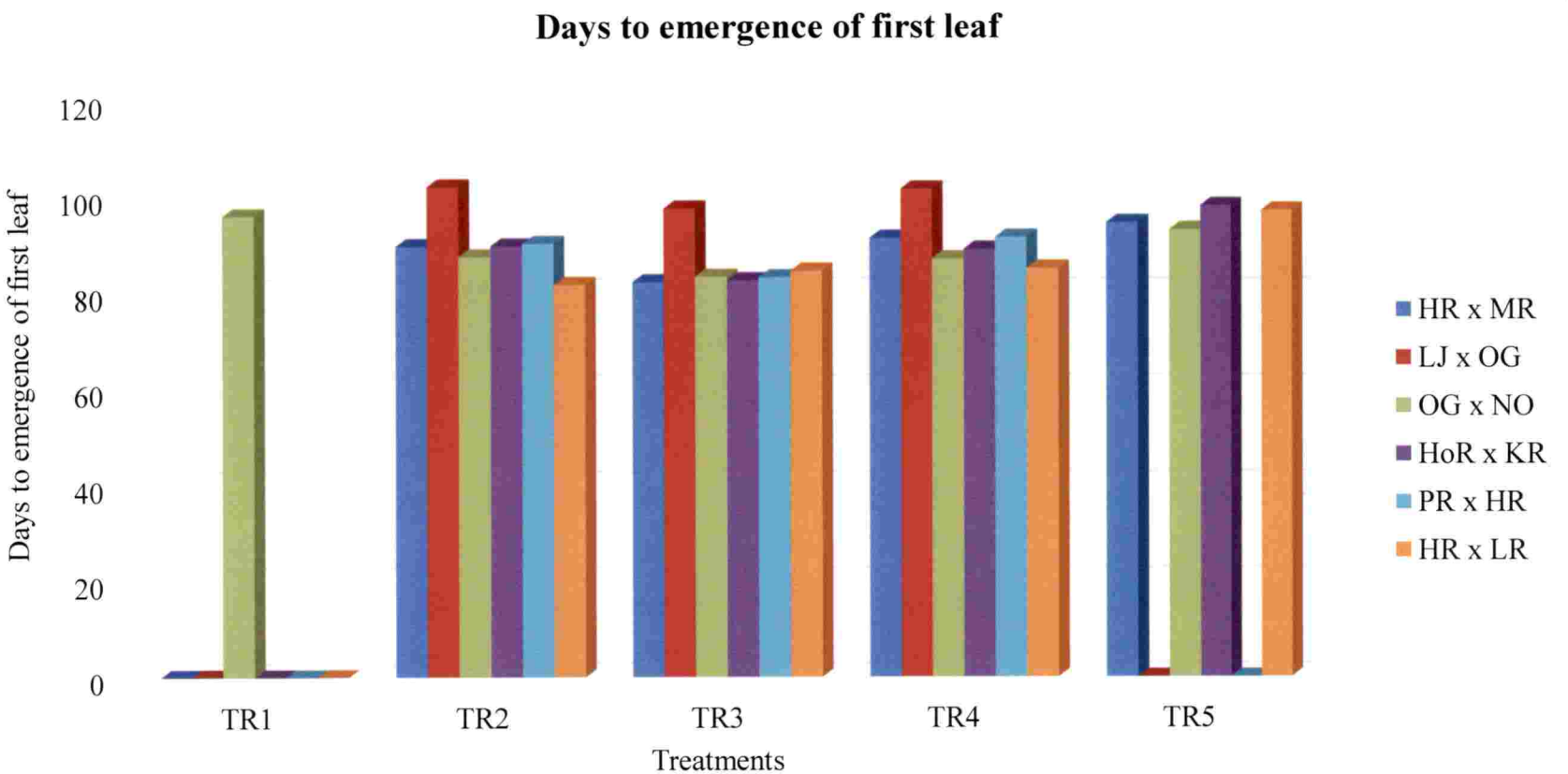
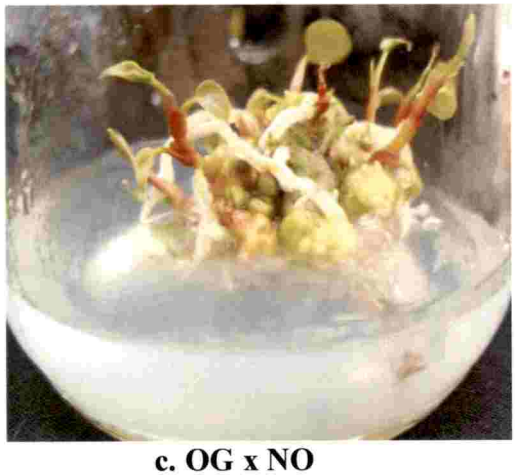


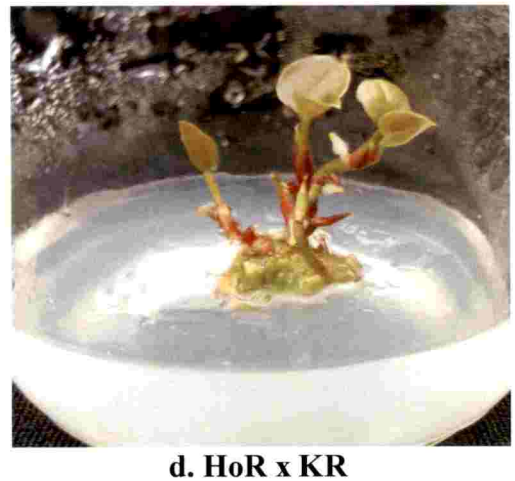
Fig. 11. Effect of media and hormones on days to emergence of first leaf in *Anthurium andreanum* hybrids (Days to emergence of first leaf)

a. HR x MR

b. LJ x OG

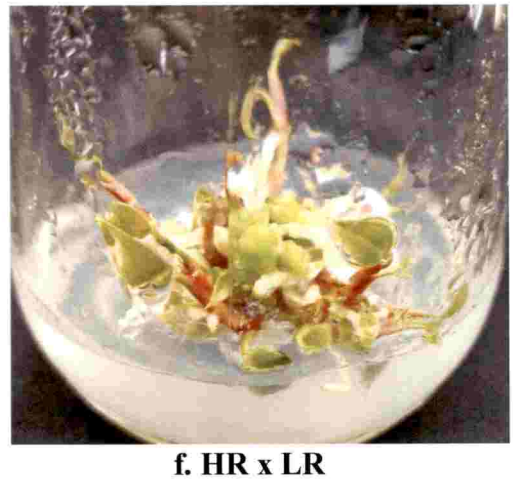


c. OG x NO



d. HoR x KR

e. PR x HR



f. HR x LR

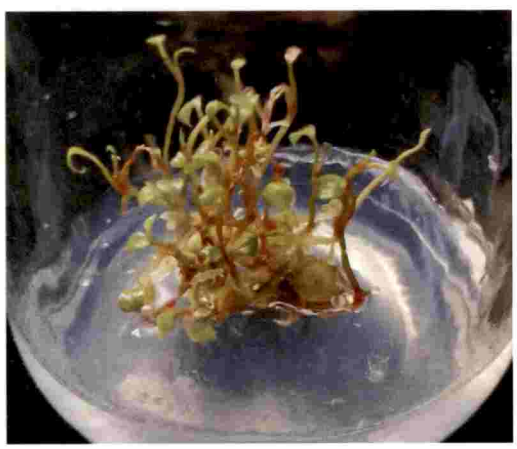
Plate 10. Plantlet regeneration of *Anthurium andreanum* Linden hybrids (15 weeks after culture in regeneration medium)

a. HR x MR

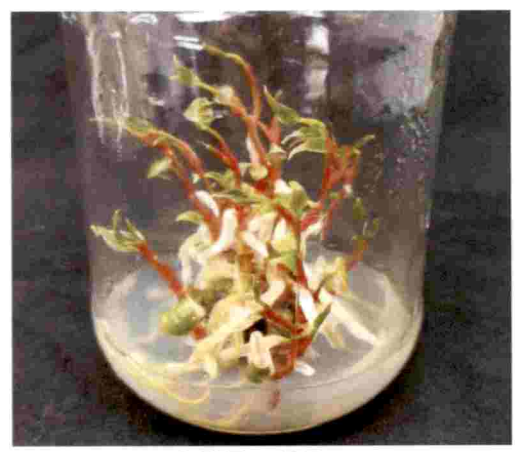
b. LJ x OG

c. OG x NO

d. HoR x KR



e. PR x HR



f. HR x LR

Plate 11. ***In vitro*** **developed plantlets of** ***Anthurium andreanum*** **Linden hybrids (20 weeks after culture in regeneration medium)**

Discussion

5. DISCUSSION

In the floriculture market the demand for unit value crops such as anthurium is booming day by day. Hybrid superiority and availability of healthy planting material must be satisfied for ensuring a strong hold in the highly competitive global floriculture trade. Development of specific *in vitro* protocols must go hand in hand with development of new hybrids as *in vitro* response varies widely among anthurium genotypes.

Considering these facts, the present investigation was taken up to assess the variability among *Anthurium andreanum* Linden hybrids for selection of superior hybrid genotypes and for developing cost effective *in vitro* mass multiplication protocol for the selected hybrids. The findings from the study based on analysis of genetic parameters and *in vitro* response of the hybrids are discussed in this chapter.

5.1 VARIABILITY ANALYSIS

5.1.1 Mean Performance

In the present study, the 20 *Anthurium andreanum* Linden hybrids were studied and wide range of variations were observed among the qualitative as well as quantitative characters.

A mean plant height of 44.8 cm was observed among the 20 hybrids. Significant variation was observed in plant height which ranged from 62.2 cm in HR x KR to 33.5 cm in the hybrid LR x OG. The genotypes LR x DT (37.4 cm) and HR x LR (34.7 cm) was found to be on par with LR x OG. Thus, the reduced plant height and compactness of the hybrids LR x OG, LR x DT and HR x LR makes them more suitable as potted plants. The effect of nutrients and integrated nutrient management methods on increase of plant height was reported by Waheeduzzama *et al.* (2007). Increased plant growth was reported in a field trail by Srinivasa (2006b) when the anthurium plants were maintained under 80 per

cent shade level. The variation observed for plant height in the present study was in accordance with the findings of Asish (2002), Pravin (2004), Anand *et al.* (2013), Sheena (2015), Gopi (2016) and Anand *et al.* (2017).

Leaf area showed wide variation based on the genotype as well as the age of the plant. The leaves were generally narrow or elongated. Among the genotypes studied the hybrid HoR x KR had the maximum leaf area of 410.85 cm^2 and the lowest leaf area of 120.20 cm^2 was recorded for the hybrid PR x HR. Leaf area of PR x HR was on par with PR x DT (1). Medium sized leaves with leaf area between 200 cm^2 and 300 cm^2 were observed in the hybrids namely LJ x OG (210.45 cm^2), OG x NO (282.60 cm^2), HR x LJ (261.05 cm^2), HR x DT (268.70 cm^2), HR x LR (203.80 cm^2) and PR x LR (208.60 cm^2). Mayadevi (2001) reported that medium sized leaves were desirable for novel anthurium hybrids. Leaf area ranging from 100.10 cm^2 to 237.24 cm^2 in the varieties Chias and Esmeralda was reported by Agasimani *et al.* (2011a). These observations were in line with the findings by Sheena (2015) and Gopi (2016).

Internodal length in the present investigation ranged from 0.70 cm to 1.60 cm in the hybrids HR x MR and HR x KR respectively. The hybrid PR x DT (1) had an internodal length of 0.92 cm which was on par with HR x MR. Anthurium hybrid with short internodes results in compact plant type which is one of the commercially desired characters as reported by Mayadevi (2001). The internodal length reported in the study is in agreement with the findings by Madhukumar (2010) and Gopi (2016).

Days from emergence to maturity of leaves ranged from 31.4 days in the hybrid HR x KR to 18.8 days in the hybrid LR x DT. On studying the variability among exotic anthurium varieties, Sheena (2015) reported wide variation in number of days from emergence to maturity of leaves ranging from 24.55 to 35.95 days. Similar results were also reported by Mayadevi (2001), Madhukumar (2010) and Gopi (2016).

The hybrid HR x MR (15.8) had the highest number of leaves spadices^{-1} plant^{-1} year^{-1} while the least was recorded in the hybrid HR x P (3.6) and LR x OG (3.6). HR x MR (15.8) and HR x LJ (10.6) had higher number of leaves spadices^{-1} plant^{-1} year^{-1} than all the hybrids and varieties studied by Premna (2003), Madhukumar (2010), Islam *et al.* (2013) and Gopi (2016). All the other hybrids had number of leaves spadices^{-1} plant^{-1} year^{-1} in compliance with the above investigations.

In the present study, sucker production was found to be the maximum in the hybrid OG x NO (2.2) and no suckers were produced by the hybrids HR x LJ, HR x P and PR x DT (1.0). All the hybrids except LR x OG, OG x DT and CR x KR were found to be on par with HR x LJ, HR x P and PR x DT (1.0). The results were in accordance with the studies conducted by Agasimani *et al.* (2011a), Gopi (2016) and Anand *et al.* (2017).

A significant variation in days from emergence to maturity of inflorescence was noticed among the anthurium hybrids studied. The maximum mean number of days from emergence to maturity of inflorescence was recorded in the hybrids HR x LJ and PR x LR (30.8 days) while the minimum was recorded for the hybrid HR x LR (21.6 days). This was in line with the conclusions made by Madhukumar (2010), Sheena (2015) and Gopi (2016). Mayadevi (2001) reported much higher duration from emergence to maturity of inflorescence ranging from 44.60 to 50.60 days among 100 anthurium genotypes while a lower duration ranging from 19 to 25.33 days was reported by Ravidas (2003).

The smallest spathe size among the 20 anthurium hybrid genotypes was recorded in HR x MR (27.60 cm^2) which was on par with HR x LJ, DT x HR, P x LR, LG x OG, PR x HR and KR x LR. The hybrid HoR x KR recorded the largest spathe size of 112.30 cm^2. Similar variations in spathe size was also reported by Madhukumar (2010), Islam *et al.* (2013), Sheena (2015) and Gopi (2016).

Anthuriums with short and slender spadix (candle) is preferred in the floriculture market. In the present study most of the hybrids namely KR x LR (3.64 cm), OG x DT (4.30 cm), LJ x OG (4.30 cm), DT x HR (4.14 cm), HR x MR (4.04 cm), CR x KR (4.04 cm), PR x HR (3.98 cm), PR x DT (1) (3.82) and PR x LR (3.74) had preferable short candles. The hybrids HR x LJ (6.76) and HR x KR (8.58) had longer candles which are not ideal. Pravin (2004) identified two hybrids MO x KR (1) (3.83 cm) and PR x LR (1) (4.97 cm) with short candles. Agasimani *et al.* (2011a) also reported spadix length ranging from 3.35 cm (Grace) to 8.24 cm (Esmeralda). The result of the present study were also supported by the findings of Madhukumar (2010), Islam *et al.* (2013) and Gopi (2016).

Number of flowers produced on the candle increases with candle length. As short spadix is preferable, the hybrids with less number of flowers per candle must be selected. In the present investigation the hybrid HR x MR (223.6) had the least number of flowers spadix^{-1}. The hybrid HR x KR (678.0) recorded the largest number of flowers among the 20 hybrids studied and was not commercially preferred. This was in line with the conclusions made by Madhukumar (2010), Sheena (2015) and Gopi (2016). Among 100 genotypes of anthurium studied Mayadevi (2001) reported the minimum number of flowers per spadix for the cross Pink x Liver Red (400.0) while the maximum was exhibited by Pink x Kalympong Red and Honeymoon Red x Dragon's Tongue (600.0).

In anthuriums, fertilized inflorescence had increased life (4-7 months) than that of unfertilized spadix (2 months) (Mercy and Dale, 1994). In agreement to this Premna (2003) reported spadix life of 59.5 to 101.5 days in unfertilized spadix. In the present study, the life of spadix from emergence to senescence varied from 58.8 days (HR x P) to 95.0 days (OG x NO) which was on par with HoR x KR (90.2 days). Senescence occurs with yellowing of peduncle followed by withering of candle and the spathe. The maximum spadix life span of 101.33 days was reported in the hybrid PR x DT, among 40 anthurium genotypes studied

by Madhukumar (2010). Similarly, Gopi (2016) observed the longest life of spadix in the variety Tropical Red (91.2 days) followed by Liver Red (88.4 days).

Female phase initiation was identified almost two weeks after flower emergence. Anthurium inflorescence develops female flowers in the initial stage in acropetal succession (Croat, 1980). The observations made in the present study also clearly indicates protogynous nature in anthurium flowers. Among the 20 hybrids studied, days to initiation of female phase from the base of the spadix was observed to be the slowest for the hybrid HoR x KR (9.4 days) and the fastest for HR x KR, CR x KR and HR x LR (4.4 days). Duration of female phase was accounted by observing honeydew secretion on the stigma. Duration of female phase also varied widely among the hybrids with HR x P (10.4 days) showing the longest duration and HR x MR (5.4 days), the shortest. Similar variations in initiation and duration of female phase were also observed in the studies by Premna (2003), Madhukumar (2010) and Gopi (2016).

Interphase is the period between female phase and male phase in anthurium. Initiation of interphase is marked by the drying up of stigmatic surface of the flowers on the candle. The hybrid PR x DT (9.4 days) had the longest interphase duration while the shortest was recorded in HoR x KR, HR x DT, CR x KR, HR x LR, LR x OG and DT x HR (4.4 days). Interphase duration ranging from 2.33 to 6.83 days was reported by Pravin (2004) on studying 14 genotypes of anthurium. Madhukumar (2010) observed that the variety Nitta Orange (9.22 days) had the longest interphase duration while the genotype Vezuvious Red (4.5 days) had the shortest. Similar reports were also made in the variability study by Gopi (2016) in 25 anthurium genotypes.

Interphase was followed by male phase. Initiation of male phase is identified by the extrusion of anther from the base of the candle. Anther production starts from the base of the candle and proceeds upwards. From the study on 25 anthurium hybrids, the longest male phase duration was reported in HR x LJ (10.8 days) and the shortest in PR x LR (6.2 days). Among 100

anthurium genotypes studied by Mayadevi (2001), the duration of male phase ranged between 5.0 days and 7.2 days while Madhukumar (2010) reported longer male phase duration in the hybrid Tropical Red x Meringue White (10.89 days), Acropolis White (10.36 days) and Arun Gold (10.33 days). The results of present study was also in accordance with the findings by Renu (2000) and Gopi (2016).

Ideal anthurium genotypes should have short candles with inclination of candle to the spathe less than 45° which facilitates it for suitable packing (Mercy and Dale, 1994). Such ideal spadix inclination was observed in the hybrids P x LR (37.2°), PR x DT (1) (39.0°), DT x HR (39.0°), OD x NO (42.6°), PR x DT (43.2°) and HR x MR (43.6°). The highest spadix angle of 103.2° was observed in the hybrid LR x DT which is less desirable. Much lower angle of inclination of candle to the spathe was reported for a genotype Kalympong Red (21°) by Mayadevi (2001) and for the variety Aymara (30°) in a study conducted by Islam *et al.* (2013). The observation of the current study is supported by the findings of Madhukumar (2010), Sheena (2015) and Gopi (2016).

Anthocyanins are the key factors that contribute towards spathe colour in anthurium. In the present study, total anthocyanin content showed wide variation ranging from from 36.184 mg g^{-1} in the hybrid LR x OG to 326.434 mg g^{-1} in LR x DT. Premna (2003) identified the lowest mean anthocyanin content in the variety Acropolis White (10.09 mg g^{-1}) and the highest in Honduras (259.18 mg g^{-1}). Similar findings showing higher anthocyanin content in red spathe cultivars compared to pink, orange and white spathe genotypes were reported by Mayadevi (2001), Madhukumar (2010), Sheena (2015) and Gopi (2016).

Vase life of cut flower is the ultimate requirement of any successful flower production technology (Anand *et al.,* 2017). Anthurium genotypes with the maximum vase life are to be selected to fetch market acceptance. On comparing the vase life of 20 anthurium hybrids using water as vase solution, wide variation among the genotypes was observed. The longest vase life was observed for the hybrid HoR x KR (24.4 days) followed by LJ x OG (23.4 days). Vase life was the

lowest for the genotype KR x LR (10.0 days) which was not preferred. Similarly, the highest vase life of 21.0 days was reported in the variety Esmeralda and the lowest for Ivory (10 days) was reported in a study by Shriram (2008). The variations observed in the present study were in accordance with the findings of Anand *et al.* (2013), Harishshivalingappa *et al.* (2013) and Islam *et al.* (2013).

Number of inflorescence year^{-1} or the flower yield in anthurium is the most important character to be considered along with flower quality. The observations made from 20 hybrid genotypes of anthurium revealed that the genotypes showed wide variation in flower yield. The maximum number of inflorescence in a year was observed in the hybrid HR x MR (6.4) while the minimum was observed in CR x KR (2.2). The hybrids LJ x OG (5.0), OG x NO (5.0), PR x HR (4.6), HoR x KR (4.4) and HR x LR (4.4) also showed high flower yield with more than four inflorescence per year. Similar variations in flower yield were also reported in the studies conducted by Madhukumar (2010), Lima *et al.* (2014) and Gopi (2016). Very few anthurium varieties with higher flower yield namely Liver Red (7.00), Titicaca (7.2), Lady Jane (7.6) and Esmeralda (9.33) were reported in various studies conducted by Pravin (2004), Islam *et al.* (2013), Renu (2000) and Agasimani *et al.* (2010).

The present study evaluated qualitative characters in anthurium such as colour of petiole and young leaves, spathe colour, spadix colour, type of inflorescence axis, pollen emergence pattern, pollen shape and pollen colour. The colour of petiole ranged from green to dark green to greenish brown and from brownish green to brown. Similarly, colour of young leaves varied from light green to green and to greenish brown, and from brown to brownish green. Similar variations were observed in the studies conducted by Asish (2002), Madhukumar (2010), Sheena (2015) and Gopi (2016).

Spathe colour is the most important qualitative character that drives consumer's attention towards cut flowers. Kamemoto *et al.* (1988) reported that in anthurium, the inheritance of spathe colour is controlled by two major genes, M

and O which were responsible for expression of major colours namely pink, red, coral, white and orange. Pink to red colour is expressed due to presence of both M and O genes together. M gene controls cyanidin 3-rutinoside production while O gene controls pelargonidin 3-rutinoside production. Variations in red colour ranging from maroon to dark red and to red and pink occur due to incremental effect of M gene over O gene, thereby a decrease in colour intensity was observed form MMOO, MMOo, MmOO and MmOo. The 'mmOO' genotype exhibited a light orange colour i.e., coral coloured spathe. Orange colour of spathe was found to be true breeding, with the genotype 'mmOO'. As recessive 'oo' was epistatic to M, the spathe colour of recessive (mmoo) genotypes or genotypes with M in combination with 'oo' (Mmoo and MMoo) exhibited true white coloured spathe. The spathe was purple when the genotype was 'M_O_ pp', as recessive 'p' allele modifies the expression of M and O allele loci. Dominant P allele was found to have no effect on spathe colour in any combinations.

The present study also proved the presence of wide variations in spathe colour such as dark red, red, light red, orange, light orange, light pink and pinkish white. On studying 50 anthurium genotypes, Asish (2002) observed variations in spathe colour ranging from deep maroon to white. The variability study of 25 anthurium genotypes, Gopi (2016) grouped the anthurium varieties into deep maroon, dark red, bright red, red, pink, peach, green, bright orange, orange and chocolate brown based on spathe colour. Similar variations in spathe colour among anthurium genotypes was reported by Madhukumar (2010), Sheena (2015) and Anand *et al.* (2017).

Spadix colour showed variations ranging from yellowish red, whitish red, greenish red, whitish yellow, yellowish green, yellowish white, reddish white, to white. All the hybrids except LJ x OG and HR x LJ (white) had blending of two or more colours on the candle. A range of spadix colours namely light yellow, yellow, pink, maroon, yellowish white, greenish yellow to creamy white was identified among 40 anthurium genotypes studied by Madhukumar (2010).

Significant variations among the spadix colour were also reported by Renu (2000), Sheena (2015), Gopi (2016) and Anand *et al.* (2017).

Inflorescence axis of anthurium needs to be long, straight and strong so as to fetch market value. Inflorescence axis among the 20 hybrids showed significant variation. Long curved thick, long straight thick, medium straight thin, medium curved thin, medium curved thick, short curved thick and short curved thin inflorescence axis were observed. Sheena (2015) categorized the type of inflorescence axis in anthurium as short to long, thin to thick and curved to straight. Similar variation and categorization of inflorescence axis were also reported in studies conducted by Madhukumar (2010) and Gopi (2016).

Anthurium hybrids in the present study showed variation in pollen shape ranging from round to oval. All the hybrids except LJ x OG, HR x LJ and OG x DT had round pollen. Predominance of round pollen was reported by Madhukumar (2010) on studying 40 anthurium genotypes. Similar variations were also reported by Sheena (2015) and Gopi (2016). Hybrids showed pollen colour variation from white to cream colour. The hybrids LJ x OG and HoR x KR had cream coloured pollen while all the other hybrids had white pollen. Gopi (2016) also reported similar pollen colour variants among 25 anthurium genotypes. Emergence of pollen in anthurium was highly influenced by seasonal variations. Pollen emergence was found to be higher in winter months and was found to be lower during summer months. The hybrids HR X KR and HR X MR showed the highest pollen emergence while it was the lowest for OG X DT and OG X NO. Madhukumar (2010) observed much lower pollen production from March to June season. Similar pattern of pollen emergence was also reported by Sheena (2015) and Gopi (2016).

5.1.2 Variability Components

Variability present in a crop is considered as the basis for effective selection. Beyond analysis of variance, absolute assessment of variability can only be done by computing the genotypic and phenotypic coefficients of variability.

Genotypic coefficients of variation (GCV) and phenotypic coefficients of variation (PCV) help to separate out environmental influence on the genotype from the total variability. GCV and PCV were analysed based on coefficients of variation and these parameters were used for the comparison among the 20 hybrid genotypes. PCV measures the extent of total variation while GCV is effective in providing the range of genetic diversity of the quantitative traits. Moreover, GCV and PCV are better indices for the comparison of quantitative traits with different units of measurement.

Variability analysis in the present study showed that PCV was higher in magnitude than GCV for all the analysed characters. High GCV and high PCV were observed for the characters namely number of suckers plant^{-1}, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size, leaf area, number of flowers spadix^{-1}, spadix length, duration of interphase, inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1}. Therefore, improvement of these traits could be achieved by practicing direct selection. The highest GCV and PCV values were observed for the character anthocyanin content followed by pollen fertility, leaf area, spathe size and spadix length in a study conducted by Madhukumar (2010). Gopi (2016) reported high GCV and PCV for the traits such as number of suckers plant^{-1}, anthocyanin content, number of inflorescence year^{-1} and leaf area. The results of the present study were also in accordance with the findings of Asish (2002), Pravin (2004) and Sheena (2015).

The minimum PCV and GCV values were recorded for the character days from emergence to maturity of inflorescence. The finding was similar to the observation made by Gopi (2016). Days from emergence to maturity of leaves, life of spadix and duration of male phase showed medium range of GCV and PCV values. The observations were supported by Pravin (2004), Madhukumar (2010) and Sheena (2015). All the characters except number of suckers plant^{-1} showed GCV values close to their corresponding PCV values, indicating relatively less influence of environmental components on these characters. Environmental influence on the trait number of suckers plant^{-1} was also reported by Madhukumar

(2010) and Gopi (2016).

5.1.3 Heritability and Genetic Advance

The transmissibility of a character from one generation to the next is represented as heritability. Heritability helps plant breeder in effective selection and thereby attaining maximum genetic gain in less time. Heritability alone fails to indicate the response to selection. So heritability estimates along with the estimates of genetic advance as percentage mean can be used in predicting the resultant effect which could be used for selection of superior genotypes.

High heritability was noticed in characters such as plant height, leaf area, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size, spadix length, days from emergence to maturity of inflorescence, life of spadix, number of flowers spadix^{-1}, days to initiation of female phase, duration of female phase, inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1}. Selection based on these characters will result in significant improvement in the next generation. The traits such as internode length, days from emergence to maturity of leaves, number of suckers plant^{-1}, duration of interphase and male phase exhibited medium heritability ranging from of 30 to 60 per cent. High heritability for the character anthocyanin content was reported by Asish (2002). The characters like number of leaves spadices^{-1} plant^{-1} year^{-1}, internode length, days from emergence to maturity of leaves, leaf area, plant height, inclination of spadix with the spathe, days from emergence to maturity of spathe, days to initiation of female phase and duration of female phase exhibited high heritability (Sheena, 2015). Similar findings were also reported by Premna (2003), Pravin (2004), Madhukumar (2010) and Gopi (2016).

In the present investigation, traits such as plant height, leaf area, internode length, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size, spadix length, number of flowers spadix^{-1}, number of suckers plant^{-1}, days to initiation of female phase, duration of male phase, duration of interphase, duration of female phase,

inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1} depicted high genetic advance values. High genetic advance for the trait anthocyanin content was reported by Asish (2002). The results were in accordance with the findings of Pravin (2004), Madhukumar (2010) and Sheena (2015).

High heritability coupled with high genetic advance was observed for the traits namely plant height, leaf area, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size, spadix length, number of flowers spadix^{-1}, days to initiation of female phase, duration of female phase, inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1}. This indicated that these characters are governed by additive gene action and direct selection for these characters may be effective (Panse and Sukhatme, 1967). Low heritability along with high genetic advance were observed for these traits such as internode length, number of suckers plant^{-1}, duration of male phase and duration of interphase. These characters were also governed by additive gene action and low heritability was due to effect of environment. Selection may be rewarding in this case also. High heritability and genetic advance was reported for the trait anthocyanin content by Madhukumar (2010) and for leaf area and spathe size in a study conducted by Anand *et al.* (2013). The present study was also in line with the findings of Sheena (2015), Tamuli *et al.* (2015) and Gopi *et al.* (2016).

5.1.4 Correlation Studies

Correlation analyses the extent of association and interrelationship of quantitative characters within a population. When selection is carried out for a trait of interest in a population, it is associated with the improvement of other traits associated with the trait of interest. This helps in simultaneous improvement of more than one character which moves in the same direction of selection. Correlated responses cannot be analysed for the traits that are governed by one or few genes. Genotypic and phenotypic correlations were worked out for 19 quantitative characters of the anthurium hybrids. In the present study, the

character number of inflorescence year^{-1} or the flower yield of anthurium had a significant positive correlation with number of leaves spadices^{-1} plant^{-1} year^{-1}, vase life and number of suckers plant^{-1}.

Due to positive correlation, improvement in any one of these characters will simultaneously result in improvement of all the other correlated characters. In genetic improvement programmes based on a character of interest, selection can be practiced by the plant breeder by considering the correlated characters. Similar correlated responses were observed in the studies conducted by Premna (2003), Shiva and Nair (2008) and Anand *et al.* (2017).

The genotypic correlation existing between the characters helps to make clear differentiation between vital associations useful in plant breeding from the non-vital characters. On analysing the genotypic correlation among 19 quantitative characters in anthurium, the trait plant height showed significant positive genotypic correlation with the characters except number of leaves spadices^{-1} plant^{-1} year^{-1}, number of suckers plant^{-1}, days from emergence to maturity of inflorescence and days to initiation of female phase, duration of interphase, inclination of candle with spathe and number of inflorescence year^{-1}. Madhukumar (2010) on studying 40 anthurium genotypes reported positive genotypic correlation of plant height with anthocyanin content, number of flowers per spadix, life of spadix, spadix length, leaf area and internodal length. Similar correlations were also reported by Premna (2003), Gopi (2016) and Anand *et al.* (2017).

Life of spadix in anthurium hybrids had significant positive correlation with traits such as plant height, leaf area, internode length, spathe size, spadix length, number of flowers spadix^{-1}, days to initiation of female phase, duration of female phase, duration of interphase, duration of male phase and vase life. Gopi (2016) also reported positive correlation of life of spadix with leaf area, plant height, spathe size, number of flowers spadix^{-1}, spadix length, days from initiation of female phase, duration of female phase and duration of interphase. Similar

reports were also made by Asish (2002), Premna (2003), Madhukumar (2010) and Anand *et al.* (2017).

Spadix length had significant positive correlation with plant height, leaf area, internode length, days from emergence to maturity of leaves, spathe size, number of flowers spadix^{-1}, life of spadix, duration of female phase, duration of male phase, inclination of candle with spathe and vase life. Similar results were reported by Premna (2003) and Madhukumar (2010).

Inclination of candle with spathe, which is one of the commercially important characters showed a significant positive correlation with leaf area, days from emergence to maturity of inflorescence, internode length, spadix length, spathe size, anthocyanin content and number of flowers spadix^{-1}. A positive significant correlation existes between anthocyanin content and inclination of candle with spathe, and spathe size. The character inclination of spadix had a positive phenotypic and genotypic correlation with the character life of spadix, as reported by Premna (2003).

Post-harvest life of anthurium i.e., in cut flowers, the vase life was found to show a positive and significant correlation with traits such as plant height, spathe size, spadix length, number of leaves spadices^{-1} plant^{-1} year^{-1}, number of flowers spadix^{-1}, life of spadix, duration of male phase, duration of female phase and number of inflorescence year^{-1}. The results of the present study was supported by the findings of Anand *et al.* (2013).

Flower yield in anthurium or number of inflorescence year^{-1} had significant and positive correlation with number of leaves spadices^{-1} plant^{-1} year^{-1}, number of suckers plant^{-1} and vase life. Reports of Premna (2003), Shiva and Nair (2008) and Anand *et al.* (2017) were in agreement with the present study. Number of inflorescence year^{-1} was negatively correlated with internode length and days from emergence to maturity of leaves. As new inflorescence arises from leaf axils, maturity of leaves becomes fast. This promotes production of new leaves and thereby increases the flower yield.

5.1.5 Path Analysis

Path analysis was done to confirm whether the correlation of component characters with the dependent character was due to their direct effect or due to indirect effect through some other character. Path coefficient analysis splits the genotypic correlation coefficients into direct and indirect component characters which contributes to yield (Dewey and Lu, 1959). Based on these characters an effective and reliable crop improvement programme can be developed. If the correlation between flower yield and a component character is due to direct effect, it indicated true direct association between these traits. Thus, direct selection for this component character can be rewarding to attain improved flower yield. If the correlation is due to indirect effect of the component character through another component character then the plant breeder should go for selection of the latter character through which the indirect effect was found to influenc the flower yield.

In the present study, considering number of inflorescence year^{-1} or the flower yield of anthurium as the dependent character, the highest positive direct effect was shown by the trait plant height followed by anthocyanin content, number of leaves spadices^{-1} plant^{-1} year^{-1}, days to initiation of female phase, duration of male phase, internode length, inclination of candle with spathe, duration of female phase, number of suckers plant^{-1}, vase life and leaf area. The three characters namely, number of leaves spadices^{-1} plant^{-1} year^{-1}, number of suckers plant^{-1} and vase life were found to have direct and significant positive correlation towards flower yield. This indicated that selection for the above traits will result in improvement of flower yield in anthurium hybrids. Shiva and Nair (2008) reported that sucker yield in anthurium had positive and direct effect on number of leaves per plant and leaf area which was in accordance with the present study. Positive and direct effect of leaf diameter, vase life and number of leaves on flower yield in anthurium was evident form the findings of Anand *et al.* (2013). Similarly flower yield in anthurium was found to have positive and direct relationship with characters such as number of suckers, leaf length, leaf breadth, number of leaves and plant height (Anand *et al.*, 2017).

The trait internode length exhibited direct, significant negative correlation towards flower yield, indicating that selection for this character will result in reduction of flower yield in anthurium hybrids. Madhukumar (2010) reported that days to initiation of female phase had significant negative correlation and direct effect on number of flowers per spadix, while in the present study the negative direct effect was found to be insignificant.

Path analysis revealed that 70.35 per cent of the variation in flower yield in anthurium hybrids was attributed to the quantitative traits considered in the current study. About 29.65 per cent of the variation in flower yield was due to the effect of characters that are not considered in the study and the environment. Low residual value of path analysis reveals that the cause and effect system was well explained by the characters in the study.

5.2 *IN VITRO* MULTIPLICATION

The young leaf explants of six superior hybrids selected from experiment I namely HR x MR, LJ x OG, OG x NO, HoR x KR, PR x HR and HR x LR were used for *in vitro* multiplication. Leaf lamina was reported to be the best explant for anthurium tissue culture based on studies conducted by Puchooa (2005), Bejoy *et al.* (2008), Zhou *et al.* (2012) and Thokchom and Maitra (2017). The basal medium used was half strength Modified MS medium (Annexure 1) with quarter strength of ammonium nitrate (200 mg L^{-1}) as reported by Nirmala and Singh (1993) and Singh (1994). The efficiency of half strength MS medium as basal medium was supported by the findings of Lan *et al.* (2003), Cui *et al.* (2007), Jeshima (2007) and Duan *et al.* (2009).

5.2.1 Surface sterilization

Contamination caused by bacteria and fungi was the major bottle neck faced during the establishment of tissue culture explants. Microbes harbor the plant tissues since the mother plants had been exposed in field conditions for long time which resulted in systemic infection during the culture of explants collected

from them. In the present study high rate of fungal contaminations were observed during initial stages of culture. To obtain contamination free culture, various surface sterilization treatments were experimented.

Among the five treatments compared in the present investigation, the percentage survival of leaf explants on half strength MS medium was found to be the highest (87.5 per cent) when double surface sterilization was practiced using five per cent sodium hypochlorite in combination with 0.1 per cent mercuric chloride for 10 and five minutes respectively. Irrespective of the hybrids, double sterilization treatment was found to be much effective. Double sterilized explant showed improved survival percentage in the culture medium, compared to surface sterilization using single surface sterilant. This was supported by the findings of Hu and Wang (1983), Jeshima (2007), Jahan *et al.* 2009, Atak and Celik (2009), Thokchom and Maitra (2017) and Bhavana (2018).

In a study conducted by Gantait *et al.* (2008), the combination of five per cent sodium hypochlorite and 0.1 per cent mercuric chloride were found effective in surface sterilization of anthurium explants. Double sterilization using 5.0 per cent sodium hypochlorite for 10 minutes, in combination with 0.1 per cent mercuric chloride for five minutes and the sterilization treatment using 0.1 per cent mercuric chloride alone for seven minutes resulted in 62.5 per cent explant survival in the genotype HR x LR. Successful surface sterilization using 0.1 per cent mercuric chloride for seven minutes was supported by the findings by Bejoy *et al.* (2008).

Variation in explant survival within the same treatment was observed among the genotypes. Double sterilization treatment was successful in attaining the highest explant survival percentage of 87.5 per cent for the hybrids HR x MR, LJ x OG and OG x NO while 62.5 per cent explant survival was observed for the hybrids PR x HR and HR x LR. The lowest explant survival percentage (50.0 per cent) was recorded for the hybrid HoR x KR in the above treatment. This proves the effect of genotypic difference on explant survival percentage. Significant

effect of explant during culture was in accordance with the findings of Geier (1990) and George and Debergh (2008).

5.2.2 Callus Induction and Multiplication

Callusing response of a crop is highly influenced by the media composition, culture conditions, type of explant and the genotype used for *in vitro* culture. In case of anthurium, less lignified leaf tissues facilitates easy de-differentiation compared to other plant parts. The physiological and morphological status of the mother plant and the collected explant can also resulted in variation of *in vitro* responses.

In the present study, young leaf explants showed successful callus induction but a faster response was observed for explants excised from the proximal part of tender leaf lamina compared to distal part of the leaf. This could be due to increased number of dedifferentiating cells at the proximal end. The observation was in support to the findings by Puchooa (2005). Callus induction was found to occur from the cut edges of pale greenish brown, young leaf lamina but faster callusing response was obtained from the midrib and vein region of the explant. Similar responses were observed in the studies conducted by Bejoy *et al.* (2008) and Thokchom and Maitra (2017).

Callus initiation occurred at various frequencies among the hybrids in about seven to eight weeks of culture, incubated in continuous dark condition at 25°C. Successful callusing responses in anthurium were achieved by following similar culture conditions in various studies by Xia *et al.* (2005), Jiang *et al.* (2006), Jeshima (2007), Bejoy *et al.* (2008), Bhattacharya *et al.* (2015), Thokchom and Maitra (2017) and Bhavana (2018). The leaf explants turned brown when exposed to light period of 16 hours and the response may be due to the oxidation of phenolic compounds under light conditions. Similar inhibitory effect of light on callus induction was recorded by Jiang *et al.* (2006) and Bejoy *et al.* (2008). In contradiction to the present study, Lan *et al.* (2003) observed no significant effect of light and dark treatments on callus induction in anthurium.

The present investigation analysed the effect of cytokinins and auxins on callus induction and callus multiplication of anthurium hybrids. Among the five treatments tried, only three were found to be responsive and the callus induction percentage ranged from 25.0 per cent to 50.0 per cent. Modified half strength MS medium supplemented with 1.0 mg L^{-1} BA and 0.5 mg L^{-1} 2,4-D was found to be the most effective with respect to callus induction and callus multiplication for all the six anthrium hybrids studied. Faster proliferation of callus may be due to the presence auxins in the culture medium. Atak and Celik (2009), Bejoy *et al.* (2008) and Prakash *et al.* (2017) also obtained successful callusing using similar plant growth regulator compositions.

The best differentiation response of 50.0 per cent was recorded for the hybrids HR x MR and OG x NO in nine weeks of culture in the same medium while the hybrids LJ x OG and HoR x KR showed slower callusing response. The slow response of the hybrid HoR x KR may be attributed to the diverse parentage of the hybrid compared to other hybrids while slow response by LJ x OG can be due to miniature plant type. In genetic control of morphogenetic response, some gene expressions are regulated by the effect of plant growth substances which may vary with difference in growth habit and nature of a particular genotype.

Comparatively slower callusing response ranging from 25.0 to 37.5 per cent was observed in modified half strength MS medium supplemented with 1.0 mg L^{-1} BA. Anthurium tissue culture studies by Yuan *et al.* (2004) and Bejoy *et al.* (2008) also ensured that callus induction can be achieved with 1.0 mg L^{-1} BA alone in the basal medium. Yang *et al.* (2008) and Liu *et al.* (2009) reported organogenic response with a reduced BA concentration of 0.5 mg L^{-1}. Based on a study by Wu (2010), further higher concentration of BA i.e., 2.0 mg L^{-1} was also found to induce callusing respone from anthurium leaf explants. Cui *et al.* (2007) opined that single plant growth regulators were unable to produce callus in anthurium which was contradictory to the findings of the current study. Even though the callus induction percentage was low, callusing response was observed in same basal medium supplemented with 1.0 mg L^{-1} BA and 1.0 mg L^{-1} 2,4-D.

On comparing the response from all the treatments, callusing was found to be higher when the medium was supplemented with 2,4-D. These responses clearly indicated that the proportion of auxins and cytokinins in the culture medium had significant impact on callus induction and multiplication responses in anthurium as reported by Skoog and Miller (1957).

The hybrid HR x MR showed callusing in modified half strength MS medium supplemented with 1.0 mg L^{-1} BA and 1.0 mg L^{-1} 2,4-D while the hybrid HoR x KR showed the fastest callusing in same basal medium supplemented with 1.0 mg L^{-1} BA alone. All the other hybrids responded in callusing media provided with 1.0 mg L^{-1} BA and 0.5 mg L^{-1} 2,4-D. Increased callusing response observed even in presence of low auxin concentration of 0.5 mg L^{-1} 2,4-D may be due to high potency of auxin or due to the increased level of endogenous auxin in the young developing apical leaf tissues.

From the present study, it is obvious that requirement of plant growth regulators for each of the hybrids in the study varied considerably. The present study also proved the nutrient and hormone specificity of the anthurium hybrids for callus induction and proliferation. The calli obtained from the callus initiation medium were subcultured for further proliferation in the same medium itself. A two fold increase in size of compact, creamy and pale yellow coloured callus was attained after a period of six to eight weeks. Similar callus multiplication responses were reported by Bejoy *et al.* (2008), Thokchom and Maitra (2017) and Prakash *et al.* (2017).

5.2.3 Shoot Regeneration and Proliferation

The dediffentiated calli can be directed towards organogenetic response such as shoot induction by subculturing the callus on an auxin free shoot regeneration medium. For inducing organogenesis, the callus was subjected to a photoperiod of 16 hours light and eight hours dark in the culture room as the light period will facilitate the pigment in the tissues absorb radiations of a particular wavelength and thereby induce photomorphogenesis. The pronounced effect of

light period on induction of multiple shoots were supported by the findings of Lan *et al.* (2003), Bejoy *et al.* (2008) and Chen *et al.* (2013). In the present study, initial development of meristamoids was marked by greening of pale yellow callus followed by the development of greenish pink protuberances on the callus surface after two months of culture on regeneration medium. These protuberances gave rise to small shoots.

The present study analysed the effect of various concentrations of BA on shoot induction response in anthurium hybrids. All the six hybrids exhibited the highest shoot induction percentage and the fastest shooting response on modified half strength MS medium supplemented with 0.5 mg L^{-1} BA. Similarly a high frequency of shoot regeneration was observed by Yakandawala *et al.* (2000) in *Anthurium andreanum* variety Avo Nette cultured on modified MS medium supplemented with 0.5 mg L^{-1} BA. The mean number of days for regeneration varied from 62.20 to 77.25 days among these hybrids. The highest shoot induction percentage of 87.5 percent was recorded for the hybrids OG x NO and HoR x KR in two months of culture. Emergence of first leaf was also the fastest for the same treatment in all the hybrids except HR x LR. Initial leaves appeared to be pale brown in colour which later changed to dark green. In all the six hybrids, development of shoots and formation of leaves were found to follow the same trend as that of shoot initiation. Similar time duration for shooting response was reported by Bejoy *et al.* (2008).

Slower callusing response was also found to occur when the same basal medium was supplemented with 0.3 and 0.8 mg L^{-1} BA. In half strength MS medium supplemented with 0.3 mg L^{-1} BA, the average number of days to emergence of first leaf ranged from 81.67 days for the hybrid HR x LR to 102 days for the hybrid LJ x OG. Bejoy *et al.* (2008) also observed that half strength MS medium supplemented with 0.3 mg L^{-1} BA was found to be optimum for shooting response in anthurium cultivar Agnihothri. Similar findings were also made by Bhavana (2018).

Low shooting response was also observed in the media supplemented with 0.1 and 1.0 mg L^{-1} BA. Only the genotype OG x NO showed shooting response when modified half strength MS medium was supplemented with 0.1 mg L^{-1} BA. In a study by Liendo and Mogollon (2009), an average of 4.17 shoots per explant was observed on MS medium supplemented with 1.0 mg L^{-1} 6-BAP. Paola *et al.,* (2014) reported improved shooting response with 1.0 mg L^{-1} BA in half strength MS medium. The variation in shooting response was attributed to the genotypic difference among the anthurium hybrids.

Shoot proliferation was observed on modified half strength MS media supplemented with 0.3 to 0.5 mg L^{-1} BA and development of more number of shoots occurred two to three weeks after shoot initiation. Multiple shoot induction and proliferation were reported by Dhananjaya and Sulladmath (2003), Martin *et al.* (2003), Bakhsi-Khaniki *et al.* (2011) and Thokchom and Maitra (2017) on half strength and full strength MS medium in presence of higher levels of BA in combination with low concentrations of auxins. In the current study, comparable multiple shoot induction was attained with 0.3 to 0.5 mg L^{-1} BA concentrations in modified half strength MS medium and this in turn reduced the use of high cost plant growth regulators.

5.2.4 Root Initiation and Proliferation

Shooting response was followed by rooting response during anthurium tissue culture in the same regeneration medium. Pale yellowish roots develop near the base of shoots from the callus. Though days to root initiation was longer than days to shoot initiation, an increase in rate of root development was observed in the later stages of development. All the hybrids that initiated shoots showed rooting response irrespective of the regeneration medium used. Average number of days to root generation followed similar pattern as that of shoot induction and ranged from 63.20 days to 93.00 days among the hybrids.

Half strength MS medium supplemented with 0.5 mg L^{-1} BA was observed to show the best rooting response. The fastest and the maximum root initiation

percentage of 87.5 per cent was observed for the genotype OG x NO and HoR x KR followed by 62.5 per cent for the hybrid HR x MR and PR x HR in the regeneration medium supplemented with 0.5 mg L^{-1} BA. Compared to other treatments with various levels of BA, the genotype LG x OG showed the fastest rooting response in the same basal medium supplemented with 0.3 mg L^{-1} BA. The most delayed root generation response was observed on basal medium supplemented with 1.0 mg L^{-1} BA for all the hybrids except LJ x OG and PR x HR. Thus, an increase in concentration of BA beyond 0.5 mg L^{-1} in the regeneration medium was found to delay the root generation response in the anthurium hybrids. Alex (2006), Jeshima (2007) and Paola *et al.* (2014) also reported that separate rooting medium was not necessary for *in vitro* mass multiplication in anthurium. According to Bhavana (2018), the highest root initiation occurred in the medium supplemented with 1.3 µM 6-BAP which was a much high cytokinin concentration and this was contradictory to the findings of the present study. Liendo and Mogollon (2009) opined that anthurium cultures does not require any phytohormones for root development.

Auxins such as indole acetic acid (IAA), indole butyric acid (IBA) and naphthalene acetic acid (NAA) were reported to have significant positive impact on root development in anthurium based on studies conducted by Mohanta and Paswan (2001), Zhang *et al.* (2001), Dhananjaya and Sulladmath (2003), Bejoy *et al.* (2008) and Thokchom and Maitra (2017). However, the use of auxin supplements and labour intensive subculturing of the cultures could be avoided as adequate rooting was obtained in the shoot regeneration medium itself.

The present investigation was successful in generating a tissue culture protocol for anthurium hybrids eliminating the requirement of special rooting medium for root initiation and proliferation. Thus the current *in vitro* multiplication protocol for anthurium hybrids less labour intensive and cost effective than most of the existing tissue culture protocols for anthurium.

5.2.5 Hardening and Acclimatization

The *in vitro* developed plantlets with more than 2.5 to 3.0 cm length, three to four leaves and more than two roots, after five months of culture in the regeneration medium were taken for hardening. Similar criteria were followed to obtain healthy hardened plants in studies conducted by Ajithkumar and Nair (1998) and Dhananjaya and Sulladmath (2003). Plantlets from the culture bottles were taken out and the adhering culture medium was washed off in running water. These were then planted on hardening medium containing sterilized river sand and charcoal in the ratio 3:1 as recommended by Bejoy *et al.* (2008). Later plantlets were kept for hardening in the mist chamber.

Summary

6. SUMMARY

The present study entitled "Genotypic evaluation and *in vitro* multiplication of anthurium (*Anthurium andreanum* Linden) hybrids" was carried out at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2017-19. The study was undertaken to evaluate anthurium hybrids for commercial qualities and to address their mass multiplication through *in vitro* techniques. The investigation consisted of two experiments. Experiment I involved the evaluation of twenty hybrid genotypes of anthurium for commercial qualities and selection of six superior hybrids with desirable characters. Experiment II involved the development of specific *in vitro* tissue culture protocols for mass multiplication of the selected superior hybrids.

For variability analysis, 20 *Anthurium andreanum* Linden hybrid genotypes maintained at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani were utilized. The selected hybrids were evaluated in completely randomized design with five replications. Observations on vegetative and floral quantitative traits as well as qualitative traits were documented. Analysis of variance showed significant variation among the 20 anthurium hybrids for quantitative characters.

Among the 20 hybrids, plant height ranged from 62.2 cm in HR x KR to 33.5 cm in the hybrid LR x OG. The hybrid HoR x KR had the maximum leaf area and the lowest was recorded for the hybrid PR x HR. The leaves were generally narrow or elongated type. Desirable medium sized leaves were observed in the hybrids LJ x OG, OG x NO, HR x LJ, HR x DT, HR x LR and PR x LR. Internodal length was the shortest in HR x MR while the maximum internode length was recorded in HR x KR. Days from emergence to maturity of leaves ranged from 31.4 days in the hybrid HR x KR to 18.8 days in the hybrid LR x DT. The hybrid HR x MR had the maximum number of leaves spadices^{-1} plant^{-1} year^{-1} while the least was recorded in the hybrid HR x P and LR x OG.

In the present study sucker production was found to be the maximum in hybrid OG x NO and no suckers were produced by the hybrids namely HR x LJ, HR x P and PR x DT (1). The maximum mean number of days from emergence to maturity of inflorescence was recorded in the hybrids HR x LJ and PR x LR while the minimum was recorded for the hybrid HR x LR.

The smallest spathe size among the 20 anthurium hybrid genotypes was recorded in HR x MR which was on par with HR x LJ, DT x HR, P x LR, LG x OG, PR x HR and KR x LR. The hybrids namely KR x LR, OG x DT, LJ x OG, DT x HR, HR x MR, CR x KR, PR x HR, PR x DT (1) and PR x LR had ideal short candles. The least number of flowers spadix^{-1} was recorded in the hybrid HR x MR while HR x KR recorded the highest which was not commercially preferred. The life of spadix from emergence to senescence varied from 58.8 days in the hybrid HR x P to 95.0 days in OG x NO which was on par with HoR x KR (90.2 days).

Days to initiation of female phase from the base of the spadix in anthurium flowers was observed to be the slowest for the hybrid HoR x KR and the fastest for HR x KR, CR x KR and HR x LR. Duration of female phase also varied widely among the hybrids with HR x P (10.4 days) showing the longest duration and HR x MR (5.4 days) the shortest. Initiation of interphase, marked by the drying up of stigmatic surface was the longest for the hybrid PR x DT while the shortest was recorded in HoR x KR, HR x DT, CR x KR, HR x LR, LR x OG and DT x HR. Male phase indicated by extrusion of anther was the longest in the hybrid HR x LJ and the shortest in PR x LR.

Ideal anthurium genotypes with inclination of candle to the spathe less than 45° was observed in the hybrids P x LR, PR x DT (1), DT x HR, OD x NO, PR x DT and HR x MR. The total anthocyanin content showed wide variation among the anthurium hybrids ranging from from 36.184 mg g^{-1} in the hybrid LR x OG to 326.434 mg g^{-1} in LR x DT.

The longest post-harvest vase life was observed for HoR x KR (24.4 days) which was on par with LJ x OG (23.4 days). Number of inflorescence year^{-1} or the flower yield in anthurium was observed to be the highest in the hybrid HR x MR (6.4) while the minimum flower yield was observed in CR x KR (2.2).

The qualitative characters in anthurium such as colour of petiole, young leaves, spathe colour, spadix colour, type of inflorescence axis, pollen emergence pattern, pollen shape and pollen colour were evaluated for the 20 hybrids. Wide variations in spathe colour such as dark red, red, light red, orange, light orange, light pink and pinkish white colours were recorded among the hybrids. Variations in spadix colour such as yellowish red, whitish red, greenish red, whitish yellow, yellowish green, yellowish white, reddish white, and white were exhibited by the hybrids.

The inflorescence axis of the hybrids were long curved thick, long straight thick, medium straight thin, medium curved thin, medium curved thick, short curved thick and short curved thin type. Predominant round shaped pollen was reported in all the hybrids and only the hybrids LJ x OG, HR x LJ and OG x DT had oval shaped pollen. The pollen colour of the hybrids LJ x OG and HoR x KR was cream while all the other hybrids had white pollen. Pollen emergence in anthurium was highly influenced by seasonal variations and was found to be higher in winter months and lower during summer months.

The components of variation namely genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were analysed. High PCV and GCV were observed for the characters number of suckers plant^{-1}, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size, leaf area, number of flowers spadix^{-1}, spadix length, duration of interphase, inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1}. Thus, selection for these characters would result in improvement of the genotype.

High heritability coupled with high genetic advance were observed for plant height, leaf area, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size,

spadix length, number of flowers spadix^{-1}, days to initiation of female phase, duration of female phase, inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1}. This indicated that expression of these traits were controlled by additive gene action and improvement could be achieved for these traits by direct phenotypic selection.

Correlation analysis with genotypic correlation coefficients revealed significant positive correlation of number of inflorescence year^{-1} with characters such as number of leaves spadices^{-1} plant^{-1} year^{-1}, number of suckers plant^{-1} and vase life. An improvement in positively correlated characters would enhance the number of inflorescence year^{-1}. Life of spadix in anthurium hybrids had significant positive correlation with traits such as plant height, leaf area, internode length, spathe size, spadix length, number of flowers spadix^{-1}, days to initiation of female phase, duration of female phase, duration of interphase, duration of male phase and vase life. Post-harvest vase life showed a positive and significant correlation with traits such as plant height, spathe size, spadix length, number of leaves spadices^{-1} plant^{-1} year^{-1}, number of flowers spadix^{-1}, life of spadix, duration of male phase, duration of female phase and number of inflorescence year^{-1}.

Path coefficients were worked out with number of inflorescence year^{-1} as the dependent variable and other characters as component variables. The three characters namely number of leaves spadices^{-1} plant^{-1} year^{-1}, number of suckers plant^{-1} and vase life were found to have direct effect and significant positive correlation towards flower yield. This indicated that selection for the above traits will result in improvement of flower yield in anthurium hybrids. Path analysis revealed that 70.35 per cent of the variation in flower yield in anthurium hybrids were attributed by the quantitative traits considered in the current study. About 29.65 per cent of the variation in flower yield was due to the effect of characters that are not considered in the study and the environment.

From experiment I, six hybrid genotypes namely HR x MR, LJ x OG, OG x NO, HoR x KR, PR x HR and HR x LR with superior flower yield attributing

traits and commercial qualitative characters were selected for *in vitro* mass multiplication study. For *in vitro* culture, pale greenish brown young leaf lamina, 5 to 10 days after unfolding of leaf, collected from healthy and mature plants were used as explant. Proper control measures were taken for control of bacterial blight and anthracnose diseases so as to obtain disease free explants. Leaf lamina explant of 1 to 1.5 cm^2 were used for inoculation in the culture medium

Surface sterilization of leaf explants with 5.0 per cent sodium hypochlorite for 10 minutes followed by 0.1 per cent mercuric chloride for five minutes was found to be the best and resulted in 87.5 per cent explant survival. For all the hybrids the highest callus induction percentage was recorded in modified half strength MS medium supplemented with 200 mg L^{-1} NH_4NO_3 + 1.0 mg L^{-1} BA + 0.5 mg L^{-1} 2,4 D + 30 g L^{-1} sucrose + 6.0 g L^{-1} agar. Faster proliferation of callus may be due to the presence of auxins in the culture medium. The best differentiation response of 50.0 per cent was recorded for the hybrids HR x MR and OG x NO in nine weeks of culture in the same medium while the hybrids LJ x OG and HoR x KR showed slower callusing response. The present study also proved the nutrient and hormone specificity of the anthurium hybrids for callus induction and proliferation. The explants were cultured in darkness for callus induction and the calli were subcultured for further proliferation on the same medium itself. A two fold increase in size of compact, creamy and pale yellow coloured callus was attained after a period of six to eight weeks.

For shoot regeneration, the multiplied callus was subcultured to regeneration medium and a photoperiod of 16 hours light and eight hours dark was provided. Of the various regeneration treatments, half strength MS medium supplemented with 0.5 mg L^{-1} BA showed shoot initiation response ranging from 50.0 (LJ x OG and HR x LR) to 87.5 (OG x NO and HoR x KR) per cent among the hybrids. The fastest shoot regeneration was observed for the hybrid HR x MR (62.20 days) and the slowest for LJ x OG (77.25 days). Slower callusing response was also found to occur when the same basal medium was supplemented with 0.3 and 0.8 mg L^{-1} BA. Only the genotype OG x NO showed shooting response when

modified half strength MS medium was supplemented with 0.1 mg L^{-1} BA. Shoot proliferation was observed on modified half strength MS media supplemented with 0.3 to 0.5 mg L^{-1} BA and development of more number of shoots occurred two to three weeks after shoot initiation.

Shooting response was followed by rooting response in all the hybrids in the same regeneration medium. All the hybrids that initiated shoots showed rooting response irrespective of the regeneration medium used. Half strength MS medium supplemented with 0.5 mg L^{-1} BA was observed to show the best rooting response. The fastest and the maximum root initiation percentage of 87.5 per cent was observed for the genotype OG x NO and HoR x KR followed by 62.5 per cent for the hybrid HR x MR and PR x HR in the regeneration medium supplemented with 0.5 mg L^{-1} BA. Compared to other treatments with various levels of BA, the genotype LG x OG showed the fastest rooting response in the same basal medium supplemented with 0.3 mg L^{-1} BA. An increase in concentration of BA beyond 0.5 mg L^{-1} in the regeneration medium was found to delay the root generation response in the anthurium hybrids.

The present investigation was successful in generating a tissue culture protocol for anthurium hybrids eliminating the requirement of special rooting medium for root initiation and proliferation. Thus the current *in vitro* multiplication protocol for anthurium hybrids less labour intensive and cost effective than most of the existing tissue culture protocols for anthurium. The *in vitro* developed plantlets with more than 2.5 to 3.0 cm length, three to four leaves and more than two roots, after five months of culture in the regeneration medium were taken for hardening. The plantlets were planted on hardening medium containing sterilized fine river sand and charcoal in the ratio 3:1 and kept for hardening in the mist chamber. Large scale multiplication of the superior hybrids and profit generation can be achieved through the tissue culture protocol that was standardized in the present study. The promising commercially superior anthurium hybrids identified in the study can be used in further crop improvement programmes.

References

7. REFERENCES

Abdussammed, K.P. 1999. Regulation of flowering and post harvest behaviour of *Anthurium andreanum* cv. 'Hawaiian Red'. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 135p.

Agasimani, A.D., Harish, D.K., Imamsaheb, S.J. and Patil, V.S. 2011a. Anthurium varieties performance in rainy and winter seasons under greenhouse. *Res. J. Agric. Sci.* 2: 337-339.

Agasimani, A.D., Harish, D.K., Imamsaheb, S.J. Patil, V.S., Kamati, C. and Preveenkumar, D.A. 2011b. Anthurium varieties performance and economics under greenhouse. *Res. J. Agric. Sci.* 2: 226-229.

Agasimani, A.D., Patil, V.S., Patil, A.A., Basavaraj, B., Uppar, D.S., Patil, B.C. and Biradar, M.S., 2010. Performance of anthurium varieties under greenhouse. *Karnataka J. Agric. Sci.* 23(3): 540-541.

Ajithkumar, P.V. and Nair, S.R. 1998. Establishment and hardening of *in vitro* derived plantlets of *Anthurium andreanum*. *J. Ornmental Hortic.* 4: 9-12.

Alex, J. 2006. *In vitro* studies on selected genotypes in *Anthurium adreanum* Linden. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 69p.

Allard, R.W. 1960. *Principles of Plant Breeding.* John Wiley and Sons, Inc., New York, 485p.

Anand, M., Sankari, A. and Arulmozhiyan, R. 2013. *Per se* performance of Anthurium (*Anthurium andraeanum* Linden Ex André) cultivars for yield and quality under shevaroys condition -Eastern Ghat.M. *Asian J. Hortic.* 8(2): 625-630.

Anand, M., Sankari, A., Arulmozhiyan, R. and Kayalvizhi, K. 2017. Research Article Evaluation of different varieties of anthurium (*Anthurium andraeanum* Linden Ex André) for cut flower product ion under Shevaroys Hills. *Electr. J. Plant Breed.* *8*(3): 792-798.

Asaduzzaman, M. and Asao, T. 2012. Autotoxicity in beans and their allelochemicals. *Sci. Hortic.* 134(1): 26-31.

Asish, K.B. 2002. Genetic variability and character association in *Anthurium andreanum* Linden. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 115p.

Asish, K.B. and Mayadevi, P. 2006. Anthocyanins affecting differential spathe colour expression in diverse genotypes of *Anthurium andreanum* Linden. *Indian J. Genet.* 66: 69-70.

Atak, C. and Celik, O. 2009. Micropropagation of *Anthurium andraeanum* from leaf explants. *Pakist. J. Bot.* 41(3): 1155-1161.

Bakhsi-Khaniki, G., Ghasemi, M and Bairamizadeh, E. 2011. Study of micropropagation of Anthurium using tissue culture. *New Cell Mol. Biotechnol. J.* 1(4): 79–87.

Bejoy, M., Sumitha, V. R., and Anish, N. P. 2008. Foliar regeneration in *Anthurium andreanum* Hort. cv. *Agnihothri. Biotechnol.* 7 (1): 134–138.

Bhattacharya, C., Dam, A., Karmakar, J. and Bandyopadhyay, T.K. 2015. Efficient organogenesis from the induced meristemoid of *Anthurium andraeanum* Linden cv. Tinora. *Plant Sci. Today*, 2(2): 82-86.

Bhavana, G.P., Satyan, K.B. and Aswath, C. 2018. A regenerative protocol and SEM study for *in vitro* propagation of Anthurium crossed lines via indirect somatic embryogenesis. *Biosci. Biotechnol. Res. Commun.* 11(1): 31-40.

Cabral, P.D.S., Soares, T.C.B., Gonçalves, L.S.A., Amaral Júnior, A.T.D., Lima, A.B.P., Rodrigues, R. and Matta, F.D.P. 2010. Quantification of the diversity among common bean accessions using Ward-MLM strategy. *Pesquisa Agropecuária Brasileira*, 45(10): 1124-1132.

Cai, W.F. 2002. Tissue culture of *Anthurium scherzerianum*. *Subtrop. Plant Sci.* 31(3): 66–68.

Chen, F.C., Kuehnle, A.R. and Sugii, N. 1997. Anthurium roots for Micropropagation and Agrobacterium-mediated gene transfer. *Plant Cell Tissue Organ Cult.* 49: 71–74.

Chen, Y., Wang, Z., Ji, S.Y., He, S.L., and Xia, Y.L. 2013. Effects of different light quality ratios of light emitting diode (LED) on the growth of *Anthurium andraeanum* plantlets *in vitro*. *Acta Agric. Univ. Zhejiangensis.* 35(2): 375–380.

Cristiano, G., Talia, M.A.C., Mustich, M. and Sancilio, A. 2007. Evaluation of Anthurium cultivars in soilless culture. *Colture Protette,* 36 (12): 99-102.

Croat, T.B. and Bunting, G.S. 1978. Standardisation of Anthurium descriptions. *Aroideana*. 2: 5-25.

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

Cui, Y.L., Lu, Q.Y. and Xu, Y.C. 2007. Study on the building technique of *in vitro* sterility culture system of *Anthurium andraeanum*. *Shandong Agric. Sci.* 2: 28-36.

Cuquel, F.L., Polack, S.W., Favaretto, N. and Possamai, J.C. 2012. Fertigation and growing media for production of anthurium cut flower. *Horticultura Brasileira,* 30: 279-285.

Dewey, D.R. and Lu, L.H. 1959. A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agron. J.* 51: 515-518.

Dhaduk, B.K., Kumari, A. and Desai, J. R. 2007. Response of gibberellic acid on growth and flowering attributes in anthurium (*Anthurium andreanum* Lind.). *J. Ornamental Hortic.* 10(3): 187-189.

Dhananjaya, M.V. and Sulladmath, V.V. 2003. Callus mediated regeneration from petiole explants of *Anthurium andreanum* cv. Singapore hybrid. *J. Ornamental Hortic.* 6: 217-221.

dos Santos, M.A., Timbó, A.D.O., de Carvalho, A.C.P.P. and Morais, J.P.S. 2005. Callus induction and plant regeneration from *Anthurium andraeanum* Lindl. fruits. *Plant Cell Cult. Micropropagation*, 1(2): 77-79.

Duan, P.H., Li, X.Z., Wang, Y.S. 2009. Study on relative factors of the callus differen-tiation culture of *Anthurium andraeanum*. *Chinese. Agric. Sci. Bull.* 25 (24): 341-343.

Dufour, L. and Guerin, V. 2005. Nutrient solution effects on the development and yield of *Anthurium andreanum* Lind. In tropical soilless conditions. *Scientia Hortic.* 105: 269-282.

Ehrenberger, J.A., Kuehnle, A.R. and Amore, T.D. 2003. *Evaluations of University of Hawaii Anthurium Accessions 1986-2001.* Cooperative Extension Service, College of Tropical Agriculture & Human Resources, University of Hawaii at Manoa. 20p.

Elibox, W. and Umaharan, P. 2012. A Study of Morphophysiological Descriptors of Cultivated *Anthurium andraeanum* Hort. *Hortic. Sci.* 47(9): 1234-1240.

Farsi, M., Taghavizadeh Yazdi, M. E. and Qasemiomran, V. 2012. Micropropagation of *Anthurium andreanum* cv. *Terra. Afr. J. Biotechnol.* 11(68): 13162-13166.

Femina, V. and Rajeevan, P.K. 2006. Performance of anthurium (Anthurium *andreanum* Lind.) cultivars under different systems of growing in humid tropical plains. *J. Ornamental Hortic.* 9(4): 274-277.

Gantait, S., Mandal, N., Bhattacharyya, S., and Das, P. K. 2008. *In vitro* Mass Multiplication with pure genetic identity in *Anthurium andreanum* Lind. *Plant Tissue Cult. Biotech.* 18: 113-122.

Gantait, S. and Mandal, N. 2010. Tissue culture of *Anthurium andreanum*: A significant review and future prospective. *İnt. J. Bot.* 6(3): 207-219.

Geier, T. 1990. Anthurium. *Hand book of Plant Cell Cultures: 4. Ornamental Species.* (eds. Ammirato, P.V., Evans, D.A., Sharp, W.R. and Bajaj, Y.P.S.), Mc Graw-Hill Publishing Co., New York. 228-252.

Geier, T. and Reuther, G. 1981. Vegetative Vermehrung plants of *Anthurium scherzerianum* Durch Gewebekultur. *Zierpflanzenbau,* 21: 476-477.

George, E.F. and Sherrington, P.D. 1984. *Plant Propagation by Tissue Culture, Handbook and Directory of Commercial Laboratories*, Eastern Press, Reading Berks, Great Britian. 10-22.

George, E.F. and Debergh, P.C. 2008. Micropropagation: uses and methods. In: George, E. F., Hall, M. A., and de Klerk, G. J. (eds), *Plant Propagation by Tissue Culture* (3rd ed.). Springer, Dordrecht, Netherlands, pp. 29-64.

Gopi, R. 2016. Varietal evaluation and genetic improvement of anthurium (*Anthurium adreanum* Linden) through hybridization. M. Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 55p.

Gopi, R., Thomas, B., and Sathishkumar, S. 2016. Genetic variability among diverse genotypes of *Anthurium andreanum* Linden. *Adv. Life Sci.* 5(12): 4886-4888.

Gu, A., Liu, W. F., Ma, C., Cui, J., Henny, R. J., and Chen, J. J. 2012. Regeneration of *Anthurium andraeanum* from leaf explants and evaluation of microcutting rooting and growth under different light qualities. *Hortic. Sci.* 47: 88-92.

Harishshivalingappa, Reddy, B.S., Yathisha, S.M., Nataraj, M., Unikrishnappa, S.K. and Rabuling, P. 2013. Effect of holding solutions with different combinations of chemical on post harvest physiology of anthurium (*Anthurium adreanum* L.) cv. Tropical. *Int. J. Proc. Post Harvest Technol.* 4(2): 114-117.

Ho, L.C. and Nichols, R. 1975. The role of phloem transport in the translocation of sucrose along the stem of carnation cut flowers. *Ann. Bot.* 39(3): 439-446.

Hu, C.Y. and Wang, P.J. 1983. Meristem, shoottip and bud cultures. In: *Handbook of Plant Cell Cultures*: 1. *Techniques for propagation and breeding*. (eds.

Evans. D.A., Sharp, W.R., Ammirato, P.V. and Yamada, Y.). Macmillan Publishing Co., New York, 177-277.

Islam, M.S., Mehraj, H., Roni, M.Z.K., Shahrin, S. and Jamaluddin, A.F.M., 2013. Varietal study of anthurium (*Anthurium andreanum*) as a cut flower in Bangladesh. *J. Bangladesh Acad. Sci.* 37: 103-107.

Iwata, R.Y., Tang, C.S. and Kamemoto, H. 1979. Anthocyanins of *Anthurium andreanum.* Lind. *J. Am. Hortic. Sci.* 104: 464-466.

Jadhav, G., Ambad, S.N., Hongal, S. and Hiremath, V. 2012. Effect of different levels of fertigation on performance of cultivars of Anthurium. *Asian J. Hortic.* 7(2): 276-280.

Jahan, M.T., Islam, M.R., Khan, R., Mamun, A.F.K., Ahmed, G. and Hakim, L. 2009. *In vitro* clonalpropagations of Anthurium (*Anthurium andreanum* L.) using callus culture. *Plant Tissue Culture Biotechnol.* 19(1):61-69.

Jain, J.P. 1982. *Statastical Techniques in Quantitative Genetics*. Tata McGraw Hill Co., New Delhi, 281p.

Jeshima, K.Y. 2007. *In vitro* multiplication and DNA fingerprinting of selected hybrids and their parents in *Anthurium andreanum* Linden. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 117p.

Jiang, L., Lan, T.W., Li, Y.H., Yi, M.S., Liang, C.H., and Zhang, Z.S. 2006. Factors influencing callus induction, proliferation and bud differentiation of *Anthurium andraeanum* Lind. *Seed*, 25(11): 26–30.

Kamemoto, H. 1962. Some factors affecting the keeping quality of anthurium flowers. *Hawaii Farm Sci.* 11(4): 204.

Kamemoto, H., Marutani, M. and Wannnakrairoj, S. 1988. Chromosome studies on anthurium amnicola and its hybrids. *Aroideana,* 11: 9-14.

KAU. 2007. *Package of Practices Recommendations 'Crops'*. Thirteenth edition. Directorate of Extension, Kerala Agricultural University, Thrissur, 278 p.

Keshav, K. and Prashant, D. 2008. Effect of substrates on anthurium culture. *Asian J. Hortic.* 3(1): 165-166.

Kuehnle, A.R. and Sugii, N. 1991. Callus induction and plantlet regeneration in tissue cultures of Hawaiian anthuriums. *Hortic. Sci.* 26(7): 919-921.

Kuehnle, A.R., Chen, F.C. and Sugii, N. 1992. Somatic embryogenesis and plant regeneration in *Anthurium andraeanum* L. hybrids. *Plant Cell Rep.* 11: 438-442.

Kunisaki, J.T. 1980. *In vitro* propagation of *Anthurium andreanum* Lind. *Hortic. Sci.* 15: 508-509.

Lan, Q.Y., Li, Q.R., He, H.Y., Zhang, Y.J., and Xie, X.Y. 2003. The callus induction of *Anthurium andraeanum* Linden and bud differentiation. *Acta Hortic. Sinica*, 30(1): 107-109.

Leffering, L., Hoogstrate, J. and Braster, M. 1976. Tissue culture of anthuriums: research into improved methods. *Vakblad Bloemisterij,* 31: 17p.

Liendo, M. and Mogollon, N. 2009. Multiplicacion clonal *In vitro* delanturio (*Anthurium andreanum* Lind. cv. Nicoya). *Bioagro,* 21: 179-182.

Lima, J.D., Ansante, N.F., Nomura, E.S., Fuzitani, E.J. and Silva, S.H.M.G.D. 2014. Growth and yield of anthurium in response to gibberellic acid. *Ciência Rural*, 44(8): 1327-1333.

Liu, G.F., Zhao, Q.Q., and Bao, M.Z. 2009. Factors affecting callus induction and plant regeneration from leaf explants of pot anthurium (*Anthurium andraeanum* Lind.). *J. Huazhong Agric. Univ.* 28(3): 356-360.

Madhukumar, K. 2010. Cross compatibility analysis and production of hybrids in *Anthurium andreanum* Linden. Ph.D. thesis, Kerala Agricultural University, Thrissur, 201p.

Mahanta, S. and Paswan, L. 2001. In-Vitro propagation of Anthurium from axillary buds. *J. Ornamental Hortic.* 4(1): 17-21.

Maira, O., Alexander, M. and Vargas, T.E. 2010. Micropropagation and organogenesis of *Anthurium andreanum* Lind cv Rubrun. In: *Protocols for In Vitro Propagation of Ornamental Plants*, Humana Press Edition, 3-14.

Maitra, S., Ghosh, P.D., Roychowdhury, N., and Satya, P. 2012. Effect of culture media on *in-vitro* regeneration of anthurium (*Anthurium andraeanum* Lind.) from axillary bud explants. *Int. J. Bio-Res. Stress Manag.* 3(1): 35–39.

Martin, K.P., Joseph, D., Madassery, J. and Philip, V.J. 2003. Direct shoot regeneration from lamina explants of two commercial cutflower cultivars of *Anthurium andreanum. Hort. In vitro Cellular Dev. Biol. Plant.* 39: 500-504.

Mayadevi, P. 2001. Genetic divergence in *Anthurium andreanum* Linden. Ph.D. thesis, Kerala Agricultural University, Thrissur, 242p.

Mercy, S.T. and Dale, B. 1994. *Anthurium.* St. Joseph Press, Thiruvananthapuram, 64p.

Miller, P.A., Williams, V.C., Robinson, H.P. and Comstock, R.E. 1958. Estimates of genotypic and environmental variances and co-variances in upland cotton and their implication in selection. *Agron. J.* 5: 126-131.

Mohanta, S. and Paswan, L. 2001. *In vitro* propagation of anthurium from axillary buds. *J. Ornamental Hortic.* 4: 17-21.

Mujaffar, S. and Sankat, C.K., 1993. Effect of waxing on the water balance and keeping qualities of cut anthuriums. *Int. Agrophysics*, 17(2): 77-84.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15: 179-473.

Nirmala, K.S and Singh, F. 1993. Micropropogation of *Anthurium andreanum* Lind. *Golden Jubilee symp. Abstr.*, Indian Institute of Horticultural Research, Banglore, 78p.

Panse, V.G. and Sukhathme, P.V. 1985. *Statistical Methods for Agricultural Workers* (2nd Ed.). Indian Council of Agricultural Research, New Delhi, p.381.

Paola A., Murillo-Gomez, P.A., Naranjo, E., Callejas, R., Atehortua, L. and Urrea, A. 2014. Micropropagation of the native species *Anthurium antioquiense* for conservation purposes. *Agronomia Colombiana,* 32(3): 334-340.

Paswan, L. and Mahanta, S. 2001. Effect of growth regulators on germination of *Anthurium andreanum* seeds under *in vitro* conditions. *J. Agric. Sci. Soc. N. E. India,* 14: 274-277.

Pierik, R.L.M., Steegmans, H.H.M., and Van der Meys, J.A.J. 1974a. Plantlet formation in callus tissues of *Anthurium andreanum* Lind. *Sci. Hortic.* 2: 193-198.

Pierik, R.L.M., Van der Meys, J.A.J. and Steegmans, H.H.M. 1974b. Vegetative propagation of *Anthurium andreanum* in propagating tubes. *Vakblad Bloemisterij*, 29: 12-15.

Pierik, R.L.M. 1975. Callus multiplication of *Anthurium andreanum* Lind. in liquid media. *Neth. J. Agric. Sci.* 23: 299-302.

Pierik, R.L.M., Van Leeuwen, P. and Rigter, G.C.C.M. 1979. Regeneration of leaf explants of *Anthurium andreanum* Lind. *In vitro. Neth. J. Agric. Sci.* 27: 221-226.

Prakash, D., Choudhary, M.L., Prasad, K.V. and Nagesh, N. 2001. Regeneration of plants from petiole explants of *Anthurium andreanum* Lind. cv "Mauritius Orange". *Phytomorphology* 51: 83-85.

Prakash, D., Sujatha, K. and Sangma. 2006. Anthurium. In: Bhattacharyam, S.K (Ed.) *Advances in Ornamental Horticulture*, Pointer Publishers, Jaipur. pp. 109-129.

Prakash, D. P., Ramya, G and Srinivasalu, G. B., 2017. Morphogenetic responses in *Anthurium andreanum* (Hort.) cultivars. *Curr. Agric. Res. J.* 5(1): 134-140.

Praneetha, S., Jawaharlal, M. and Vijayakumar, M. 2002. Performance of anthuriums under shade net condition at Yercaud. In: Misra, R.L. and Misra, S. (eds), *Proceedings of the National Symposium on Indian Floriculture in the New Millennium*, 12-14 November 2002, Indian Society of Ornamental Horticulture, LalBagh, Bangalore, pp.328-329.

Prasad, K., Prakash, D., Choudary, M.L. and Pandey, R. 2001. The art of growing anthuriums. In: Choudhary, M.L., Singh, Kanwar, P. and Hussain, C.T.S. (eds). *Advances in floriculture*. Division of floriculture and Land scaping, IARI, New Delhi, pp.116-126.

Pravin, R.S. 2004. Genetic improvement of F_1 Hybrids in *Anthurium andreanum* Linden. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 117p.

Premna, V. 2003. Compatibility studies of three way crosses in *Anthurium andreanum* Linden. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 93p.

Puchooa, D. 2005. *In vitro* mutation breeding of anthurium by gamma radiation. *Int. J. agric. Biol.* 7: 11-20.

Rajasekaran, P. and Mohankumar, P. 1994. Somatic embryogenesis and *in vitro* plant development of *Anthurium andreanum* Lind [Abstract]. In: *Abstracts, First National Seminar on Anthuriums*, 3-4 March, 1994, Tropical Botanic Garden and Research Institute and Anthurium Growers Society, Trivandrum, p.29.

Rangana, S. 1977. *Manual of Analysis of Fruit and Vegetable Products.* Tata Mc Graw-Hill Pub. Co. Ltd., New Delhi, 634p.

Ravidas, L. 2003. Improvement of *Anthurium andreanum* Linden by *in vivo* and *in vitro* methods. Ph.D. (Ag.) thesis, Kerala Agricultural University, Thrissur, 163p.

Renu, R.S. 2000. Intervarietal hybridization in *Anthurium andreanum* Linden. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 119p.

Robinson, H.F., Comstock, R.E. and Harvey, P.H. 1949. Estimation of heritability and the degree of dominance in corn. *Agron. J.* 14: 352-359.

Sahare, H.A. and Alka, S. 2015. Effect of pulsing on post postharvest life and quality of cut anthurium flowers (*Anthurium andraeanum* L.) cv. Xavia. *Trends Biosci.* 8(2): 305-307.

Sathyanarayana, B.N. 2007. *Plant Tissue Culture: Practices and New Experimental Protocols*. I. K. International Pvt Ltd. 106p.

Sheela, V.L. 2008. *Flowers for Trade*. New India publishing agency, New Delhi, 379p.

Sheena, S. 2015. Performance analysis and combining ability studies in anthurium cultivars. Ph.D. (Ag.) thesis, Kerala Agricultural University, Thrissur, 119p.

Shiva, K.N. and Nair, S.A. 2008. Performance of anthurium cultivars in Andamans. *Indian J. Hortic.* 65(2): 180-183.

Shriram, N., Ambad, Anita, R., Shetye, M.T. and Patil, 2008. Varietal performance of anthurium (*Anthurium andreanum* L.) under cost effective Polyhouse. *Proc. Indian Hortic. Congress*, Bhubaneshwar, India, 360p.

Singh, F. 1987. Anthurium – Vyeing for a place among commercial flower crops. *Indian Hortic.* 4: 14-16.

Singh, F. 1994. New strategies in Tissue Culture propagation of *Anthurium andreanum*. [Abstract] In: *Abstracts, First National Seminar on Anthuriums*; 12-15 June, 1994, Tropical Botanic Garden and Research Institute and Anthurium Growers Society, Trivandrum, 54p.

Singh, R.K. and Chaudhary, B.D. 1977. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Pub., Ludhiana, 78p.

Skoog, F. and Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant tissues cultivated *in vitro*. In: Biological action of Growth Substances. *Symp. Soc. Exp. Biol.* 11: 118-131.

Sreelatha, U. 1992. Improvement of Propagation efficiency of *Anthurium* species *in vitro*. Ph.D. thesis, Kerala Agricultural University, Thiruvananthapuram, 124p.

Srinivasa, V. 2006a. Effect of fertilizers on growth and flowering of *Anthurium andreanum*. *Res. Crops*, 7 (1): 282-284.

Srinivasa, V. 2006b. Influence of shade on growth and flowering of *Anthurium andreanum*. *Environ. Ecol.* 24: 117-119.

Srinivasa, V. and Reddy, T.V. 2005. Evaluation of different varieties of anthurium under hill zone of Coorg District, Karnataka. *Mysore J. Agric. Sci.* 39(1): 70-73.

Stancato, G.C. and Tucci, M.L.S.A. 2010. Monitoring the end of the *in vitro* phase of *Anthurium andreanum* Lindl. plantlets. *Brazilian J. Plant Physiol.* 22(1): 61-68.

Talia, M.A.C., Cristiano, G. and Forleo, L.R. 2003. Evaluation of new anthurium cultivars in soilless culture. (eds. Malfa, G., Lipari, V., Noto, G. and Leonardi, C.). *Proceedings of the Sixth International Symposium on Protected Cultivation in Mild Winter Climate: Product and Process Innovation,* Ragusa Sicilia, Italy, 5-8 March, 2002, Acta Horticulturae, 1 (614): 223-226.

Tamuli, P.S., Talukdar, M.C. and Talukdar, P. 2015. Extent of genetic variation in Anthurium (*Anthurium andreanum* linden ex Andre) cultivars for growth, flowers and physiological characters under soil-less culture. *J. Agric. Vet. Sci. 8*(31): 07-10.

Thokchom, R. and Maitra, S., 2017. Micropropagation of *Anthurium andreanum* cv. Jewel from leaf explants. *J. Crop Weed*, 13(1): 23-27.

Thomas, T.D. 2008. The role of activated charcoal in plant tissue culture. *Biotechnol. Adv.* 26: 618-631.

Thorpe, T.A. 1980. Organogenesis *in vitro*: structural, physiological and biochemical aspects. *Int. Rev. Cytol.* 1A: 71-111.

Valsalakumari, P.K., Geetha, C.K., Musthafa, M.S., Rajeevan, P.K. and Abdussammed, K.P. 1998. Response of cutflowers of *Anthurium andreanum* Lind. to pulsing treatments[Abstract]. In: *Abstracts, national seminar on anthurium production*, 2-3 June, 1998, Indian Institute of Horticuture Research, Bangalore, p. 34. Abstract 36.

Vargas, T.E., Mejias, A., Oropeza, M. and De Garcia, E. 2004. Plant regeneration of *Anthurium andraeanum* cv. Rubrun. *Electron. J Biotechnol.* 7(3): 282-286.

Viégas, J., da Rocha, M.T.R., Ferreira-Moura, I., Corrêa, M.G.S., da Silva, J.B., dos San-tos, N.C. and Teixeira da Silva, J.A. 2007. *Anthurium andraeanum* (Linden ex André) culture: *in vitro* and *ex vitro*. *Floriculture Ornamental Biotechnol.* 1 (1): 61-65.

Waheeduzzama, M., Jawaharlal, M., Arulmozhiyan, R. and Indhumathi, K. 2007. Intergreated nutrient management practices to improve flower yield in anthurium (*Anthurium andreanum* Lind.). *J. Ornamental Hortic.* 10(1): 42-45.

Wainwright, H. 1988. Overcoming problems in establishing micropropogules-guidelines for growers. *Prog. Hort.* 2: 67-72.

Weiming, G., YunPeng, Z. and FangDe, W. 2004. Relative physiological and biochemical features of redifferentiation difference in three types of calli subculture in *Anthurium andreanum*. *Acta horticulturae Sinica*. 3: 69-72.

Williams, C.A., Harborne, J.B. and Mayo, S.J., 1981. Anthocyanin pigments and leaf flavonoids in the family Araceae. *Phytochemistry*, 20(2): 217-234.

Winarto, B., 2013. Effect of basic medium and ammonium nitrate on formation and regeneration of calli and shoot multiplication derived from anther culture of anthurium. *J. Hortic.* 23(1): 9-20.

Winarto, B., Mattjik, N.A., Silva, J.A.T.D., Purwito, A. and Marwoto, B. 2010. Ploidy screening of anthurium (*Anthurium andreanum* Linden ex André) regenerants derived from anther culture. *Scientia Horticulturae*, 127(1): 86-90.

Winarto, B. and da Silva, T.J.A. 2012. Influence of isolation technique of half-anthers and of initiation culture medium on callus induction and regeneration in *Anthurium andreanum*. *Plant Cell Tissue Organ Cult.* 110(3): 401–411.

Wright, S. 1954. The interpretation of multivariate systems. In: Kempthrone, O., Bancroft, T.A., Gowen, J.W. and Lush, J.L. (eds) *Statistics and Mathematics in Biology,* State University Press, Iowa, 11-33pp.

Wu, A.L., 2010. Tissue culture *in vitro* and rapid propagation of *Anthurium andreanum.Gemomics. Appl. Biol.* 29(1): 185-190.

Xia, S.Y., Mai, Y.L., Xu, J.Y., Zhang, W., Ti, Q., Lin, S.H., and Huang, W.X. 2005. Study on technique of improving leaf callus and buds differentiation and strengthen seedling in *Anthurium andraeanum. Chinese. Agric. Sci. Bull.* 21(2): 45-48.

Yakandawala, G., Peiris, S.E and Yakandawala, Y.L.N. 2000. Transient expression of *uida* reporter gene in regenerable callus tissues of *Anthurium andreanum* Lind. by Agrobacterium mediated transformation. *Tropical Agricultural Research*. Peradeniya, Sri Lanka, Post graduate Institute of Agriculture, University of Peradeniya. 12: 50-55.

Yang, Y.H., Chen, F.C. and Tsai, C.T. 2002. Effect of cytokinins on plant regeneration from *in vitro* lamina of Anthurium. *J. Chinese Soc. Hortic. Sci.* 48: 371-377.

Yang, X.L., Hou, Z.F., Ji, J., Gui, S.J., and Wang, G. 2008. Effect of culture medium and temperature on the ratio of callus of Anthurium leaf. *J. Shenyang Agric. Univ.* 39: 15-18.

Yao, Z., Ji, J., Wang, P., and Wang, G. 2006. Induction of callus and plantlet regeneration of *Anthurium andraeanum. J. Jilin Agric. Univ.* 28(1): 43-46.

Yu, Y.X., Liu, L., Liu, J.X., and Wang, J. 2009. Plant regeneration by callus-mediated protocorm-like body induction of *Anthurium andraeanum. Hortic. Agric. Sci. China.* 8(5): 572-577.

Yuan, L.W., Yuan, Z.F., Zhang, S.F., and Zhang, W. 2004. Tissue culture and rapid propagation of *Anthurium andraeanum. J. Xinyang Normal Univ.* 17(3): 338-340.

Zhang, G.H., Xu, B.Y., Peng, C.Z., and Lu, L. 2001. Shoot cutting tissue and propagation *in vitro* of *Anthurium andreanum* Lind. *Acta Agric. Shanghai,* 17: 13-16.

Zhao, Y., Guo, W., Wang, G. and Wen, F. 2004. Asceptic plantlet hardening of *Anthurium andreanum in vitro* culture. *Plant Physiol. Commun.* 40(1): 48-50.

Zhou, Y., Gao, Z., Gao, S., Sun, F., Cheng, P. and Li, F. 2012. *In vitro* adventitious shoot regeneration *via* indirect organogenesis from inflorescence explants and peroxidase involvement in morphogenesis of *Populus euphratica* Olivier. *Appl. Biochem. Biotechnol,* 168(8): 2067-2078.

Appendix

Appendix 1. Media composition for half strength Modified Murashige and Skoog medium

Chemical composition	Quantity in mgl^{-1}
KNO_3	950.00
$NH_4 NO_3$	200.00
$MgSO_4.7H_2O$	185.00
KH_2PO_4	85.00
$CaCl_2.2H_2O$	220.00
$FeSO_4.7H_2O$	13.90
Na_2 EDTA.$2H_2O$	18.65
$MnSO_4.4H_2O$	11.15
$ZnSO_4.7H_2O$	4.30
H_3BO_3	3.10
KI	0.415
$Na_2MoO_4.2H_2O$	0.125
$CuSO_4.5H_2O$	0.0125
$CoCl_2.6H_2O$	0.0125
Nicotinic acid	0.25
Pyridoxine HCl	0.25
Thiamine HCl	0.05
Glycine	1.00
Sucrose	30000.00

GENOTYPIC EVALUATION AND *IN VITRO* MULTIPLICATION OF ANTHURIUM (*Anthurium andreanum* Linden) HYBRIDS

by

ANAND S

(2017-11-039)

Abstract of the thesis
Submitted in partial fulfilment of the
requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANT BREEDING AND GENETICS
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695522
KERALA, INDIA

2019

ABSTRACT

The present study entitled "Genotypic evaluation and *in vitro* multiplication of anthurium (*Anthurium andreanum* Linden) hybrids" was carried out at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2017-19. The study was undertaken to evaluate anthurium hybrids for commercial qualities and their mass multiplication through *in vitro* techniques.

For variability analysis, 20 *Anthurium andreanum* Linden hybrid genotypes maintained at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani were utilized. When the selected hybrids were evaluated in completely randomized design with five replications, wide range of variations were observed among the qualitative as well as quantitative traits. The mean number of inflorescence year^{-1} ranged between 6.4 (HR x MR) and 2.2 (CR x KR). Spathe size was maximum for HoR x KR (112.30 cm^2) and the minimum for HR x MR (27.60 cm^2). The longest post-harvest vase life was observed for HoR x KR (24.4 days) which was on par with LJ x OG (23.4 days).

The components of variation namely genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were analysed. High PCV and GCV were observed for the characters number of suckers plant^{-1}, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size, leaf area, number of flowers spadix^{-1}, spadix length, duration of interphase, inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1}. Thus, selection for these characters would result in improvement of the genotype.

High heritability coupled with high genetic advance were observed for plant height, leaf area, number of leaves spadices^{-1} plant^{-1} year^{-1}, spathe size, spadix length, number of flowers spadix^{-1}, days to initiation of female phase, duration of female phase, inclination of candle with spathe, anthocyanin content, vase life and number of inflorescence year^{-1}. This indicated that expression of

these traits were controlled by additive gene action and improvement could be achieved for these traits by direct phenotypic selection.

Correlation analysis with genotypic correlation coefficients revealed significant positive correlation of number of inflorescence year^{-1} with characters such as number of leaves spadices^{-1} plant^{-1} year^{-1}, number of suckers plant^{-1} and vase life. An improvement in positively correlated characters would enhance the number of inflorescence year^{-1}. Path coefficients were worked out with number of inflorescence year^{-1} as the dependent variable and other correlated characters as component variables revealed that all the three positively significant, correlated characters had positive direct effect with the dependent variable. Path analysis further proved direct association of traits such as number of leaves spadices^{-1} plant^{-1} year^{-1}, number of suckers plant^{-1} and vase life with flower yield of anthurium hybrids accounting for more than 70 per cent of variation in flower yield.

From experiment I, six hybrid genotypes namely HR x MR, LJ x OG, OG x NO, HoR x KR, PR x HR and HR x LR with superior flower yield attributing traits and qualitative characters were selected for *in vitro* mass multiplication study. For *in vitro* culture, pale greenish brown young leaf lamina, 5 to 10 days after unfolding of leaf, collected from healthy and mature plants were used as explant. Proper control measures were taken for control of bacterial blight and anthracnose diseases so as to obtain disease free explants. Surface sterilization of leaf explants with 5.0 per cent sodium hypochlorite for 10 minutes followed by 0.1 per cent mercuric chloride for 5 minutes was found to be the best and resulted in 87.5 per cent explant survival. For all the hybrids the highest callus induction percentage was recorded by modified half strength MS medium supplemented with 200 mg L^{-1} NH_4NO_3 + 1.0 mg L^{-1} BA + 0.5 mg L^{-1} 2,4 D + 30 g L^{-1} sucrose + 6.0 g L^{-1} agar. The explants were cultured in darkness for callus induction and later the callus was subcultured in the same culture medium for two months for multiplication.

For shoot regeneration, the multiplied callus was subcultured to regeneration medium and a photoperiod of 16 hours light and eight hours dark was provided. Of the various regeneration treatments, half strength MS medium supplemented with 0.5 mg L^{-1} BA showed shoot initiation response ranging from 50.0 (LJ x OG and HR x LR) to 87.5 (OG x NO and HoR x KR) per cent among the hybrids. The fastest shoot regeneration was observed for the hybrid HR x MR (62.20 days) and slowest for LJ x OG (77.25 days). Rooting response preceded shooting response in all the hybrids in the same regeneration medium.

To summarize the research results revealed the presence of wide range of variability among the 20 anthurium hybrid genotypes for the 29 characters studied. Most of the quantitative traits were controlled by additive gene action permitting direct selection for improvement. Traits such as number of leaves spadices $^{-1}$ $plant^{-1}$ $year^{-1}$, number of suckers $plant^{-1}$ and vase life had positive significant correlation and direct association with flower yield in anthurium hybrids. Genotypic differences were evident from *in vitro* mass multiplication studies on the six superior hybrids. Modified half strength MS medium supplemented with 200 mg L^{-1} NH_4NO_3 + 1.0 mg L^{-1} BA + 0.5 mg L^{-1} 2,4 D + 30 g L^{-1} sucrose + 6.0 g L^{-1} agar was the most suitable for callus induction and callus multiplication while shoot initiation, proliferation of shoot and root were the highest and faster in the same basal medium supplemented with 0.5 mg L^{-1} BA. Large scale multiplication of the superior hybrids and profit generation can be achieved through the tissue culture protocol that was standardized in the present study. The promising commercially superior anthurium hybrids identified in the study can be used in further crop improvement programmes.