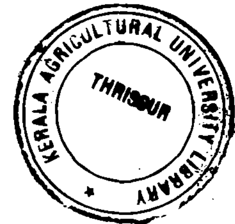
172549

EVALUATION, MOLECULAR CHARACTERISATION AND IN *VITRO* PROPAGATION OF HELICONIAS



SMITHA BABU

Thesis submitted in partial fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN HORTICULTURE

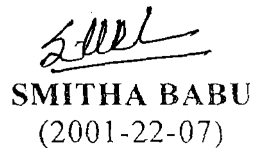
Faculty of Agriculture
Kerala Agricultural University, Thrissur

2005

DEPARTMENT OF POMOLOGY AND FLORICULTURE
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522

DECLARATION

I hereby declare that this thesis entitled '**Evaluation, molecular characterisation and *in vitro* propagation of Heliconias**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

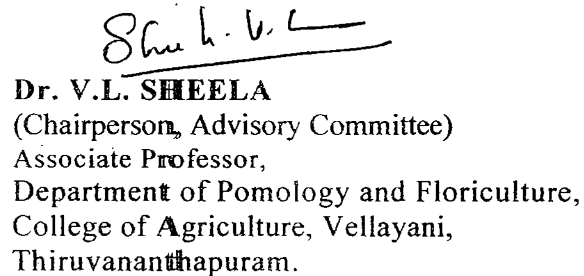


Vellayani,
-2005.

SMITHA BABU
(2001-22-07)

CERTIFICATE

Certified that this thesis entitled **'Evaluation, molecular characterisation and *in vitro* propagation of Heliconias'** is a record of research work done independently by Mrs. Smitha Babu (2001-22-07) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



Vellayani,
5.10-2005.

Dr. V.L. SHEELA
(Chairperson, Advisory Committee)
Associate Professor,
Department of Pomology and Floriculture,
College of Agriculture, Vellayani,
Thiruvananthapuram.

Approved by

Chairperson :

Dr. V.L. SHEELA
Associate Professor,
Department of Pomology and Floriculture,
College of Agriculture, Vellayani,
Thiruvananthapuram - 695 522

Members:

Dr. C.S. JAYACHANDRAN NAIR
Associate Professor and Head,
Department of Pomology and Floriculture,
College of Agriculture, Vellayani,
Thiruvananthapuram - 695 522

6/3/06

Dr. K. RAJMOHAN
Associate Professor and Head,
Department of Plant Biotechnology,
College of Agriculture, Vellayani,
Thiruvananthapuram-695 522.

4/3/06

Dr. K.B. SONI
Assistant Professor,
Department of Plant Biotechnology,
College of Agriculture, Vellayani
Thiruvananthapuram-695522.

6/3/06

Dr. VIJAYARAGHAVAKUMAR
Associate Professor,
Department of Agricultural Statistics,
College of Agriculture, Vellayani
Thiruvananthapuram-695522.

6/3/06

External Examiner :

4/3/06
(M. JAWAHARLAL)
Professor & Head,
Dept. of Floriculture & LS,
TNAU, Coimbatore.

ACKNOWLEDGEMENT

It is a pleasure and privilege for me to express my profound sense of gratitude to Dr. V.L. Sheela, Associate Professor, Department of Pomology and Floriculture and chairperson of my advisory committee for her valuable guidance, constant encouragement and care rendered during the research period which gave me a sense of comfortness and enabled me to complete the thesis successfully.

My sincere thanks to Dr. C.S. Jayachandran Nair, Associate Professor and Head, Department of Pomology and Floriculture and member of my advisory committee for his critical suggestions and timely help during the course of this research.

My profound gratitude to Dr. K. Rajmohan, Associate Professor and Head, Department of Plant Biotechnology and member of my advisory committee for his critical suggestions and valuable advice.

My sincere thanks to Dr. K.B. Soni, Assistant Professor, Department of Plant Biotechnology and member of my advisory committee for her sincere help and whole hearted co-operation.

I wish to place on record my sincere thanks to Dr. Vijayaraghavakumar, Associate Professor, Department of Agricultural Statistics and member of my advisory committee for the kind help rendered during the statistical analysis and interpretation, valuable suggestions and thorough scrutiny of the manuscript.

I wish to place on record my deep sense of gratitude and indebtedness to Late Dr. G. Sreekandan Nair, former Dean and member of my Advisory Committee for his vision, unstinted interest, expert guidance, inspiring words, valuable suggestions and kindness evinced during the part of my study.

I gratefully acknowledge the help rendered by the Associate Professor and Head, Kayamkulam for permitting me to take photographs of the DNA profiles in the gel doc. system.

I would like to express my heartfelt gratitude to Mr. C.E. Ajithkumar, Junior Programmer, Department of Agricultural Statistics for executing the statistical analysis for my study.

The teaching and non-teaching staff of Department of Horticulture, for their co-operation and assistance rendered during the period of study.

Staff members and trainees especially Dr. Anitha and Sri. Pradeep of Plant Molecular Biology and Biotechnology Centre for all their help and co-operation during the tissue culture work.

All my classmates, friends and juniors, who were of great help at times of need.

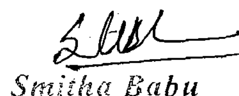
My sincere thanks to Mr. Biju for his patience and co-operation in neat execution of the manuscript.

I sincerely acknowledge Kerala Agricultural University for awarding the Senior Research Fellowship.

I thank Late Dr. Datsun Dani Philip for the love and affection evinced during the period of the research.

I wish to express my gratitude and indebtedness to my husband and my son, in laws, parents, sister, Ammachi and Appachan for their interest, encouragement, forbearance and patience during the period of my study.

Above all, I thank God Almighty for being with me always.



Smitha Babu

CONTENTS

	Page No.
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
3. MATERIALS AND METHODS	59
4. RESULTS	81
5. DISCUSSION	130
6. SUMMARY	153
7. REFERENCES	
APPENDICES	
ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Different levels of plant growth regulators tried for culture establishment in *H. psittacorum* cv. Deep Orange	77
2	Different levels of plant growth regulators tried for shoot proliferation and elongation in *H. psittacorum* cv. Deep Orange	78
3	Different levels of plant growth regulators tried for rooting in *H. psittacorum* cv. Deep Orange	80
4	Plant height of 12 *Heliconia* species and varieties	82
5	Number of leaves of 12 *Heliconia* species and varieties	82
6	Leaf area (cm^2) of 12 *Heliconia* species and varieties	84
7	Number of shoots of 12 *Heliconia* species and varieties	84
8	Inflorescence characters of 12 *Heliconia* species and varieties	87
9	Difference in pollen morphology of 12 *Heliconia* species and varieties	89
10	Crop duration of 12 *Heliconia* species and varieties	91
11	Number of flowering shoots of 12 *Heliconia* species and varieties	91
12	Genotypic and phenotypic coefficient of variation for the characters of 12 *Heliconia* species and varieties	93
13	Heritability and genetic advance for the characters of 12 *Heliconia* species and varieties	95
14	Phenotypic correlation coefficient for the characters of 12 *Heliconia* species and varieties	97

LIST OF TABLES CONTINUED

Table No.	Title	Page No.
15	Genotypic correlation coefficient for the characters of 12 *Heliconia* species and varieties	99
16	Environmental correlation coefficient for the characters of 12 *Heliconia* species and varieties	101
17	Economics of cultivation of 12 *Heliconia* species and varieties	103
18	Quantity and yield of DNA from *Heliconia psittacorum* cv. Deep Orange, Lady Di and Petra Orange	104
19	Effect of storage of leaves on the quantity and quality of the DNA of *Heliconia psittacorum* cv. Deep Orange	106
20	Quantity and yield of DNA from 12 *Heliconia* species and varieties	106
21	Primer associated banding patterns with the DNA of *Heliconia psittacorum* cv Deep Orange using 40 primers belonging to kit A and kit B of Operon Inc, CA, USA	108
22	Nucleotide sequence of primers and total number of informative RAPD markers amplified by them in the 12 *Heliconia* species and varieties	110
23	Similarity matrix of 12 *Heliconia* species and varieties obtained by RAPD analysis using three primer combinations	112
24	Effect of explants of *Heliconia psittacorum* cv. Deep Orange on days taken for bud initiation and survival rate in MS medium	114
25	Effect of surface sterilization on contamination in shoot tip explants of *Heliconia psittacorum* cv. Deep Orange	115
26	Effect of season of *Heliconia psittacorum* cv. Deep Orange on explants collection on rate of microbial contamination	116

LIST OF TABLES CONTINUED

Table No.	Title	Page No.
27	Effect of BA, auxins and gibberellins alone and in combination on culture establishment of *Heliconia psittacorum* cv. Deep Orange	118
28	Effect of Kinetin, auxins and gibberellins alone and in combination on culture establishment of *Heliconia psittacorum* cv. Deep Orange	119
29	Effect of 2-ip, auxins and gibberellins alone and in combination on culture establishment of *Heliconia psittacorum* cv. Deep Orange	121
30	Effect of BA and 2,4-D on culture establishment of *Heliconia psittacorum* cv. Deep Orange	121
31	Effect of Basal media on culture establishment of *Heliconia psittacorum* cv. Deep Orange	123
32	Effect of BA auxins and gibberellins alone and in combination on shoot proliferation and elongation of *Heliconia psittacorum* cv. Deep Orange	123
33	Effect of kinetin, auxins and gibberellins alone and in combination on shoot proliferation and elongation of *Heliconia psittacorum* cv. Deep Orange	125
34	Effect of 2-ip, auxins and gibberellins alone and in combination on shoot proliferation and elongation of *Heliconia psittacorum* cv. Deep Orange	127
35	Effect of plant growth regulators on rooting of *Heliconia psittacorum* cv. Deep Orange	128

LIST OF FIGURES

Sl. No.	Title	Between pages
1	Spike length (cm) of 12 *Heliconia* species and varieties	87-88
2	Petiole length (cm) of 12 *Heliconia* species and varieties	87-88
3	Number of bracts of the 12 *Heliconia* species and varieties	87-88
4	Bract size of 12 *Heliconia* species and varieties	89-90
5	Crop duration of 12 *Heliconia* species and varieties	91-92
6	Number of flowering shoots of 12 *Heliconia* species and varieties	91-92
7	Coefficient of variation of characters of 12 *Heliconia* species and varieties	93-94
8	Heritability and genetic advance of characters of 12 *Heliconia* species and varieties	95-96
9	Phenotypic correlation coefficient of characters in 12 *Heliconia* species and cultivars	97-98
10	Genotypic correlation coefficient of characters in 12 *Heliconia* species and cultivars	99-100
11	Amplification profiles (total bands) of the DNA of *Heliconia psittacorum* cv. Deep Orange using 40 primers of Kit A and Kit B	108-109
12	Amplification profiles (intense and faint bands) of the DNA of *Heliconia psittacorum* cv. Deep Orange using 20 primers of Kit A	108-109

LIST OF FIGURES CONTINUED

Sl. No.	Title	Between pages
13	Amplification profiles (intense and faint bands) of the DNA of *Heliconia psittacorum* cv. Deep Orange using 20 primers of Kit B	108-109
14	Total number of informative RAPD markers amplified by the eight primers in 12 *Heliconia* species and cultivars	108-109
15	Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPA-01	108-109
16	Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPA-14	108-109
17	Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPA-17	108-109
18	Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPA-18	108-109
19	Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPB-07	110-111
20	Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPB-12	110-111
21	Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPB-18	110-111
22	Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPB-20	110-111
23	Dendrogram obtained from RAPD analysis using UPGMA method	112-113

LIST OF PLATES

Plate No.	Title	Between pages
1	Field view of the experimental plot	60-61
2	*Heliconia* species ad varieties used for the experiment	60-61
3	Pollen characteristics of 12 *Heliconia* species and varieties	89-90
4	Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPA-01	109-110
5	Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPA-14	109-110
6	Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPA-17	110-111
7	Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPA-18	110-111
8	Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPB-07	110-111
9	Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPB-12	110-111
10	Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPB-18	110-111
11	Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPB-20	110-111
12	Stages of *in vitro* propagation of *H. psittacorum* var. Deep Orange	129-130

LIST OF ABBREVIATIONS

µl	–	Micro litre
µm	–	Micro molar
AC	–	Activated charcoal
AFLP	–	Amplified Fragment Length Polymorphism
BA	–	Benzyl adenine
BAP	–	Benzyl aminopurine
bp	–	Base pair
CD	–	Critical difference
cm	–	Centimetre
CPCRI	–	Central Plantation Crop Research Institute
CRD	–	Completely Randomized Design
CTAB	–	Hexa decyl trimethyl ammonium bromide
DNA	–	Deoxy ribonucleic acid
dNTPs	–	Deoxy nucleotide
EDTA	–	Ethylene diamino tetra acetic acid disodium salt
GA	–	Genetic Advance
GA_3	–	Gibberellic acid
GCV	–	Genotypic coefficient of variation
H^2	–	Heritability
IAA	–	3- indole acetic acid
IBA	–	3-Indole butyric acid
KCl	–	Potassium chloride
kg	–	Kilogram
KIN	–	Kinetin
M	–	Molar
m	–	Metre
$MgCl_2$	–	Magnesium chloride
ml	–	Millilitre
MS	–	Murashige and Skoog

LIST OF ABBREVIATIONS CONTINUED

1/2 MS	_	Half strength Murashige and Skoog
ISSR	_	Inter simple sequence repeats
NAA	–	α - Naphthalene acetic acid
NaCl	–	Sodium chloride
ng	–	Nanogram
NS	–	Not significant
OD	–	Optical density
PCR	–	Polymerase chain reaction
PCV	–	Phenotypic coefficient of variation
PM	–	Pico Molar
PVP	–	Polyvinyl pyrrollidone
RAPD	–	Random Amplified Polymorphic DNA
RBD	–	Randomized Block Design
RFLP	–	Restriction fragment length polymorphism
SCAR	–	Sequence characterized amplified region
SD	_	Standard deviation
SE	–	Standard error
SH	_	Schenk and Hilderbrandt
SSR	–	Simple sequence repeats
STMS	–	Sequence tagged micro satellite sites
TAE	–	Trisacetic acid EDTA
TE	-	Tris HCL-EDTA
Tris-HCL	–	Tris (hydroxy methyl) amino methane hydrochloride
TSS	–	Total soluble solids
UPGMA	–	Unweighted pair group method arithmetic average

Dedicated to

My Beloved Son

& Ammachi

Introduction

1. INTRODUCTION

The world trade in floriculture is reported to be around US $ 50 billion, out of which 60 per cent is from cut flowers. As a result of liberalized economic policy and encouragement of Government of India for floriculture produce exports, in the recent years the floriculture scenario in India is changing fast.

India especially Kerala, which is blessed with favourable climatic conditions and skilled manpower, could become a major production centre for flowers. Besides earning large foreign exchange through export, this sector could provide non traditional employment to a very large number of people. With the growing population, lack of open space and development of multistoried housing systems, people have to depend largely on indoor plants for decorating their surroundings.

Heliconias belonging to family Heliconiaceae are tropical flowering plants native to Central and South America and some islands of South Pacific. Their easy cultivation and spectacular presence have made them as a favourite garden plant throughout the world. Their brilliant colour, exotic form, long straight peduncle and excellent post harvest characteristics make them as an outstanding flower for florist trade. They stand out with striking visual effect in flower arrangement and also possess long vase life enhancing their value as cut flowers. They last in the field without losing their visual appeal from several days to several months extending the saleable stage for a considerable period of time. They flourish in warm humid tropics and do equally well under direct sun and shade. Major Heliconia producing nations include Barbados, Hawaii, Brazil, and Venezuela. It is reported that about one lakhs stems of Heliconia are produced annually in India, fifty per cent of which is from coconut farm located in Andhra Pradesh.

The importance of Heliconia as a newly introduced cut flower is increasing, since in the international and national market there is high demand for newly introduced cut flowers. Consumers are interested in newer and different types of flowers. Though many species and varieties are grown, their proper evaluation is not carried out to determine the most suitable cultivars for Kerala's agro climatic and economic conditions. Hence, it is necessary to evaluate the morphological, cytological and inflorescence characters and also to determine the economics of cultivation.

In India, floriculture development is slow and unplanned. Several varieties and number of flowers have been developed within the country by the universities, research institutes and amateurs. However the commercial value of such materials has not been evaluated properly. Lack of availability of quality planting materials is another major drawback. The conventional methods of vegetative propagation are laborious and time consuming. Hence it is necessary to develop a suitable method for mass multiplication of Heliconias to meet the future demand. Under such circumstances, tissue culture methods of plant propagation has got immense importance.

The discovery of molecular and polymerase chain reactions has now made the DNA finger printing easier in crop plants. A wide variety of techniques to reveal the DNA sequence, polymorphism have been developed in the past years and several molecular markers like RFLP (Restriction fragment length polymorphism), RAPD (Random Amplified Polymorphic DNA), microsatellites etc. have been utilized in various fields of phylogenic studies, genetic diversity analysis, forensic science, paternity determination, plant varietal characterization etc. Results of RAPD analysis are independent of environmental influences, tissue types etc. and provided greater resolution than the other techniques. The procedure is faster and easier than other molecular analysis. In Heliconia

too, it is necessary to carry out grouping of various species and varieties to enable future breeding programmes.

The relevance of the present study becomes evident in this context.

The objectives of the study are:

- To evaluate *Heliconia* sp. and varieties as cut flowers
- To standardise *in vitro* propagation procedures in *H. psittacorum*
- To undertake molecular characterization of *Heliconia* sp. and varieties using RAPD (Random Amplified Polymorphic DNA) markers.

Review of Literature

2. REVIEW OF LITERATURE

Heliconias are banana like plants with rhizomes or under ground stems having distribution of nutrients and water, like the true stems. There are about 89 species under the genus Heliconia and more than 350 varieties. They are propagated by bits of rhizomes as well as suckers or side shoots arising from the clumps and rarely from seeds (Tom, 1997).

There are two main types of heliconias, erect heliconia and pendent heliconia. Erect heliconias stand straight with bracts pointing up. Pendent heliconias hang with bracts pointing down. Their inflorescence has colourful bracts which curve upwards and downwards in alternate patterns along a thick stem (Endre, 1996). When heliconias were first discovered they were included in the Musaceae family along with bananas. But now they are included in the family Heliconiaceae. Heliconia is the only genus in the plant family Heliconiaceae, which is a member of a larger taxonomic order Zingiberales. There are several characteristics by which they can be recognized, including large leaves and large colourful bractic inflorescences. Most taxonomists recognize eight separate families in the Zingiberales *viz*., Musaceae, Sterilitziceae, Lowiaceae, Heliconiaceae, Zingiberaceae, Costaceae, Cannaceae and Marantaceae. The Heliconiaceae and Musaceae may be distinguished by characteristics of their lamina anatomy and by the fact that Musaceae blades have an irregular apex (Triplett and Kirchoff, 1991).

This review highlights the research on the various aspects of evaluation, molecular characterization and *in vitro* propagation of heliconias and related crops.

2.1 EVALUATION OF HELICONIAS

Some of the most important species of *Heliconia* are described below.

2.1.1 *Heliconia stricta*

The strictas have exotic inflorescence with colour ranging from red, gold, orange, maroon and green singly or in combination. These exotic tropicals are ideal for small arrangements as their inflorescences range from 12.5 – 30.0 cm long and are not too heavy (Charleston, 1997).

***H. stricta* cv. Dwarf Jamaican**

It is a small plant grows up to a height of 0.45 – 0.90 m. It grows well in pots. The inflorescence is rose coloured and evenly graded from pale to deep hues. Each bract is ridged with green on its upper edge, matching the tiny green and white-stripped sepals. It blooms throughout the year. Peak flowering is observed in winter. It adapts to variable temperatures and grows in full sun to 60 per cent shade (Charleston, 1997). Lekawatana and Criley (1999) studied various aspects such as propagation, general culture, temperature response and the use of growth retardants (ancymidol, paclobutrazol and flurprimidol) in the production programme for Dwarf Jamaican.

***H. stricta* cv. Sharonii**

They have broad foliage, which is borne on stiff red stalks. Flowering starts from late July to February. It grows well at low light up to 80 per cent shade. It grows up to a height of 0.90 – 1.80 m. It possesses red and yellow inflorescence that stands erect. The foliage is broad and has red vine colouration beneath it (Charleston, 1997).

2.1.2 *Heliconia rostrata*

They are beautiful tropical herbs with banana like leathery green leaves, commonly known as hanging lobster claws. It is a native of Peru

and generally distributed in tropical America. They have pendent inflorescence of alternating bracts each 6-10 cm long, scarlet red tipped with cream to yellow colour. The bract has deep red colour with yellow green tips, boat shaped. Each inflorescence has 6 to 20 bracts. It grows well at full sun to 50 per cent shade. Height ranges from 0.90 to 5.40 m. It blooms throughout the year. It is one of the hardiest varieties (Goel, 2004).

2.1.3 *Heliconia psittacorum*

They originated from the Coast of Guyana. The psittacorum (or parrot's beak) heliconias are small, dainty and exotically tropical. It resembles the plant commonly known as Bird-of-Paradise. They bloom throughout the year. The flowers are greenish yellow with black spots near apex. The psittacorums rarely exceed 0.90 – 1.80 m in height. It grows well under tropical conditions (Juan, 1997). According to Geertsen (1988) *H. psittacorums* are the most useful flowers for cut flower production in glass house cultivation because of its upright stems, moderately vigorous growth habit, long flowering season and long-lasting flowers.

***H. psittacorum* cv. Andromeda**

It possess very attractive reddish-orange to pink bracts. Flowers are very long lasting. It grows up to a height of 1.20 to 1.80 m. Broschat *et al.* (1984) had given the following description about Andromeda. Height ranges from 1.0 to 1.8 m and it produce 5 leaves/shoot followed by a terminal inflorescence with 3 or 4 bracts. The bracts are red, fading to light orange at their bases. The lower half of each bract and the upper 2 to 3 cm of the peduncle are covered with a waxy white bloom. The florets are orange with black tips and the main axis of the inflorescence is orange.

***H. psittacorum* cv. Lady Di**

Lady Di may be the most beautiful among the psittacorums. It has dark rose red bracts and cream yellow sepals with dark green bands and

white tips. Height of the plant ranges from 0.60 to 0.90 m with an erect habit. It grows well in full sun to 40% shade. Peak flowering is during April to November (Juan, 1997).

***H. psittacorum* cv. Choconiana**

They bloom throughout the year and produce 4 to 6 orange bracts and orange sepals with distal black bands and yellow white tips. It grows well under full sun to 50% shade. Height ranges from 0.30 to 2.4 m.Flowers are long lasting (Juan, 1997).

***H. psittacorum* cv. Sassy**

It is a dwarf variety. Bracts are pale green or cream at base and reddish pink distally. Sepals are orange with distal green-black bands and white tips. It blooms from April to November. It grows up to a height of 0.90 – 1.80 m and in full sun to 40% shade (Juan, 1997).

2.1.4 *H. psittacorum* × *H. spathocircinata* cv. Golden Torch

They have large golden boat-shaped bracts with golden yellow flower. It posses rigid flowers which were produced through the selective breeding for colour, longevity and durable texture. They are larger and sturdier than other psittacorums. Height ranges from 0.75 to 2.40 m. It grows well in full sun to 40% shade (Alan, 2004). According to Broschat *et al.* (1984) Golden torch plants ranges in height from 1.0 to 1.8 m, and produce 4 or 5 leaves/shoot followed by a terminal inflorescence with 3 or 4 bracts. The entire inflorescence is uniformly orange-yellow in colour. Trials conducted in south-eastern Florida revealed that under higher dose of NPK application (3.6 kg 18-6-12 NPK/m^2/ year) flower production was increased up to an average of 84 flowers m^{-1} $year^{-1}$. The optimum temperature range for flower production was 21° to 35° C. Growing plants under 63.00 per cent shade reduced flower production by about 50%. The postharvest life of cut inflorescences placed in deionized water ranged from 14 to 17 days at 23° C.

***H. psittacorum* × *H. spathocircinata* cv Guyana**

These are perennial plants having a height of 1.0 to 1.4 m. It grows well in partial shade. It prefers moist soil. Bracts are wide and stout with orange red coloured edges. Sepals are light yellow with emerald green tips. Bracts are arranged spirally around reddish orange peduncle. It blooms throughout the year (Alan, 2004).

2.1.5 *Heliconia humilis*

They are the native of Trinidad and Brazil. They have shiny green leaves, and erect flower heads. The bracts are boat shaped with salmon red colour changing to green towards tip. It has greenish yellow flowers (Timothy, 1996).

2.1.6 *Heliconia wagneriana*

These are erect heliconias, similar to *H. humilis*. But they are stouter than *H. humilis*. Their inflorescence is also stouter and paler in colour (Timothy, 1996).

***H. wagneriana* Red**

The height of *H. wagneriana* red ranges from 3 to 4.5 m. They possess 5 to 10 red yellow and green coloured bracts. It takes 10 months to flower and is having vase life of 15 days (Timothy, 1996).

***H. wagneriana* Yellow Peterson**

These are erect plants having a height of 3 to 4.5 m. Bracts are 5 to 10 in number and are yellow and green coloured. It also takes 10 months to flower and has a vase life of 15 days (Timothy, 1996).

2.1.7 *Heliconia latispatha*

They are the native of Central and South America. The leaves are broad and oblong having 1m length and 30 cm width. It has erect inflorescence with well separated boat shaped bract, 15 cm long and

orange yellow at base near axis and red towards the tip. They are tropical plants having green flowers (David, 1985).

2.1.8 *Heliconia bihai*

It is commonly known as Wild plantain or Fire bird. It is a large perennial herb having oblong smooth textured pointed green leaves. The bracts are crimson red with pointed tip and arranged in two rows on erect inflorescence (David, 1985).

2.1.9 *Heliconia collinsiana*

They are robust tropical perennial plants with lush growth. The inflorescence is pendent. The bracts are crimson red and covered with waxy powder yellowish towards tip. The flowers are cream in colour (David, 1985).

2.1.10 *H. collinsiana X H. bourgena* cv Pedro Ortiz

These are natural hybrids between the pendent *H. collinsiana* and the erect *H. bourgena*. It is an erect inflorescence, but has the tendency to twist and hang down like a pendent. Bracts are pink reddish coloured. It grows well in partial shade to full sun. Height ranges from 1.80 to 2.40 m (David, 1985).

Artificial hybridization among 14 species of neotropical Heliconia was studied by Kress (1983) at two sites in Costa Rica. At Las Cruces Tropical Botanical Garden, individuals in cultivation was used as parents in crosses primarily between species with pendent inflorescences that are normally distributed allopatrically. At Finca La Selva normally sympatric species with either pendent or erect inflorescences were crossed in their natural habitats. Observation of pollen tube growth by means of fluorescence microscopy and seed set were used to determine the extent of crossability. Crossability barriers between the majorities of species were strong and foreign pollen tubes were inhibited at the stigmatic surface, within the stylar tissue or within the ovary. The site of inhibition was

consistent for each pair of species, and depended on the parentage and the direction of the cross. Although additional isolating mechanisms, such as pollinator specificity and phenological separation were present, pre-fertilization crossability barriers acted as the ultimate mechanism to prevent hybridization. The type of barrier (stigmatic, stylar or ovarian) that existed between two species was not dependent upon the geographical distribution of the parental species or the specific types of pollinators that visit them, but in some cases might indicate taxonomic relationships.

2.1.11 Floral Biology

Heliconias derive their beauty from highly modified leaves or bracts. The flowering bracts may be upright or pendulous depending on the variety and may exhibit the shape of a lobster claw, bird's beak or fan shape. The flowers are pollinated by humming birds and bugs. However some pollen may be carried from one flower to another, by insects. These insects are not specialists, they feed from the flower or nectar and pollination rarely occurs. South East Asian heliconias are pollinated by bats (David, 1985).

Watson and Dallwitz (1991) had given the following descriptions about the floral biology of heliconia. Each inflorescence bract contains varying number of flowers upto 15 depending on the species. Each flower inturn is subtended by a small floral bract. The floral bracts of some species are opaque and leathery and persist through fruit development to protect maturing ovaries. In other species they are plumy and translucent and quickly decompose after the flower close. Flowers are hermaphroditic possessing both male and female sexual parts. Perianthes made up of three outer sepals and three inner petals united at the base and to each other in various phase. When the flower opens, a single sepal become free from the outer perianth part and allows pollinators to enter the flower. The colour of the perianth is species specific. The flowers are opened only for a single day after which the perianth falls from the ovary. The flower

contains high fertile stamens that produce viable pollens. A sixth stamen is replaced by a sterile stamenoid that does not produce pollen but may function in some species as a guide leading the pollinators tongue to the floral nectaries situated at the base of the style. Anthers are basifixed, tetrasporangiate. Ovary lies below the sepals and petals and can be variously coloured. It is usually smooth in most species, but hairy in others. Gynoecium is three carpelled, carpels rhizomerous with the perianth. The pistil is three celled. Placentation is basil to exile and there is one ovule per locule, which is anatropous. The mature fruit of heliconia is a drupe with a hard inner layer enclosing each of the four seeds, which are triangular (1 – 3 per fruit). The outer layer of fruit is fleshy and at maturity the surface layer becomes blue in American species or red to orange in South Pacific species. The colourful fruits are very attractive to the birds and mammals that disperse the seeds.

Cross-fertilization between species is generally unsuccessful because pollen of one species is usually inhibited by other species.

In Heliconia thread-like structures connecting the pollen grains are described by Rose and Barthlott (1995). These threads are decay products of the walls separating the pollen chambers, and products of the rupture of the mature anthers in the stomium region. The pliable cell threads mix with the pollen and entangle individual grains to form aggregates. This ensures that the pollen becomes embedded in the feathers or attached to the smooth, unsculptured beak of pollinating hummingbirds (Trochilidae).

Six cultivars of *H. psittacorum* were selected by Lee *et al.* (1994) for studies on their natural fruit-bearing ability, pollen formation and pollination under the tropical climatic conditions of Singapore. Three of them, namely Tay, Andromeda and Lady Di, were partially fertile with a very low rate of fruit set, ranging from 2.8 to 4.7%. They were found to be diploid with 2n = 24 chromosomes. The process of pollen formation (microsporogenesis) was normal, and pollen grains were all uniform in

size and appeared normal. The poor fruit set of these 3 cultivars was attributed to poor pollen germination on stigmas rather than poor pollination or self-incompatibility. The other 3 cultivars, namely Petra, Sassy and Iris, were completely sterile. Their pollen grains were of variable sizes and appeared to be abnormally fragmented. Over 80% of the pollen grains aborted 1-2 days before pollination. These abnormal features were consistent with irregular distribution of chromosomes during meiosis in microsporocytes. All 3 cultivars were confirmed to be triploid (2n = 3x = 36).

2.1.12 Flowering in Relation to Environmental Factors

Most of the species of heliconias can be found in moist or wet regions, but some are found in seasonally dry areas. Although Heliconias flourish in the humid lowland tropics at elevations below 1500 feet, the greatest number of species is found in middle elevation rain and cloud forest habitats. Many of the *Heliconia* species flourish well at open sites like roadsides, riverbanks and also in patches of light in the forest (Tom, 1997).

The peak flowering period is from September to December in the first year of planting when planted in January. In the subsequent years it flowers in April and continues up to December. However flowers are produced almost throughout rest of the year. During winter, partial shading of leaves occurs and flowering is arrested. The natural flowering season for *Heliconia* species in their natural habitats may be influenced locally by rainfall and drought periods as well as by photoperiod. With more than three dozen species of *Heliconia* grown and exported in international trade, the seasonality of flowering is important to the supply and marketing of this bold tropical flower (Criley, 2000).

Heliconias grow well at a temperature of 21 to 35^{0}C. Plants grown in full sun produce much more flowers than in partial shade. The influence of irradiance on photosynthesis under natural conditions was studied by Jie-He *et al.* (2000) using *Heliconia rostrata*, *H. psittacorum* x *H. spathocircinata*

cv. Golden Torch and *H. psittacorum* cv. Tay. When grown under full sunlight, all three taxa exhibited reduced photosynthetic capacities and chlorophyll content per leaf area compared with those grown under intermediate and deep shade. In heliconia, the top leaves (particularly leaf tips) experienced sustained decreases in PS II efficiency upon exposure to full sunlight. Although all three taxa exhibited sustained decreases in photosynthetic capacity in full sunlight, the sun leaves of Tay showed higher photosynthetic capacity than those of the other two taxa. This could be due, at least in part, to the vertical leaf angle and smaller lamina area. When the upright leaves of Tay were constrained to a horizontal angle, they exhibited lower PS II efficiency, while horizontal leaves of Rostrata and Golden Torch inclined to near-vertical angles showed increased efficiency. Thus, an increase in leaf angle helps to achieve a reduction in the sustained decrease in PS II efficiency by decreasing the levels of incident sunlight and subsequently the leaf temperature.

Geertsen (1989) found that by increasing the minimum air temperature from 15^0 to 21^0C the number of shoots emerging and the number of flowering stems produced per m^2 could be doubled in *H. psittacorum* cv. Tay. Also, stem length was increased and the quality was noticeably improved. Photoperiod had only a slight effect on growth and flowering.

A seasonal pattern of flowering was observed in field production of *Heliconia stricta* cv 'Dwarf Jamaican' by Criley and Kawabata (1986). This seasonality could be photoperiod-related because greater yields for plants were obtained when grown under 8 hours daylengths for 6 weeks than those plants grown under natural day lengths (about 13.5 hours). Depending on the capacity of the plant to respond to photoperiod, 3 or 4 weeks of short daylength (SD) were sufficient for flower initiation. Geertsen, (1990) observed that by exposing plants to a photoperiod of 8 hours, flowering was more advanced and more abundant. Raising the

temperature from 15^0 to 21^0 C flowering percentage increased by 20%; the flowering stems were 40 cm longer and the number of leaves subtending the inflorescence increased by 2.5 cm.

Growth and development of *H. bihai* cv. Lobster Claw One and *H. latispatha* were studied under 3 shade levels (0, 40 and 60%) for 20 months, during which 5 generations of shoots were developed. Plants without shade produced the maximum pseudostems. The number of shoots per clump was greater in *H. bihai* than in *H. latispatha*. In *H. bihai* the first three generations flowered simultaneously, when the clump reached the age of 12 months. This flowering period lasted 6 months with a peak during March-June, when 95% of the flowers developed. In *H. latispatha* flowering began when plants were 10 months old and showed an irregular pattern during the cycle, with the peak (82%) occurring in July and August (Maciel and Rojas 1994).

Flowering responses of *H. psittacorum x H. spathocircinata* cv. Golden Torch to temperature and photosynthetic photon flux (PPF) were studied by Catley and Brooking (1996) under controlled environmental conditions. Temperature had no significant effect on new shoot production. An average of 9.3 shoots/plant was produced over the 248 days of treatment. More shoots, however, were produced at the higher PPF level (10.1 shoots, compared with 8.3 shoots at the lower light level). The proportion of shoots that initiated flowers (85%) was similar in all treatments. The time from shoot until inflorescence emergence was significantly shorter at $32/20^0$ than at $24/20^0$ (140 and 146 days respectively) and was unaffected by PPF combination. Acceptable flower quality with at least 2 opened, well-formed, well-coloured bracts was obtained in all treatments. Overall, temperature was more dominant than light in influencing production and quality of flowers. Cut flower production of Golden Torch should be feasible in temperature controlled greenhouses in temperate regions where mean air temperatures can be

maintained at approximately equal to 20° C. Although year round flowering of *H. chartacea* is potentially possible in Hawaii as new shoots develop regularly, flowering is low in the period from late March to early June. Floral initiation occurs after shoot emergence when 4 leaves have unfurled with 2-3 leaves still within the enclosing pseudostem. This places flower initiation in the October-December timeframe for these shoots. In contrast, autumn-winter shoots initiate flowers during January-March and they produce an average of one leaf less than the summer shoots. The greater leaf count and low flowering percentage of spring shoots suggest autumn conditions are favourable for leaf initiation and unfavourable for floral initiation. An attempt to promote flower initiation during the autumn with light-break lighting was thwarted by disease (*Phytophthora)* according to Criley and Kawabata (1986).

It has been reported previously by Jie-He *et al.* (1996) that *Heliconia* cv. Golden Torch leaves grown in full sunlight exhibit a sustained decrease in PS II efficiency as compared to those grown under shade conditions. In a study by Jie-He *et al.* (1996) full sunlight plus low levels of fertilizer application caused a further reduction in photosynthesis, chlorophyll content and Fv:Fm ratio while plants grown at high nutrient levels showed higher values of all these parameters. When plants were grown under intermediate or deep shade, there was no significant difference in any of these parameters irrespective of nutrient supply. In the recovery experiments, plants without fertilizer were refertilized weekly. Maximum photosynthetic rates, chlorophyll content, and Fv:Fm ratio increased gradually after refertilizing the plants grown in full sunlight. However, no significant changes in these parameters were observed in plants grown under intermediate or deep shade over the same period. Total leaf N was measured parallel with all the parameters. Photosynthetic rates, chlorophyll content and Fv:Fm ratio showed a clear linear correlation with total leaf N in plants grown in full sunlight while no clear relationship was observed in plants grown under intermediate or

deep shade. These results suggest that acclimatization of *Heliconia* under full sunlight could be achieved by high nutrient levels.

Criley *et al.* (1999) reported that although *Heliconia* is a tropical genus, many species exhibit seasonal patterns of flowering. Some cases have been attributed to seasonal rainfall, but research has demonstrated that *H. wagneriana and H. stricta* cv. Dwarf Jamaican are short day (SD) species while *H. angusta* initiates its flowers during long days (LD). *H. stricta* cv. Dwarf Jamaican initiates its flowers when its pseudostem has 3 unfurled leaf blades. Anthesis is reached 15-19 weeks after the beginning of SD. *H. angusta* initiates its flowers when daylengths exceed 13.3 hours and requires 15-17 weeks of long days to reach anthesis. Growers shipping records have been used to identify other species with marked seasonality.

Criley, 2000 reported that the natural flowering season for *Heliconia* species in their natural habitat may be influenced locally by rainfalldrought periods as well as by photoperiod. Strong seasonal flowering patterns were reported in *H. angusta, H. bihai, H.carribea* x *H. bihai, H. collinsiana, H. lingulata, H. rostrata, H. stricta* and *H. wagneriana*

2.1.13 Harvesting and Postharvesting Technology

The flowers can be harvested with peduncles of 70 cm to one m length. Harvesting of flowers is done by cutting the entire stalk at the ground level. The cut inflorescence lasts for 2 to 3 weeks. Cutting the flowers before 8 a.m. and immediately submersing the cut end in water along with recutting every few days extends their vaselife. Zimmer and Carow (1977) reported that when cut flowers of *Heliconia* spp. were kept at temperatures between 0 and 12° C under reduced pressure, all spp. stored successfully for at least 4 weeks. According to Bredmose (1987) *Heliconia* spp. inflorescences remained in water for 2-4 weeks, with no need for additives. Vase life was shorter in winter than other times. Paull and Chantrachit (2001) reported that the vase life of *Heliconia psittacorum* cv. Andromeda, *H. chartacea* cv. Sexy Pink and red was

increased by benzyladenine (BA, 100 mg litre^{-1}), applied as a dip or as a spray.

2.2 MOLECULAR CHARACTERIZATION OF HELICONIAS

2.2.1 Molecular Markers

Traditionally various morphological, anatomical, cytological and physiological methods have been used to distinguish plant species and varieties. The limitations of these methods have prompted the identification and use of more reliable methods. During the last decades the use of molecular markers has significantly contributed to our understanding of the species at the genetic level.

Genetic studies over the past several years have led to the establishment of several different types of molecular markers which include biochemical markers like isozyme markers and DNA marker. Protein and DNA markers are together called molecular markers.

Molecular markers are genotypic markers (Bretting and Widrlechner, 1995). Biochemical markers have been used since long for the characterisation of variation in plant, now considered to be inappropriate as universal markers (Cooke, 1994).

Molecular markers have shown to be useful for diversity assessment in a number of plant species (Waugh and Powel, 1992). These markers are direct manifestations of genetic content (Weising *et al.*, 1995) and they serve as reliable indices of genetic variation. In the past decade, molecular markers have vary rapidly complemented the classical strategies.

The genetic markers are used for clonal identification, linkage mapping, population diversity, taxonomy, evolutionary studies, determining the genetic fidelity during micropropagation, germplasm conservation etc. (Bretting and Widrlechner, 1995).

2.2.2 Isozyme

The use of isozyme markers is based on protein polymorphism. These markers code for specific proteins and they can be visualized through gel electrophoresis.

Isozyme markers were reported to be used in rose for varietal identification (Kim and Byrne, 1996; Walker and Werner, 1997) and classification (Kim and Byrne, 1994). Taxonomic studies on *Calanthe* (Ryuk *et al.*, 1999) and *Lilium longiflorum* Thunb. (Wen and Hsiano, 1999) using enzyme polymorphism was also reported.

The enzyme coding loci do not constitute a random sample of genes and they are not randomly dispersed throughout the genome. Electrophoresis will detect only portion of the actual variability present in amino acid sequences (Hillis and Moritz, 1990). Some isozyme variants are not selectively neutral (De Michele *et al.*, 1991). Moreover, isozyme markers, are unstable during plant development and standardisation of sampling procedure is sometimes difficult. Therefore, the isozymes have been replaced by DNA based molecular markers (Anolles and Trigiano, 1997).

2.2.3 DNA based Markers

With the advent of molecular biology techniques DNA based markers have replaced enzyme markers in germplasm identification and characterization as well as in gene tagging. Because of its plasticity, ubiquity and stability, DNA is the ideal molecule for such analysis (Caetano-Anollees *et al.*, 1991). The advantage of the methods is greater resolution and uniformity of DNA in all cells. DNA markers directly assess the genetic differences between species at the DNA level. Various types of molecular markers are used to evaluate DNA polymprohism and are generally classified as hybridization based markers and polymarase chain reaction (PCR) based markers (Joshi *et al.*, 1999).

2.2.4 Hybridization based DNA Markers

The hybridization based DNA marker techniques utilize labeled nucleic acid molecules as hybridization probes (Anolles *et al.*, 1991). Probe molecules range from synthetic oligonucleotides to cloned DNA. Some of the important hybridization based DNA techniques are restriction fragment length polymorphism (RFLP) and hypervariable sequence.

2.2.5 Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism analysis involves digesting the genome with restriction enzymes, separating the fragments electrophoretically and then preferentially visualizing fragments containing particular homologous sequences by hybridizing them to specific DNA probe (Deverna and Alpert, 1990; Walton, 1990).

In petunia, RFLP mapping has been carried out for certain identified genes namely the actin gene families and chalcone synthase genes (Koes *et al.*, 1987 and Mc Lean *et al.*, 1990).

Wolff *et al.* (1994) developed RFLP probes and primers in chrysanthemum. In carnation, the locus controlling flower type (Double/ semi double) was identified using RAPD and RFLP markers (Scovel *et al.*, 1998).

Ballard *et al.* (1996) identified and constructed the genome map of rose cultivars using random amplified polymorphic DNA (RAPD) and RFLP markers.

Genetic diversity in Musa was documented using RFLPs (Gawel *et al.*, 1992). Chloroplast DNA RFLPs were used to study cytoplasmic genetic diversity in various *Musa* species and subspecies (Gawel and Jarret, 1991).

RFLP probes are locus specific, resulting in an easy to screen co-dominant behaviour. However, it is relatively labour intensive, expensive

and involves the use of radioactive chemicals, which are harmful to the users.

2.2.6 Polymerase Chain Reaction (PCR) based DNA Maker Techniques

Among the PCR based markers, the important ones are minisatellites, amplified fragment length polymorphism, microsatellites, sequence characterized amplified region and random amplified polymorphic DNA.

2.2.7 Minisatellites (Variable Number of Tandem Repeats) (VNTR)

Jeffreys *et al.* (1985) was the first to report on the presence of minisatellite hyper variable sequence in the human genome that could be used for DNA fingerprinting. Minisatellites are repeat sequence having repeat units ranging from 11 to 60 bp in length. The repeat sequences comprise upto grater than 90 per cent of total DNA in certain plant genomes. The conserved sequence flanking minisatellites can be amplified using a suitable primer to reveal the polymorphism. The polymorphisms are attributed to the variation in length of minisatellites. The presence of hypervariable sequences was confirmed in plants and animals by Gupta *et al.* (1996).

Vainstein and Ben-Meir (1994) reported that DNA finger printing using mini and microsatellites probes was useful for cultivar identification in roses.

Cafasso *et al.* (2001) reported the occurrence of tandem repeats in the chloroplast genome of the marsh orchid (*Orchis palustris*). The repeat unit is an AT rich, 16 bp sequence located in the chloroplast tRNALEU intron. The 16 bp repeat unit was found to be present in all *O. palustris* accessions studied, as well as closely related *O. laxiflora*.

Compared to RAPD the banding pattern yielded by minisatellites probes is highly reproductive but involves high cost and complexity.

2.2.8 Amplified Fragment Length Polymorphism (AFLP)

This technique is also called 'selective restriction fragment amplification'. It is a combination of RFLP and PCR used for obtaining highly informative fingerprints. It is based on PCR amplification of restriction fragments generated by specific restriction enzymes and oligoneucleotide adapters of few nucleotide bases (Vos *et al.*, 1995).

AFLP markers were used to identify rose varieties (Zhang *et al.*, 2000). With twelve pre-screened primer combinations 322 AFLP markers were generated in thirteen modern rose varieties and their polymorphism was studied. The intravarietal variability was also evaluated.

Lesur *et al.* (2000) compared molecular methods namely RFLP, RAPD, SCAR, STMS (PCR amplification of microsatellites motives), oligo fingerprinting, inter simple sequence repeats and AFLP in order to select appropriate techniques for cultivar identification in 23 *Pelargonium* cultivars. AFLP and STMS techniques were reported to be best methods for cultivar identification because of their minimum requirement of template DNA and high reproducibility.

RAPD, Inter Simple Sequence Repeats (ISSR) and AFLP markers were used to fingerprint and to exercise genetic diversity among 12 genotypes of the gooseberry (*Ribes grossularia*). AFLP generated unique profiles for each genotype (Sanchez *et al.*, 1998).

Crouch *et al.* (1999) while working with *Musa* breeding population found that of the different PCR-based markers systems (RAPD, VNTR and AFLP), AFLP assays had the highest multiplex ratio.

AFLP proved to be more powerful and reliable tool capable of producing a large number of genomic loci and discriminate genetic difference between phenotypically similar individuals with high reproducibility.

2.2.9 Microsatellites

The term microsatelites was introduced by Litt and Lutty (1989). Microsatellites are tandem repeats of DNA sequence of only a few base pairs (1-6 bp) in length. It consists of di-tri-tetra nucleotide repeats, which are hypervariable and ubiquitously distributed throughout the eukaryotic genomes, the most abundant being the dinucleotide repeats. Microsatellite DNA markers, which can be directly amplified by PCR, have been developed using the unique sequences that flank microsatellites (Litt and Lutty, 1989; Tantz, 1989; Weber and May, 1989).

Solivia *et al.* (2000) isolated and characterised a microsatellite loci from *Ophrys araneola* to study the influence of pollinating system or population genetic structure and on gene flow between similar co-flowering *Ophrys* species.

Microsatelites were used to study the genetic diversity in natural populations of *Dianthus* species and for the identification of carnation varieties (Smulders *et al.*, 2000).

Crouch *et al.* (1997) stressed the use of simple sequence repeat length polymorphism (SSRLP) assay for fingerprinting of *Musa* hybrids. Data on segregation at microsatelite loci in haploid and diploid gametes of *Musa* indicated the suitability of microsattelite markers for marker assisted selection system in *Musa.*

The microsatelites are highly polymorphic and thus highly informative. Being shorter in length they are easy to be cloned, sequenced and amplified through PCR. But the identification of informative microsattelite loci and consequence of suitable primer sequence is more cumbersome and expensive.

2.2.10 Sequence Characterized Amplified Region (SCAR)

SCAR analysis was developed to produce reliable PCR based results. Parent and Page (1998) used this technique to identify raspberry

cultivars. Damasco *et al.* (1998) used markers based on SCAR to detect dwarf off – types of *in vitro* grown Cavendish bananas.

2.2.11 Random Amplified Polymorphic DNA (RAPD)

Polymerase chain reaction in conjuction with random primers was used for fingerprinting genomes (Welsh and Mc Clelland, 1990), for population biology studies (Astley, 1992), identification of genome specific markers and other uses (Williams *et al.*, 1990 and Erlich *et al.*, 1991). This method utilizes single short oligonucleotide primers of arbitary sequence for the amplification of DNA segments distributed randomly throughout the genome, using polymerase chain reaction (PCR). In this reaction the primers of arbitary sequence bind to perfect or imperfect sites in the genomic DNA, so that a subset of them will be in inverted orientation to each other. As a result a number of bands are amplified through the action of DNA polymerase. The reaction products are conveniently analysed on agarose gels.

Several authors have applied the RAPD technique to investigate genetic variability and found the technique very efficient and reliable (Brown *et al.*, 1993; Munthali *et al.*, 1996).

Analysis of RAPDs offers several advantages, compared to other DNA based markers. The most important advantage is that RAPD is not a labour intensive procedure. It is not necessary to construct or maintain a genomic library. RAPD requires smaller quantities of genomic DNA and also it is less costly compared to others. Generation of RAPD is quicker and can be used to detect even single gene mutations (Williams *et al.*, 1990).

2.2.11.1 RAPD and Linkage Maps

RAPD assay has been used by several groups as an efficient tool for identification of markers linked to agronomically important traits which are introgressed during the development of near isogenic lines. Peltier *et al.*

(1994) established a linkage map for *Petunia hybrida* based on the RAPD and phenotypic markers. The map consisted of 35 RAPD loci, which covered 262.9 cM with a mean distance of 8.2 cM, dispersed over seven linkage groups.

Debener *et al.* (1996) reported that RAPD markers were used for the construction of chromosome linkage maps in *Rosa spp.* Debener and Mettiesch (1999) used RAPD and RFLP markers to construct the first linkage maps of rose genome. A total of 305 RAPD and AFLP markers were analysed in a population of 60 F_1 plants. Of these, 278 could be located on the 14 linkage groups of the two maps covering a total map length of 326 and 370 cM respectively.

Traits of interests studied also include jointless pedicel in tomato (Wing *et al.*, 1994), disease resistance (*Pseudomonas* resistance (Martin *et al.*, 1991) and spotted wilt virus resistance (Chaque *et al.*, 1996) in tomato, Anthracnose resistance in mango (Subramanian *et al.*, 1996), scab resistance in apple (Hong *et al.*, 1997 and Tartarini, 1996) and Lettuce infectious yellows virus resistance (Mc. Creight, 2000), leaf miner resistance in melon (*Cucumis melo*) (Moriera *et al.*, 1999) etc.

In an effort to map the loci affecting the cooking quality traits in basmati rice, a doubled haploid population from the basmati indica (Hasan Serai) x non-basmati japonica (Xiang Nuo 4) hybrid generated earlier was genotyped using 121 RAPD markers and a linkage map was constructed. Single factor analysis of variance revealed significant association between some of the markers and cooking quality traits (IARI, 1999).

The trait associated with the seed oil content in Indian mustard was identified with three RAPD markers *viz.*, OPH-11, OPJ-06 and OPL-15 (Sharma *et al.*, 1999). It was revealed that there was significant association of oil content with these markers.

Genetic linkage maps have been created in banana (Faure *et al.*, 1993), sweet cherry (Stockinger *et al.*, 1996), citrus (Christofani *et al.*, 1999), and in oil palm (Moretzsolm *et al.*, 2000) using RAPD.

2.2.11.2 RAPD and Species Relationships

RAPD markers have been widely used for taxonomic and related studies. RAPD markers were used to assess the relationship among species, cultivars and hybrids of *Lilac* (Marsolais *et al.*, 1993) in the classification of *Lilium* (Jongsuk *et al.*, 1994; Haesun *et al.*, 1999) the taxonomic positioning Kalanchoe (Gehrig *et al.*, 1997) and in the phylogenetic relationship between *Hibiscus syriacus* (Malvaceae) and 26 allied species (Jong-Hwa *et al.*, 1999).

Millan *et al.* (1996) analysed nineteen species of rose using RAPD markers. Each primer produced a specific banding pattern that grouped plants belonging to the same species in botanical sections. Dendrograms constructed showed a good correlation with previous classification based on morphological and karyological studies.

Taxonomy of 22 wild species of rose was analysed using isoenzymes and RAPD markers (Moreno *et al.*, 1996).

Makarevitch *et al.* (2000) constructed phytogenetic relationship of Siberian *Iris* species, based on an analysis of 56 RAPD markers in 12 Siberian *Iris* species and comparative analysis of trnL intron and trnL-trnF integeneric spacer non-coding chloroplast DNA sequences in 22 Siberian *Iris* species.

The technical simplicity of the RAPD technique has facilitated its use in the analysis of phylogenetic relationship in several plant genera, e.g. blueberry (Levi and Roveland, 1997), barley (Noli *et al.*, 1997), roses (Debener et al., 1997), Cymbidium (Okeyo and Kako 1998) etc.

Demek *et al.* (1992) investigated the potential use of RAPDs for taxonomic studies using *Brassica, Sinapis* and *Raphanus* taxa. Analysis

of the RAPD bands revealed the relationship between diploid and amphidiploid *Brassica* taxa. Results showed that the *Raphanus sativus* and *Sinapis alba* were distinct from the *Brassica* taxa.

Halward *et al.* (1992) reported the application of RAPD technique to cultivate peanut and related wild species to determine whether this approach would be feasible for the construction of a genetic linkage map in peanut or for systematic studies of the genus. No variation in banding pattern was observed among the cultivar and germplasm lines of *A. hypogea,* whereas the wild *Arachis* species were uniquely identified with most primers tested. The bands were scored (+/-) in the wild species and the PAUP computer programme for phylogenetic analysis and the Hyper RFLP programme for genetic distance analysis were used to generate dendrograms. The two analysis produced nearly identical dendrograms of species relationships.

Dunemann *et al.* (1994) investigated the use of RAPD markers for taxonomic studies in *Malus*. Eighteen accessions of wild species and 27 apple cultivars were treated with 29 preselected primers. The analysis of the bands using unweighted pair group arithmetic average showed the relationships among the cultivars which was in agreement with the known linkage. A dendrogram generated for wild species gave relationships that were in accordance with the known phylogenetic information.

Ratnaparkhe *et al.* (1995) used RAPD markers for the identification of pigeon pea (*Cajanus cajan* L. Mill sp.) cultivars and its wild species. The results showed externally high level of polymorphism among the wild species while little variation was detected within the accessions. The cultivars and wild species under study were distinguished with the help of different primers.

Lazaro and Aguinagalde (1998) carried out a study to evaluate the genetic diversity in 29 populations of wild taxa of the *Brassica oleraceae* L. group and two cultivars, using RAPDs. The results were compared

with the results of earlier study using isozymes. Genetic diversity in *Brassica* populations were estimated using the ANOVA programme.

Santalla *et al.* (1998) carried out a study to evaluate the genetic diversity in 19 races of the cultivated mungbean and three weedy and wild relatives including *Vigna mungo, V. luteola* and *V. radiata* var. *Sublabata*, using RAPD technique. Polymorphic bands of 229 numbers were obtained and the analysis of the bands resulted in a dendrogram which separated the accessions studied into three main clusters.

2.2.11.3 RAPD for Identification of Cultivars / Genotypes

RAPD assay has been used for the identification of cultivars and genotypes and for genetic finger printing and related studies in a variety of crop species.

RAPD analysis was used to detect genetic variations and similarities among *Heliconia* species, cultivars and hybrids by Goh *et al.* (1995). Significant differences in RAPD profile occurred amongst different *Heliconia* species and some distantly related plants. *H. rostrata* had three prominent bands that were absent in *H. psittacorum* cultivars.

Many *Heliconia spp.* are polymorphic with a large number of cultivars. Cultivar identification has been primarily based on morphological differences of the flower and inflorescence. A protocol was developed to extract DNA from Heliconia leaves and to analyse genetic variation using RAPD by Kumar *et al.* (1998).

Torres *et al.* (1993) differentiated rose cultivars by comparing DNA banding patterns.

RAPD markers were used to study cultivar identification in chrysanthemum (Wolff *et al.*, 1995).

Debener *et al.* (1996) reported that RAPD markers were used for the construction of chromosome linkage maps in *Rosa spp.* RAPD and RFLP banding patterns were utilized to identify and differentiate 22 cultivars of

rose (Ballard *et al.*, 1996). Taxonomy of 22 wild species of rose was analysed using isoenzymes and RAPD markers (Moreno *et al.*, 1996). Millan *et al.* (1996) analysed 19 species of rose using RAPD markers. Specific banding pattern was observed and that grouped plants belonging to the same species in to different botanical sections. Dendrograms constructed showed a good correlation with previous classifications based on morphological and karyological studies.

Aloisi *et al.* (1996) studied the molecular polymorphism in botanical and ancient roses via RAPD analysis using 10-mer Operon primers and observed lot of variation.

Debener and Mettiesch (1999) used RAPD and RFLP markers to construct the first linkage maps of rose genome. A total of 305 RAPD and AFLP markers were analysed in a population of 60 F_1 plants.

Riek *et al.* (1999) reported that variety protection could be done by use of molecular markers in ornamentals. In vegetatively propagated ornamentals, new varieties can be obtained from conventional breeding and selection or spontaneous or induced variant types of existing varieties. To detect essentially derived varieties (EDV), a criteria based on plant morphology are insufficient or even not applicable. In such case molecular markers are used for variety protection.

The genetic closeness of various species of *Vanda* was determined using RAPD markers. Strip-leaved *Vanda* sp. (including *Vanda sanderiana*) and *Ascocentrum miniatum* were more closely related to each other than to the terete leaved *Vanda* species studied. RAPD analysis supported the suggestion that terete leaved *Vanda* trees and *V. hookeriana* be classified in the separate genus *Papilionanthe* and that *V. sanderiana* should remain in the genus *Vanda* (Lim *et al.*, 1999).

Jau-Yueh *et al.* (1999) used RAPD markers to identify and to assess genetic diversity among 15 *Anthurium* cultivars. Twenty four DNA fragments

derived from eight primers were polymorphic and were used to distinguish the cultivars.

Random amplified polymorphic DNA (RAPD markers were used for the characterization of potential parents in a breeding programme on *Alstroemeria* for pot plant production by Beneditti *et al.* (2000). A similarity matrix was constructed to estimate the genetic similarity values between 12 genotypes. Cluster analysis was performed to examine the relationships among genotypes. The classification of genotypes through the use of four selected primers is in agreement with the expectations based on geographical origin and pedigree data. On the basis of RAPD data, the most distantly related genotypes were selected to start the breeding programmes. RAPD analysis thus resulted an effective tool for the genetic characterization of potential parents that may enable ornamental plant breeders to improve the efficiency of the breeding programme.

Morphological, karyological and molecular analysis was done in *Aster* spp. by Tornadore *et al.* (2003). Here two species of the genus Aster from Nepal have been studied and compared with a common European species *A. alpinus*. The aim was to verify the specific taxonomic classification and to characterize the aster population on the basis of morphological, karyological and molecular analysis.

Phylogenetic relationship among several subgenus *Yulania* species based on RAPD markers was done by Wang *et al.* (2003). Twenty materials of the subgenus Yulania (Magnolia: Magnoliaceae), including species and cultivars were analysed using RAPD markers. Fifteen primers were screened from 55 arbitrary 10-mer primers. A total of 274 DNA bands were amplified, 262 of which (95.6 %) were polymorphic. According to the data, a tree diagram of phylogenetic relationship was constructed using the UPGMA analysis of the MEGA programme. The

results showed that the genetic background of Yulania was very complex and difficult to classify.

Wen *et al.* (2003) reported genetic diversity in wild accessions of *Rosa roseburghii*. Tratt from four provinces as revealed by RAP analysis. The genetic variation among 30 wild accessions of *R. roxburghii* from four provinces of China was investigated by RAPD analysis using 16 arbitrary primers. With these primers 94 RAPD bands were detected, 62 (65.96 %) of which were polymorphic.

Detection of DNA sequence polymorphism among closely related lines of common wheat (*Triticum aestivum*) has been reported by He *et al.* (1992). A high level of polymorphism was observed among a number of commercial varieties and breeding lines of wheat. Over 38 per cent of the 65 primers, used for PCR amplification, produced readily detectable and reproducible DNA polymorphism.

Fukuoka *et al.* (1992) have reported the use of RAPDs for the identification of rice accessions. Sixteen rice accessions were assayed with 28 primers which generated 116 polymorphic bands. All accessions were uniquely distinguished by at least one RAPD and clustered into three distinct groups which corresponded to *Japonica, Javanica* and *Indica.*

Collins and Symons (1993) detected polymorphism between the nuclear DNA of grape vine (*Vitis vinifera*) cultivars and clones. Unique finger prints of a number of cultivars were distinguished using either one or two primers.

Koller *et al.* (1993) differentiated eleven apple cultivars using RAPD markers.

Wolff and Van-Riju (1993) studied the genetic variation in chrysanthemum (*Dendrauthema grandiflora*) using RAPDs. The results showed that the variation between cultivars was high and that the cultivars used could be distinguished from each other by using only two different

primers. A family of cultivars, derived from one original cultivar by vegetative propagation, had identical fragment patterns.

Mori *et al.* (1993) characterized 36 potato cultivars released in Japan and three introduced cultivars by RAPD assay. The banding patterns were highly reproducible and identical using DNA samples obtained from different locations and tissues. Five decamer primers amplified 15 polymorphic bands shared among the cultivars.

Coussirat (1994) have reported genetic diversity and varietal identification in *Nicotiana tabacum* by RAPD markers. These markers allowed differentiation of 32 varieties by calculation of genetic distance and cluster analysis.

Keil and Griffin (1994) assessed the reproducibility of the RAPD technique and its ability to discriminate between individual genotypes for varification of clonal identities in four separate studies, using samples of *Eucalyptus* supplied by several organizations. RAPD profiles unique to a genotype were generated reliably and simply and even closely related genotypes were distinguished.

N'goran *et al.* (1994) assessed the genetic diversity of 106 genotypes of *Theobroma cacoa* L. using RFLP and RAPD methods. Both RFLP and RAPD bands separated the individuals into three distinct groups. Similar results were obtained with RFLPs and RAPDs with regard to the genetic structure of *Theobroma cacao* L.

Bracaccia (1994) tried to optimize PCR variables for RAPD analysis in the genus Medicago and the evaluation of the reproducibility of genome specific fingerprints. The influence of type of thermal cycler, ramping temperatures, magnesium concentration, polymerase brand and primer performance on the optimal DNA yields and useful electrophoretic patterns was studied.

Gidoni *et al.* (1994) studied the feasibility of developing cultivar specific markers in commercial strawberries. Reproducible RAPD finger prints were generated for eight cultivars, each containing at least one polymorphic DNA product. A combination of 10 polymorphic DNA products exhibited cultivar specific patterns enabling the distinction between closely related varieties.

Virk *et al.* (1995) tested rice accessions obtained from the major world collections held at IRRI using RAPD technique to study the diversity within the large germplasm collection, at the University of Birmingham, UK. Optimization of PCR technique by testing the several factors affecting reproducibility of RAPD amplification was carried out. Variation between rice accessions was observed with 18 of the 24 primers employed.

Karihaloo *et al.* (1995) carried out RAPD analysis on 52 accessions of *Solanum melongena* and related weedy forms. Twenty two primers amplified a total of 130 fragments. *S. melongena* exhibited 117 fragments, all of which were also present in wild forms. Wild forms displayed an additional 13 fragments not found in the cultivated *S. melongena*. The RAPD results were closely concordant with the result of isozyme survey.

Bhat *et al.* (1995) carried out a study to identify the cultivars of *Musa* using RAPDs and RFLPs. Genomic DNA from 57 *Musa* cultivars were amplified with 60 random primers which generated 605 polymorphic products. Statistical analysis of the data grouped the cultivars into specific clusters depending upon their genomic similarities. The diploid ancestral species of cultivated banana and plantains, namely *M. acuminata spp., Malaccensis* and *M. balbisiana* were farthest apart from each other in the phenogram. The edible fruit yielding cultivars with the genomic constitution of AA, AAA, AB, AAB, ABB and ABBB were grouped in different clusters according to overall genetic homologies. The result of RFLP analysis wass comparable with that of RAPD analysis.

Lashermes *et al.* (1996) have successfully employed RAPD markers to analyse genetic diversity among cultivated and subspontaneous accessions of *Coffea arabica*. The narrow genetic base of commercial cultivars was confirmed by their study. On the other hand, a relatively large genetic diversity was observed within the germplasm collections. Results suggested an East-West differentiation in Ethiopia, the primary centre of diversification of *C. arabica.*

Hang *et al.* (1996) studied the DNA variation and genetic relationships among hulles barley accessions using RAPD at the National Small Grain Research Facility, Aberdeen, and USA. Thirty six hullers barley accessions from North America, China, Turkey and Central Asia were analysed and 47 reproducible fragments were produced by amplification. Cluster analysis revealed that most of the accessions from North America belonged to one well-defined cluster. Those from China, Turkey, Central Asia and one cultivar from North America belonged to another group.

Samec and Nasinec (1996) used RAPD technique for the identification and classification of *Pisum sativum* L. genotypes. Genomic DNAs of 42 genotypes representing four wild and cultivated subspecies were used for the study. Amplification with eight decamer primers generated 149 polymorphic products. Each genotype were clearly identified and separated from the others.

Mechado *et al.* (1996) carried a study in Brazil to evaluate polymorphism and genetic similarity between 39 Mediterranean mandarin genotypes, using RAPD. One hundred and eleven amplification products were identified using 21 random primers, cluster analysis revealed the low level of genetic variation between accessions of Mediterranean mandarins, whereas their hybrids with other citrus species showed greater genetic dissimilarity.

Demek *et al.* (1996) studied the genetic diversity of 28 potato genotypes using RAPD procedure. Twelve decamer primers yielded a total of 158 amplified fragments and analysis of the RAPD bands reflected the expected trends in relationships of the genotypes. However, there were important exceptions to this general trend and it appeared that the related varieties could be genetically different as varieties with no immediate relationships.

Ford and Taylor (1997) also reported the application of RAPD markers for potato cultivar identification. Of the 63 decamer primers screened, 51 produced 256 amplification products of which 33 were polymorphic between the cultivars assessed. Polymorphic bands were selected to produce cultivar specific markers to identify correctly suspect materials in commercial plantings.

Stavrakakis *et al.* (1997) used RAPD technique in order to identify and discriminate between eight cultivars of *Vitis vinifera*. Over 140 reproducible polymorphic fragments were generated by this method. Each grape cultivar showed a unique banding pattern for more than five of the primers used. The degree of genetics similarity was calculated and a dendrogram was constructed. The results showed that RAPD analysis was a reliable and very useful method for the identification and genomic analysis of grape cultivars.

Iqbal *et al.* (1997) have used RAPD analysis to evaluate the genetic diversity of elite commercial cotton varieties. Twenty two varieties belonging to *Gossypium hirsutum* and one to *G. arboreum* were analysed with 50 random decamer primers. Forty nine primers detected polymorphism in all varieties tested giving 349 bands, cluster analysis showed that 17 varieties can be placed in two groups.

Duran *et al.* (1997) analysed 48 coconut types belonging to the East African Tall types by different DNA marker techniques including RAPDs, microsattelite primed PCR and ISTR analysis. All three approaches

detected large number of DNA polymorphism among the set of genotypes and allowed the identification of single genotypes by individual-specific finger prints. The cluster and principal co-ordinate analysis were done and the observed clustering and association of individuals corroborated the expectations based on the known geographical origin and parental relationships.

Varghese *et al.* (1997) evaluated the applicability of RAPD markers in the cultivated rubber tree, *Hevea*, using 43 primers in a set of 24 clones selected in different South-East Asian countries. Out of the total 220 fragments amplified 111 were polymorphic. The statistical analysis indicated the absence of a distinct geographical grouping because of the breeding of *Hevea*.

2.2.11.4 RAPD Detection of Genetic Variability

RAPD technique has been reported to be used to study the genetic variation in chrysanthemum (*Dendranthema grandiflora*). The variation between cultivars was high and the cultivars were distinguished from each other by using only two different primers (Wolff and Van-Riju, 1993).

Aloisi *et al.* (1996) studied the molecular polymorphism in botanical and ancient roses via RAPD analysis using 10-mer operon primers and observed large variation.

Scott *et al.* (1996) examined twenty-one cultivars of chrysanthemum that belonged to several species using RAPD techniques. A few polymorphic characters were uniquely identified in closely related cultivars within each of the species. In contrast many DNA polymorphism were observed between members of different families.

Wang *et al.* (1999) suggested that RAPD markers are useful in the breeding programmes of Anthurium (*A. andreanum* x *A. amnicola*), allowing cultivar identification as well as genetic similarity among

genotypes, which will be valuable in selecting the best parents to obtain new hybrid combinations.

RAPD technique was used to study the genetic diversity and relationship between different species of *Jasminum* (Mukundan, 2000).

Wang *et al.* (2001) assessed the genetic similarity of thirty Anthurium cultivars using both ISSR and RAPD markers. The result suggested that both ISSR and RAPD markers are useful in the breeding programme of Anthurium and also for cultivar identification.

Ranamukhanachchi *et al.* (2001) utilized RAPD markers to determine the genetic relationships of nine morphologically similar pot plant cultivars of *Anthurium* sp.

Kozyrenko *et al.* (2002) carried out the investigation of callus cultures derived from six *Iris* species using RAPD analysis. The callus lines originating from one maternal plant had the highest genetic similarity indices, while the callus cultures derived from maternal plants of different species had the lowest indices.

Arafeh *et al.* (2002) observed the pattern of genetic variation in *Iris hacynei* and *I. atrofuscea* with RAPD markers. Similar studies on Iris was carried out by Johnston *et al.* (2001), Zhuravlev *et al.*, 1998; Cruzan and Arnold, 1994).

Wroblewska *et al.* (2003) used RAPDs to investigate genetic diversity and it partition within and between three populations of Iris. The results indicated that genetic diversity was independent of population size.

RAPD markers have been used successfully to detect genetic variation among lowland and upland rice cultivars and the genetic characterization and classification of Japonica cultivars into temperate and tropical groups for analysis of genetic variability in rice populations (Yu and Nguyen, 1994). In general, a higher level of polymorphism was found between upland and lowland cultivars within the *indica* subspecies. Thus

rice cultivars, genetic variation was easily detected using RAPDs.

According to Verma *et al.* (1999) RAPD analysis allows the identification and discrimination of the individual genotypes of Basmati rice including the identification of duplicates in genetic resource collections. Random amplified polymorphic DNA technique was used to detect the genetic variation at the level of DNA among the aromatic and non-aromatic cultivars by Baishya *et al.* (2000). With the objective of identifying and classifying 48 aromatic rice genotypes, RAPD profiling was employed using 58 random decamer primers. Most of these primers (96.5 per cent) detected polymorphism among the genotypes. Of the 465 amplified bands 314 were polymorphic. All the rice genotypes included in the study could be distinguished from each other at the level of 19 to 186 polymorphic bands between individuals in pair wise comparison over all the 58 primers (Choudhary *et al.*, 2001).

Random amplified polymorphic DNA markers were used for fingerprinting genotypes within and between *Annona* species (Renning *et al.*, 1995). The use of RAPD analysis for *Mangifera* germplasm classification and clonal identification was reported by Schnell *et al.* (1995). Graham and Mc Nicol (1995) generated RAPD markers from different *Rubus* species in order to access the degree of similarity between species. Inter and intra specific varietal variations were observed in the RAPD analysis of 42 accessions of *Vitis*, representing 13 species (Wang *et al.*, 1998). According to Lanham and Brennen (1999), RAPD markers were used to fingerprint and to examine genetic diversity among 12 genotypes of gooseberry. Six hazelnut (*Corylus avellana*) cultivars were identified using RAPD markers (Galderisi *et al.*, 1999). An RAPD analysis was carried out by Egashira *et al.* (2000) to investigate genetic diversity of 'peruvianum-complex' (PC) species of highly polymorphic wild tomato relatives and the genetic relationship between the PC and the 'esculentum - complex (EC) species including the cultivated species. A total of 435

RAPDs were obtained from 50 accessions of all the nine *Lycopersicon* species using only 16 random primers.

RAPD markers can be used to detect genetic variation at the intra as well as interspecific level (Aboelwafa *et al.*, 1995).

Randomly amplified polymorphic DNA analysis was done to determine intra-specific variability in *Andrographis paniculata*. The results indicated that RAPD could be effectively used for genetic diversity analysis in wild species of prospective value as it is reliable, rapid and superior to those based on pedigree information (Padmesh *et al.*, 1999).

According to Hu and Quiros (1992), RAPD markers provided a quick and reliable alternative to identify broccoli and cauliflower cultivars. RAPD markers generated by four arbitrary-10-mer primers, discriminated 14 broccoli (*Brassica oleraceae italica*) and 12 cauliflower (*B. oleraceae botrytis*) cultivars by banding profiles.

Iqbal *et al.* (1995) used RAPD markers to establish polymorphisms among local sugarcane varieties. The amplification profiles of the varieties L118, L116, BL4, BF162, CO144 and CO154 were compared and polymorphisms were detected.

The RAPD technique was used for cultivar identification of 11 aubergine cultivars. Twelve 10-mer primers were used, 9 of which revealed polymorphism in cultivars (Kochieva *et al.*, 1999).

RAPD markers have been used to characterize cocoa clones representing the three main cultivated subpopulations *viz.*, Criollo, Forestro and Trinitario (Wilde *et al.*, 1992). The use of single primers of arbitrary nucleotide sequence resulted in the selective amplification of DNA fragments, which were unique to the individual cocoa clones studied. Mulcahy *et al.* (1993) characterized twenty-five accessions of apple, representing eight cultivars (Golden Delicious, Delicious, Gala, Jonathan, Jonagold, Florina, Fior di Cassia and Imperate Dallago) with

RAPD. Using separate ten base pair primers, it was possible to obtain a distinctive fingerprint for each of the cultivars. Thus RAPD provided a simple and reliable method for cultivar identification in apple. Efforts were done in *Piper longum* to find out the genetic difference among the varieties using RAPD analysis by Philipp *et al.* (2000).

RAPD and SSR markers were used to characterize genetic relationship among 46 accessions in two *Cucumis melo* L. subsp. (*Cantaloupensis lodorus*) and subsp. *agrestis* (Conomon and Flexuous) groups (Jack *et al.*, 2000). Empirical estimation of variances associated with each marker type in the accessions examined indicated that per band, lower coefficients of variation can be attained in the estimation of genetic diversity when using RAPDs compared to SSRs. Result of RAPD marker analysis suggest that 80 marker bands were adequate for assessing the genetic variations present in the accessions examined.

Evaluation of the genetic diversity among 27 superior tea accessions (*Camellia sinensis* var. *sinensis*) accessions from Korea, Japan and Taiwan was done by Kaundun *et al.* (2000) using RAPD-PCR markers. Out of the 50 primers screened, 17 primers generated 58 polymorphic and reproducible bands. A minimum of three primers was sufficient to distinguish all the 27 accessions studied.

In order to assess genetic diversity of clones of a subset collection of wild apple which were collected at forest sites in the federal state of Herse. PCR based methods were applied by Vornam and Gebhardt (2000). RAPD analysis allowed them to distinguish clones and single tree progenies.

Using the technique of RAPD, Howell *et al.* (1994) identified 116 amplification products in *Musa* germplasm using nine primers. This enabled them to identify RAPD markers that are specific to each of the nine genotypes of *Musa* representing AA, AAA, AAB, ABB and BB genotypes. Fifty seven accessions of *Musa* including cultivated clones of

six genomic groups (AA, AB, AAA, AAB, ABB, ABBB), *M. balbisiana* Colla (BB), *M. accuminata* Colla ssp. Banksii F. Muell. (AA), *Musa accuminata* Colla ssp. Malaccensis Ridl. (AA) and *M. velutina* Wendl. and Drude were examined by Bhat and Jarret (1995) for RAPD genetic markers using PCR with sixty 10-mer random primers which generated 605 polymorphic amplification products. RAPD analysis was performed on several clones of the variety Williams by Iqbal *et al.* (1995). Results of the studies by Bhat *et al.* (1995) on DNA profiling of banana and plantain using RAPD and RFLP markers showed that the use of different kinds of molecular markers in gene banks is essential for characterization and classification of germplasm collections.

RAPD using operon primers was used to evaluate genetic variability of 66 *Musa spp.* accessions in the germplasm collections of the National Banana Corporation of Costa Rica (CORBANA) and the Agronomic Centre for Research and Training (CATIE) also in Costa Rica. PCR products were separated electrophoretically in agarose gels which were then analysed on a binary basis [presence (1) or absence (0) of bands]. The data obtained were used to generate a similarity matrix according to Jaccard's criteria, and a phenogram was built. High variability among the AA clones and a low variability among the AAA, AAB, ABB triploids were observed (Cabrera *et al.*, 1998).

With the aim of identifying RAPD markers for the A and B genomes, Pillay *et al.* (2000) used eighty 10-mer operon primers to amplify DNA from *Musa acuminata* sub sp. *burmanicoides* clone Calcutta 4 (AA genomes) and *M. balbisiana* clone Honduras (BB genomes). Three primers (A-17, A-18 and D-16) that produced unique genome specific fragments in the two species were identified. The results showed that RAPD analysis could provide a quick and reliable system for genome identification in *Musa* that could facilitate genome characterization and manipulations in breeding lines.

Genetic diversity among 76 plantain land races has been studied by Crouch *et al.* (2000) using RAPD analysis at two levels of intensity and compared with groupings based on phenotypic indices and morphotypes. There was also a poor correlation between RAPD analysis and morphotype do not provide a true reflection of overall genetic divergence.

RAPD analysis of fifteen African plantain land races by Newbury *et al.* (2000) revealed a very low proportion of polymorphic bands (13 of 276). However, further examinations of these thirteen marker bands demonstrated that they varied within land races and could not be used to distinguish between land races.

2.2.11.5 RAPD and Hybrids

Debener *et al.* (1997) reported that molecular markers were used for the identification of inter-specific rose hybrids in three cross *viz., Rosa acicularis* x *R. similes, Robturfolia x R. sherardii* and *R. stylosa* and *R. gallica.* All putative F_1 hybrids were unambiguously identified as true hybrids using 24-42 RAPD markers.

RAPD markers were used to verify inter-specific hybridization in *Alstroemeria* (Benedetti *et al.*, 2000). Five putative inter-specific hybrids and their parents were analysed by four RAPD primers. The putative parentage was confirmed in four hybrids and was excluded in one.

Takatsu *et al.* (2001) studied genetic relationship among wild gladiolus species through RAPD analysis. Out of the 140 tested primers, 32 amplified a total of 133 RAPD bands. Genetic distance was calculated and dendrogram was generated. Interspecific crosses were carried out in seven combinations within or between clusters and F_1 seedlings were obtained from most combinations. The RAPD analysis showed that these F_1 seedlings were real hybrids.

RAPD technique has been used for the identification of hybrids and their parentage determination. Wang *et al.* (1994) proposed RAPD finger

printing as a conventional tool for the identification, protection and parentage determination of plant hybrids. In their study, DNA from three families of rice plants selected in Northern China (each comprising the male sterile, the restorer, the hybrid F_1 and the maintainer lines) was extracted and amplified by RAPD technique. The results obtained were useful for identification of each single plant line.

Truksa and Prochazka (1996) reported different banding pattern based on the DNA polymerase used for testing three lines of cucumber used for production of hybrid seeds. Low level of polymorphism was obtained which indicated that RAPD was not suitable for verifying the hybridity of seeds.

Tatineni *et al.* (1996) analysed genetic diversity of 16 near homozygous elite cotton genotypes derived from inter specific hybridization. The study was conducted at the DNA level using RAPD procedure and at phenotypic level using stable and highly heritable morphological characters. Analysis of the data from both producers produced two clusters with one resembling *Gossypium hirsutium* and the other *G. barbadense*. Classification of all genotypes based on the two methods given similar results.

Harvey and Botha (1996) evaluated two PCR based methodologies in the determination of DNA diversity between 20 commercial sugarcane hybrids and six 'outgroup' varieties of *S. spontaneum*, *S. officianarum* and hybrids from early in the geneology. First method involved RAPD while the second protocol utilized specific microsatellite and telomere sequences as primers. A total of 41 RAPD primers were screened across the varieties of which 15 were used in the calculation of DNA diversity. The RAPD data indicated that there had been a gradual decline of DNA diversity from the early interspecific hybrids to the commercial hybrids.

RAPD markers have been successfully used to test the paternity of Japanese pear hybrid (Bano *et al.*, 2000).

2.2.11.6 RAPDs for Identification of Somatic Hybrids

One of the limiting factors for the efficient exploitation of protoplast fusion is the difficulty of unequivocally identifying nuclear hybrids. RAPDs have been used to characterise molecular relationship of both interspecific and intraspecific somatic hybrids.

Protoplast fusion of *Iris ensata* and *Iris germanica* by electrofusion was done and six months after cell fusion, RAPD analysis was done which yielded bands specific to both parental species. RAPD analysis confirmed that the resultant cross was somatic hybrid (Shimizu *et al.*, 1999).

Baird *et al.* (1992) proposed RAPDs for the identification of hybrids at an early stage following fusion in potato. Inter and inter-specific somatic hybrids of potato were characterized by using RAPD along with the sexual hybrids.

Xu *et al.* (1993) used RAPD assay for the identification of somatic hybrids between *Solanum tuberosum* and *Solanum brevidens*. Somatic hybrids showed a combination of the parental banding pattern with four of the five primers surveyed. Whereas regenerants from one of the parents had a similar banding pattern as that of the parent.

RAPD analysis for the confirmation of somatic hybrids in the dihaploid breeding of potato (*Solanum tuberosum* L.) was reported by Takemori *et al.* (1994). Polymorphism was detected even among closely related clones. All the bands of dihaploids were transmitted stably to the respective hybrids. Hybridity of all the fusion-derived regenerants of 32 fusion combinations were unequivocally confirmed.

Identification of somatic hybrids of tobacco was reported by Filippis *et al.* (1996) from Germany. The relationship between two parental species (*Nicotiana tabaccum* and *N. sustica*) and six somatic hybrids produced as a result of fusion of vacuolated and evacuolated protoplasts were analysed. The procedure and protocols were applicable to

species as well as to hybrids tested and just four primers gave enough polymorphic data.

2.2.11.7 RAPD and Somaclones

There are several reports on the use of RAPD technique for assessing the genetic stability of tissue culture derived plants. It will be highly advantageous if the variants among the regenerants could be identified at an early stage. RAPD method seems to be a suitable method for this.

Oropeza *et al.* (1995) have characterized somaclonal variants resistant to sugarcane virus using RAPD markers. The somaclones were obtained from a susceptible cultivar through somatic embryogenesis by increasing the number of subcultures of embryogenic callus. The selected resistant subclones have maintained the resistance over seven years of testing in the field.

Rani *et al.* (1995) used RAPD markers to assess the genetic fidelity of 23 micropropagted plants of a single clone of *Populus deltoides*. Of the eleven primers used, five distinguished a total of 13 polymorphism common across six micropropagated plants. Apart from these six plants, the amplification products were monomorphic across all the micropropagated plants, the mother plant and four additional field grown control plants.

Sangwan *et al.* (1995) employed RAPD technique to investigate molecular differences and also to generate polymorphic DNA markers in regenerated cassava plants. Twenty primers were used to generate patterns from several regenerants of two cultivars.

Schneider *et al.* (1996) have employed RAPD technique to identify the somaclonal variants in grape vine (*Vitis vinifera*) regenerants. Forty seven plants, each one regenerated from a single protoplast were analysed using sixty primers. Only one primer indicated somaclonal variation.

HuyBao *et al.* (1996) revealed molecular evidence for the occurrence of stable genomic changes in the transgenic plants by a study in Italy. RAPD approach was employed to analyze the microspore-derived embryogenic rice cells grown in suspension cultures, transgenic plants recovered from protoplasts produced from the cultured cells and self-pollination progeny of transgenic plants. Occurrence of somaclonal variation in the material used to produce protoplasts for gene transfer and the stability of the foreign gene in transgenic plants were established.

Damasco *et al.* (1996) reported detection of dwarf off types for micropropagated Cavendish banana plants (*Musa* spp. AAA). Fifty seven normal and 59 dwarf plants generated through micropropagation of cultivars New Guinea Cavendish and Williams were analysed using 60 random primers of which 19 gave polymorphism between normal and dwarf plants.

Mandal *et al.* (1996) tested seven somaclones of *Lathyrus sativus* having contrasting features and the parent cultivar for genetic identity using RAPD. Out of the 81 primers used, 24 revealed polymorphism. Differences were observed between somaclones and also between some of the somaclones and parent cultivar. It was not possible to identify a particular somaclone with a single primer and a combination of two or more primers was suggested.

Munthali *et al.* (1996) reported detection of somaclonal polymorphism using RAPD, the result of which was similar to a previously reported study employing isozyme and RFLP technologies. One hundred and twenty regenerants of beet and a parent were analysed with five primers and two polymorphism were obtained. Thirty secondary regenerants were then tested and only single band polymorphism was obtained.

Angel *et al.* (1996) have analysed the stability of cassava plants stored under *in vitro* conditions. The study was carried out at CIAT to

determine whether any DNA rearrangements resulting from *in vitro* storage under slow growth could be detected by molecular analysis in retrieved plants. RFLPs with homologous probes, RAPDs with twenty primers and DNA fingerprinting with M-13 probe were tested to detect variation in cassava plants after 10 years of *in vitro* storage. The data obtained in this study support the stability of the cassava germplasm under *in vitro* storage conditions.

Walther *et al.* (1997) described the possibility of early detection of *in vitro* mutants using RAPD analysis. Four different types of somaclonal variants were identified and characterized in banana plants generated by meristem culture.

Piccioni *et al.* (1997) reported the estimation of somaclonal variation in alfalfa by RAPD fingerprinting. Plantlets obtained through enhanced release of axillary buds on a growth regulator-free medium was compared with those obtained through indirect somatic embryogenesis. No polymorphism was exhibited by plantlets derived through enhanced release of axillary buds whereas nine out of 39 plantlets regenerated by indirect somatic embryogenesis different from that of the donor plant for at least one primer and one amplification product.

Kokaeva *et al.* (1997) have compared several lines and somaclonal variants of pea (*Pisum sativum*) using RAPD analysis. Data obtained revealed that there was considerable divergence between lines originating from different cultivars and between somaclonal variants that originated from same cultivar.

Godwin *et al.* (1997) performed RAPD analysis among eight rice somaclonal families known to vary for specific characters and somaclonal families, which were phenotypically normal. The parental cultivar was found to be homogenous and homozygous and all but one of the RAPD loci. Polymorphism was reported at 28 of the 45 bands among the

somaclonal families. All somaclonal material differed significantly from the parental material.

A report from Japan by Shoyama *et al.* (1997) showed confirmation of homogeneity among plantlets of ginseng regenerated through somatic embryogenesis by employing RAPD technique. Analysis using 21 primers gave monomorphic amplification products for all the plantlets of *Panax notoginseng*.

Hashmi *et al.* (1997) analysed peach (*Prunus persica* (L.) Batsch) regenerants from cv. Sunhigh and Redhaven. Of the 60 primers tried, 35 primers produced results with scorable bands and only 10 of them revealed polymorhism for regenerants of cv. Sunhigh and one revealed low level of polymorphism for regenerants of Redhaven.

Parani *et al.* (1997) reported RAPD fingerprinting in twenty micropropagated plants and the mother plant in *Piper longum*. The RAPD fragments were scored for presence /absence of bands to evaluate Jacard's similarity index. Further analysis of the data showed eighteen micropropagated plants forming a major cluster along with the mother plant. The other two plants could be regarded as somaclonal variants as they have shown less than 80 per cent similarity to the mother plant and other micropropagated plants.

Rout *et al.* (1998) used RAPD markers to evaluate the genetic stability of micropropagated plants of *Zingiber officinale* cv. W3S18. Fifteen primers were used for the study and all the RAPD profiles from micropropagated plants were monomorphic and similar to those of field grown control plants.

Rival *et al.* (1998) conducted RAPD analysis in order to investigate the genetic fidelity of somatic embryogenesis derived regenerants of oil palm (*Elaeis guinensis* Jacq.). Clonal variants were compared with the normal type using 387 primers. Of these, 73 primers enabled the identification of

polymorphism between clones. No intraclonal variability and no difference between mother palms on regenerants could be identified.

A study by Khanuja *et al.* (1998) at the Central Institute of Medicinal and Aromatic Plants, Lucknow proved the utility of RAPD markers for rapid isolation of somaclones of altered genotypes in *Mentha arvensis*.

Randomly amplified polymorphic DNA markers were found to be useful for confirmation of genetic fidelity in micropropagated banana plants (Gupta *et al.*, 1999). Somaclonal variant CIEN BTA-03 resistant to yellow sigatoka leaf spot was obtained from a susceptible banana clone (Williams clone) by increasing the production of adventitious buds using 6-benzyl amino purine at high concentration (Vidal and Garcia, 2000).

According to Lu *et al.* (1996), RAPDs are useful for establishing a genetic basis for somaclonal variation in rice. The results of RAPD analysis in cultured rice showed that somaclonal variation might have occurred in transfer RNA, ribosomal protein and other genes during cell culture. Also somaclonal variation was found to increase with culture age (Yang *et al.*, 1999).

Random amplified polymorphic DNA technology was applied to monitor the genetic fidelity of micropropagated, meadow fescue *viz.*, *Festuca pratensis* (Valles *et al.*, 1993), Norway spruce (Heinze and Schemidt, 1995) and Strawberries (Kumar *et al.*, 1995).

Plants regenerated by somatic embryogenesis from long term callus cultures derived from five garlic cultivars were subjected to RAPD analysis (Al-Zahim *et al.*, 1999). Certain changes were observed in the RAPD profiles of the regenerants of different cultivars, suggesting the existence of somaclonal variants.

RAPD analysis was done by Babu (2000) to access the genetic stability in tissue culture derived black pepper plants. Monomorphic banding pattern was observed for the tissue culture regenerants, compared

with their respective source plants. Uniformity was confirmed at both stages of development studied. Thus genetic stability and clonal fidelity was ensured for the tissue culture regenerants and the viability of the protocol was confirmed.

2.2.11.8 Identification of Chimera, Sports and Mutants

RAPD markers have been reported to be used to study sporting and chimerism in chrysanthemum (Shibata *et al.*, 1998).

Debener *et al.* (2000) used RAPD and AFLP markers to study the genetic difference between sports from two cut flower rose varieties as well as a garden rose variety. There was no polymorphism between the sports of the cut rose variety and the original cultivar, whereas five polymorphism were detected between the garden rose variety and its sports.

Pathania and Misra (2001) confirmed the mutability of gladiolus cv Eurovision mutants. Out of seven random primers used for PCR amplification one primer OPX-02 produced polymorphic banding pattern. A monomorphic band of 1 Kb was present in both the parent cultivar and its mutant. However a single band 0.8 Kb difference between five mutants ERM3, ERM4, ERM5, ERM6 and ERM7 was obtained.

RAPD is a fast and sensitive method, requiring only small amounts of DNA and involving no radioactivity hazards. However, RAPD's have certain quality problems like confinement to dominant gene expression and occasional low reproducibility of results.

2.2.11.9 RAPD in Sex Determination

Early identification of sex in dioecious plants like papaya (Somri, 1998) and nutmeg (Shibu *et al.*, 2000) was possible with the help of RAPD markers.

Genotypic and morphogenetic differences among three female varieties of *P. longum,* one variety each from Assam and Calicut and are

variety released from Kerala, were investigated using RAPD analysis and it was revealed that those varieties were genetically different. In *Piper longum* RAPD technique was used to investigate the molecular basis of genotypic differentiation between the male and female parents (Banerjee *et al.*, 1999). As a result male sex associated RAPD markers were identified for the first time in *Piper longum*.

2.3 *IN VITRO* PROPAGATION OF HELICONIA

Micro propagation is attracting considerable attention for obtaining large number of genetically pure elite propagule (Anand and Bir, 1984). According to Murashige (1974) there are three possible routes available for *in vitro* propagule multiplication (a) enhanced release of axillary buds (b) production of adventitious shoots through organogenesis and (c) somatic embryogenesis.

Vegetatively propagated rhizomes of heliconia cannot be imported into several countries without stringent quarantine control due to discovery of *Pseudomonas solanacearum* in diseased plants grown from rhizomes. However, *in vitro* propagation techniques have been developed recently for production of disease-free propagules (Lalrinawmi and Talukdar, 2000b). This review highlights the research on the various aspects of *in vitro* propagation in heliconias and related crops.

Axillary and apical shoots contain quiescent or active meristems depending on the physiological state of the plant. Only a limited number of axillary meristems have the capacity to develop *in vitro*, if the type of branching of a particular species displays apical dominance. Since the mechanism of apical dominance has been demonstrated to be under control of various growth regulators, the proportion of these substances in the media can be so manipulated as to induce each meristem to regenerate a shoot in culture (Razdan, 1993).

2.3.1 Explants

Axillary buds have been used together with shoot apex in most of the tissue culture works of Heliconia. Loh *et al.* (1978) were of the opinion that there was generally a decrease in the generative capacity when the buds were derived farther away from the apex. Success of *in vitro* propagation always depends on the proper selection of explants. The response varies according to the type, stage and physiological age of the explants.

In axillary shoot proliferation, cytokinin is utilized to overcome the apical dominance of shoots and to enhance the branching of lateral buds from leaf axils. This enhanced release of axillary buds with cytokinins was discovered by Wickson and Thimann (1958).

A protocol was developed by Nathan *et al.* (1992) for *in vitro* propagation of *H. psittacorum* by using terminal and axillary buds of rhizomes.

In *H. psittcorum* cv. Choconiana, dwarf rhizomes tips shorter than 4 cm, from fast growing stock plants, resulted in the lowest contamination (Pedersen and Brandt, 1992).

Shiau *et al.* (1998) conducted a study to identify and control the contaminants in tissue culture of *H. psittacorum*. Explants from different parts of *H. psittacorum* were tested for the percentage of contamination. The lowest contamination rate was obtained from leaf tip explants (57.7) after 21 days of culture. Contamination rates of 80, 83.3, 90, 96.7, and 100% were observed for petioles, leaf base, ovaries, peduncle and terminal buds respectively.

In vitro propagation of *H. psittacorum* cv. Rhizomatosa, was done by Shiau *et al.* (1999) using terminal shoot tip explants.

Lalrinawmi and Talukdar (2000b) reported that explants for shoot proliferation was obtained in *H. psittacorum* should contain terminal and axillary buds.

Talukdar *et al.* (2002) obtained shooting and rooting in *H. psittacorum* rhizomes when shoot buds were used as explants.

A three-fold increase in length and more than double in breadth was obtained in *H. psittacorum* when axillary buds were used as explants (Bora and Paswan, 2003).

Shoot tip culture of *Iris* sp. cv. 'Ovation' stimulated early lateral bud formation (Lucretti *et al.*, 1984).

Linde and Hol (1988) recorded multiplication within seven days when basal parts of the shoots were used as explants.

Bulb segments of *Iris xiphium* var. Hybridium when cultured on MS medium supplemented with different growth regulators, adventitious bud proliferation occurred easily (Huang *et al.*, 1999).

Clonal propagation *via* enhanced release of axillary buds was successful in many bulbous ornamental plants belonging to the family Iridaceae and Liliaceae (Hussey, 1976a and 1976b). In gladiolus, Hussey (1977) reported precocious shoot formation using axillary buds from corms as explant material. Apical buds from corm (Takatsu, 1982; Lilien-Kipnis and Kochba, 1987; Ziv, 1979) and cormel (Lilien-Kipnis and Kochba, 1987) and axillary buds from corm (Ziv, 1979; Takatsu, 1982) were also reported as explants.

Corm production in *Gladiolus dalenii* and *Gladioulus tritis* was increased when axillary buds with corm tissues were used as explants (De-Bruyn and Ferreira, 1992).

Dantu and Bhojwani (1995) reported optimum elongation rate for shoot cultures of *Gladiolus spp.* cv. 'Friendship', initiated from axillary buds.

Shoot and root proliferation was obtained in terminal or axillary stem scale sections from *Polianthes tuberosa* cultivars Shrinager (Single type) and Suvasini (double type) (Krishnamurthy *et al.*, 2001).

2.3.2 Surface Sterilants

The explants collected from the field harbour numerous microorganisms which when inoculated into the nutrient media contaminate the entire *in vitro* system. Hence surface sterilization is resorted prior to inoculation of explants. While sterilizing the explant minimum damage should occur to the plant or part to be cultured. The efficiency of the sterilants depends upon the type of chemical, concentration, duration of exposure etc.

A procedure for the disinfection of rhizome tips, based on trimming of scale leave three times alternating with short (1-10 min) immersions in disinfectant was used with *Heliconia psittacorum* cv. Choconiana Dwarf. The per cent of growing, uninfected explants obtained after three weeks of culture on MS medium supplemented with growth regulators was 60. Rates of infection increased significantly when one of the three steps was omitted. Rhizome tips shorter than 4 cm, from fast growing stock plants, resulted in the lowest contamination (Pedersen and Brandt, 1992).

According to Shiau *et al.* (1999), lower rates of contamination of *H. psittacorum* cv. Rhizomatosa were obtained in liquid MS medium supplemented with 500-mg/litre cefotaxime for three days.

Bora and Paswan (2003) recommended sodium hypochlorite as a surface sterilant for heliconia. They excised tissue blocks of 1 cm containing axillary buds and washed in running tap water for 15 minutes and then rinsed in 80 per cent ethanol for 30 seconds and immersed for 15 minutes in 0.8 per cent sodium hypochlorite solution with continuous agitation. After that 2 – 3 rinses of buds with sterile distilled water for five minutes each time were done. Outer tissues of each bud were further trimmed to a size of 4 mm, which was used as explant. Before culturing, they were again surface sterilized with sodium hypochlorite solution for 10 minutes and rinsed with distilled water for two to three times.

Misra and Singh (1999) recommended 0.1% $HgCl_2$ for 10 minutes for surface sterilization of *in vitro* propagation of *Gladiolus spp.*

Priyakumari (2001) recommended 0.08% of $HgCl_2$ as the best sterilant for *Gladiolus spp.*

Krishnamurthy *et al.* (2001) reported that 0.1 per cent Hg Cl_2 was the best sterilant for terminal or axillary stem scale sections of *Polianthes tuberosa*.

2.3.3 Culture Media

Composition of media influences the growth and morphogenesis of plant tissues. The principal components of plant tissue culture media are inorganic nutrients (macronutrients and micronutrients), carbon sources or organic supplements, growth regulators and gelling agent. Murashige and Skoog's (1962) developed MS medium for *in vitro* culture of explant species. Because of the desired salt composition MS medium is widely ısed (Razdan, 1993).

ℑasal media	Species / cultivars	Reference
MS medium	*H. psittacorum*	Nathan *et al.* (1993)
MS medium	*H. psittacorum*	Goh *et al.* (1995)
MS medium	*H. psittacorum* cv. Choconiana	Pedersen and Brandt (1992)
MS medium	*H. psittacorum* cv. Rhizomatosa	Shiau *et al.* (1998, 1999)
MS medium	*Heliconia* sp.	Sato *et al.* (1999)
MS medium	*H. psittacorum*	Lalrinawmi and Talukdar (2000b)
MS medium	*H. psittacorum*	Talukdar *et al.* (2002)

MS medium as the ideal medium for *in vitro* propagation of gladiolus was also reported by various workers [Sutton, (1978); Logan and Zettler, (1985); Dickens *et al.*, (1986); Bhojwani and Dantu, (1986); Lilien-Kipnis and Kochba, (1987); Rao *et al.*, (1991); Gosal and Grewal, (1991); Steinitz *et al.*, (1991); De Bruyn and Ferreira, (1992); Prasad *et al.*, (1993), Dantu and Bhojwani, (1995); Hussain *et al.*, (1995); Nagaraju and Parthasarathy, (1995); Misra and Singh, (1999); Babu and Chawla, (2000) and Pathania *et al.*, (2001)].

MS medium was recommended as the best culture medium for Iris by Lucretti *et al.* (1984), Linde and Hol (1988), Bach (1988), Linde *et al.* (1988) and Huang *et al.* (1999).

In *Polianthes tuberosa* MS medium was recommended by Bose *et al.* (1987) and Krishnamurthy *et al.* (2001).

2.3.4 Plant Growth Substances

Plant growth substances are certain complex organic additives, which influence the establishment and growth of *in vitro* cultures. Apart from the inorganic constituents of the media, which give consistent results, the organic medium supplements often do not give any definite results. A major achievement in modifying composition of the culture medium was the incorporation of growth substances which include auxins, gibberlins, cytokinins etc.

Nathan *et al.* (1992) initiated *H. psittacorum* cultures on modified MS medium containing 40 μM BA, 150 ml coconut water, 30 g sucrose and 2 g Gelrite / litre. Shoot multiplication was achieved on the above medium without coconut water, but supplemented with 10 μM BA. Shoots were rooted on MS basal medium and successfully acclimatised.

A rapid and flexible propagation system for *H. psittacorum* was developed by Goh *et al.* (1995) by culturing on MS medium in the presence of 10 μM 2,4-D, the explants produced rooted plantlets. 2,4-D at

40 – 80μM induced the formation of highly morphogenic callus. When subcultured on basal medium, thin callus produced protocorm like bodies that developed into plantlets.

Morphogenic callus cultures of *H. psittacorum* accumulated less ethylene than non-morphogenic cultures during early stages of differentiation of protocorm – like bodies leading to plantlet regeneration 10 fold higher than that during callus proliferation. It is concluded that high concentrations of ethylene inhibited regeneration, but low concentration of ethylene may be necessary for plant regeneration in callus cultures of *H. psittacorum* (Kumar *et al.*, 1996).

Shiau *et al.* (1999) obtained well developed shoots after 45 days of culture on MS medium supplemented with 4-8 mg l^{-1} benzylaminopurine (BA) and 0.5 mg l^{-1} NAA. Addition of 0.01 – 0.5 mg l^{-1} thidiazuron (TDZ) to the medium was found to be beneficial for rapid growth of shoot tip explants.

Lalrinawmi and Talukdar (2000b) obtained initial establishment of *H. psittacorum* buds, on MS medium supplemented with 0.25 mg IAA/litre and 6.0 mg BAP/litre. For shoot proliferation, it needs a lower concentration of BAP than what is required in the establishment medium. Rooting of *in vitro* developed shoots occurred in 1.0 to 1.5 mg IAA in MS basal medium. A 60 per cent survivability of the regenerated plantlets is observed when transferred to *ex vitro* condition.

Talukdar *et al.* (2002) obtained highest regeneration of *H. psittacorum* on MS medium supplemented with 6.0 mg BAP/litre. Treatment with 0.25 mg IAA/ litre + 2.0 mg BAP/litre recorded the highest shoot proliferation percentage, within 4- 5 weeks of culture. Maximum shoot length observed was 8.0 cm. Rooting was observed in medium containing 1.5 mg IAA/litre.

Bora and Paswan (2003) got three fold increase in length and more than double in breadth of axillary buds of *H. psittacorum* in the MS medium with 5.0 – 6.0 mg l^{-1} BAP, 0.05 – 0.1 mg l^{-1} IAA and 1.5 – 2.0 mg

l^{-1} GA_3. The supplementation of 2.5 mg l^{-1} BAP, 0.2 mg l^{-1} IAA and 2.5 mg l^{-1} GA_3 was ideal for initiation of multiple shoots and their multiplication rates. Medium supplemented with 0.75 mg l^{-1} IAA, 0.05 mg l^{-1} NAA gave the maximum rooting.

Iris

Lucretti *et al.* (1984) cultured axillary buds of Iris on MS medium supplemented with casein hydrolysate 10 μM, 2,4-D and 5 μM BA or 10 μM BA and 0.1 μM NAA. The best result was obtained in medium containing 10 μM BA and 0.1 μM NAA.

The desirable effect of plant growth substances for culture initiation, multiplication and rooting in Iris was recommended by Linde and Hol (1998).

Huang *et al.* (1999) cultured bulb segments of *Iris xiphium* var. *hybridium* on MS medium supplemented with different ratios of growth regulators. The highest induction frequency (70 per cent) was observed on MS medium supplemented with BA (1.0 mg l^{-1}) and NAA (0.2 mg l^{-1}). The best proliferation medium was BA (2.0 mg l^{-1}) and NAA (0.2 mg l^{-1}).

Gladiolus

Rapid *in vitro* shoot multiplication through adventitious bud formation and axillary bud proliferation has been achieved in three commercial varieties of gladiolus using MS medium supplemented with 0.5 mg per litre BA. The shoots could be readily rooted in culture (Bhojwani and Dantu, 1986).

Dantu and Bhojwani (1995) obtained optimal elongation of gladiolus axillary buds on liquid MS medium containing 0.5 mg l^{-1} BA.

Nagaraju and Parthasarathy (1995) cultured gladiolus buds on MS medium supplemented with BA and kinetin. Shoots obtained *in vitro* were excised aspectically and cultured on MS medium supplemented with 1.0 mg BA/litre that was found to be better than kinetin. Addition of NAA to

the basal medium enhanced rooting. BA at 7.5 mg l^{-1} gave the maximum number of shoots (16). The use of auxins (NAA, IBA, IAA and 2,4-D) and cytokinins (kinetin and BAP) have been reported in gladiolus for *in vitro* culture [Hussey, (1976 a and b); Ziv, (1979); Konoshima, (1980); Bajaj *et al.*, (1983); Dickens *et al.*, (1986); Lilien-Kipnis and Kochba, (1987); Rao *et al.*, (1991); Steinitz *et al.*, (1991); Gosal and Grewal, (1991); De Bruyn and Ferreira, (1992); Prasad *et al.*, (1993); Hussain, (1995); Nagaraju and Parthasarathy, (1995); Misra and Singh, (1999); Anandhi and Sekhar, (2000); Pathania *et al.*,(2001)].

Arora and Grewal (1990) reported the influence of GA on shoot elongation. The addition of GA_3 caused earlier sprouting and faster increase in sprout length than BAP alone in the culture establishment medium of cvs. Eurovision and Wild Roses (Pathania *et al.*, 2001).

Tuberose

In *Polianthes tuberosa*, the flower buds initiated shoots best on MS medium supplemented with 2.5 μM NAA and 10 μM BA. Shoot proliferation was maximum on MS medium supplemented with 0.5 μM NAA and 20 μM BA. Shoots rooted *in vitro* when cultured on MS with 0.5 μM NAA or 5.0 μM IBA (Shen *et al.*, 1991).

Krishnamurthy *et al.* (2001) cultured terminal and axillary stem scale sections of *Polianthes tuberosa* on MS medium supplemented with various growth regulators. The shoot tips from sprouted explants were transferred into a medium containing 2.0 or 4.0 mg BAP/litre singly or in combination with 0.1 mg IAA/litre. The regenerated shoots were transferred into half MS medium containing 0.5 and 0.1 mg IBA, 0.5 mg IAA or 0.25 mg IAA + 0.25 mg IBA/litre. Cytokinins induced multiple shoot formation. Auxins increased the regeneration capacity. Shoot length increased with the increasing BAP levels. A multiple shoot induction of 100 per cent was obtained with 0.2 mg IAA + 0.25 mg IBA/litre.

Materials and Methods

3. MATERIALS AND METHODS

The investigation on "Evaluation, molecular characterization and *in vitro* propagation of Heliconias" was carried out in the Department of Pomology and Floriculture and Department of Plant Biotechnology at College of Agriculture, Vellayani and at CPCRI substation, Kayamkulam during 2001-2005. The present study was conducted as three experiments. In Experiment 1: The evaluation of 12 different species and varieties of *Heliconia* for morphological characters, cytological characters, inflorescence characters and duration of the crop was studied and economics of cultivation calculated. In Experiment 2: Molecular characterization of 12 species and varieties were studied. In Experiment 3: Micropropagation of *H. psittacorum* cv. Deep Orage was carried out. The details regarding the experimental material used, methodology adopted and analytical techniques followed are described in this chapter.

3.1 EXPERIMENT 1: EVALUATION OF *HELICONIA* SP. AND VARIETIES AS CUT FLOWERS

Evaluation of 12 *Heliconia* species and varieties was done to assess their value as cut flower. The experiment was conducted in the Department of Pomology and Floriculture, College of Agriculture, Vellayani, Thiruvananthapuram located 8.5° N latitude and 76.9°E longitude and at an altitude of 29 m above mean sea level.

3.1.1 Materials

1. *H. psittacorum* cv. Lady Di
2. *H. psittacorum* cv. Deep Orange
3. *H. psittacorum* cv. Petra Orange
4. *H. psittacorum* × *H. spathocircinata* cv. Golden Torch
5. *H. psittacorum* × *H. spathocircinata* cv. Guyana

6. *H. psittacorum* × *H. marginata* cv. De Rooij
7. *H. rostrata*
8. *H. latispatha*
9. *H. humilis*
10. *H. wagneriana* cv. Wagneriana Red
11. *H. wagneriana* cv. Wagnariana Yellow(Peterson)
12. *H. collinsiana* × *H. bourgena* cv. Pedro Ortiz

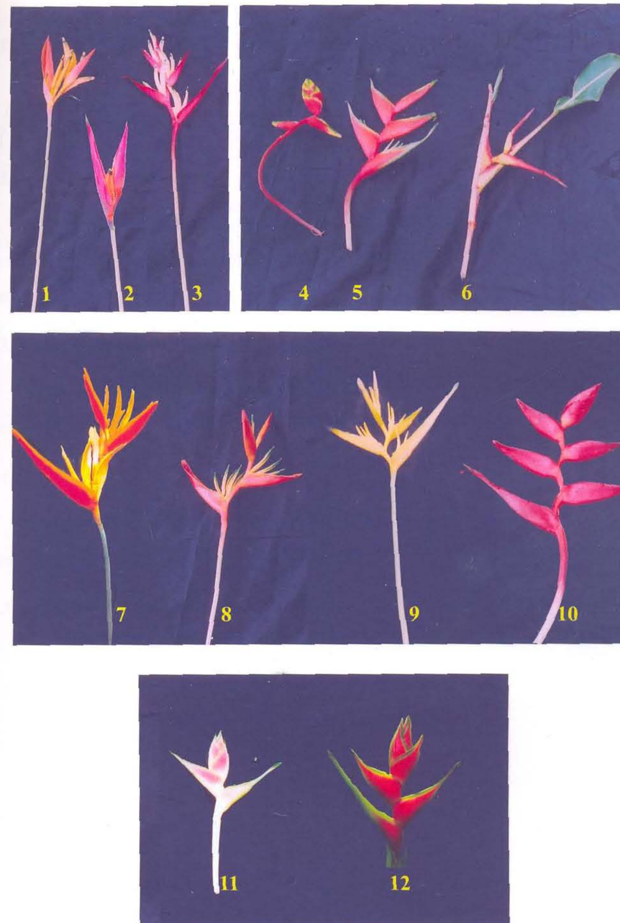
Treatment details

V_1	Golden Torch
V_2	Lady Di
V_3	*H. latispatha*
V_4	De Rooij
V_5	Petra Orange
V_6	Deep Orange
V_7	*H. rostrata*
V_8	Pedro Ortiz
V_9	*H. humilis*
V_{10}	Guyana
V_{11}	Wagneriana Red
V_{12}	Wagneriana Yellow

3.1.2 Method

The rhizomes of all selected species and varieties of *Heliconia* were planted in randomized block design with four replications. In each replication four plants were maintained. Field view and materials used are given in Plates 1 and 2. The experiment was carried out from August 2002 to July 2003. Planting was done at a distance of 1.00 x 1.00 m.

Plate 1. Field view of the experimental plot



1 - Deep Orange	**5 - *H. humilis***	**9 - Golden Torch**
2 - Petra Orange	**6 - *H. latispatha***	**10 - Pedro Ortiz**
3 - Lady Di	**7 - De Rooij**	**11 - Wagneriana Yellow**
4 - *H. rostrata*	**8 - Guyana**	**12 - Wagneriana Red**

Plate 2. *Heliconia* species and varieties used for the experiment

3.1.3 Morphological and cytological characters

Observations on vegetative characters and cytological characters were recorded and the mean values were taken.

3.1.3.1 Height of the plant

Height of the plant from base to tip was measured at bimonthly intervals for one year and their mean values were calculated and expressed in cm.

3.1.3.2 Number of leaves

Total number of leaves per shoot was measured at bimonthly intervals for one year and their mean values were calculated.

3.1.3.3 Leaf area

Average leaf area for each variety was taken at bimonthly intervals for one year and their mean values were calculated and expressed in cm^2.

3.1.3.4 Number of shoots

Total number of shoots per plant was taken at bimonthly intervals for one year and their mean values were calculated.

3.1.3.5 Chromosome number

Studies were carried out to find the chromosome number of *Heliconia*. Emerging inflorescence was selected and pollen mother cells were stained in acetocarmine solution (1 %). The slides were slightly heated and observed under the microscope under 1000 X magnification.

3.1.4 Duration of the crop

3.1.4.1 Days taken for flowering

Total number of days taken by the plant from planting to first flowering was recorded and the mean calculated.

3.1.4.2 Flowering duration

Total number of days taken by the spike from complete opening to the stage when the first bract started showing symptoms of senescence.

3.1.4.3 Vase life

Number of days taken for the appearance of symptoms of senescence in the first flower was recorded as vase life. The vase solution was replaced on alternate days after cutting 0.5 cm of the stalk base. This process was repeated until the cessation of vase life. Vase life was expressed in days.

3.1.4.4 Number of flowering shoots per year

Total number of flowering shoots produced by each plant after planting for one year was counted and the mean values calculated.

3.1.5 Inflorescence Characters

3.1.5.1 Length of spike

It was measured from base of first bract to tip of inflorescence and the mean calculated and expressed in cm.

3.1.5.2 Length of petiole

Length of petiole was measured from the point of emergence from shoot to the base of the first bract and the mean calculated and expressed in cm.

3.1.5.3 Number of bracts

Number of bracts in each spike was counted and the mean value calculated.

3.1.5.4 Size of bracts

The length and breadth of the second bract from base was recorded and the size of the bract was obtained by multiplying length by breadth and the mean calculated and expressed in cm^2.

3.1.5.5 Pollen morphology

From fully opened flowers pollen grains were selected and stained in 1: 1 glycerin, acetocarmine solution (2 %). Diameter of ten normal shaped and well-stained pollen grains were measured at random using a standard occular micrometer after calibrating the occular divisions under the high power (10 x 40 x) of a microscope. The mean diameter was recorded in microns.

Pollen fertility

Acetocarmine staining technique was employed for studying pollen fertility. Pollen fertility was estimated by counting fertile and sterile pollen grains separately in the microscope. Pollen, which was fully stained, was considered as fertile. Unstained, small or shriveled pollen grains were considered as sterile (Zirkle, 1937). Three slides were prepared and five random fields from each slide were observed in each species and variety. Fertility of each variety was estimated as percentage of the number of fertile pollen grains to the number of pollen grains scored.

3.1.6 Statistical analysis

The data collected were subjected to the following statistical analysis after testing the homogeneity of error variances.

Analysis of Variance (ANOVA) technique was used to test the significance of genotypic differences among the 12 species and varieties. Mean, variance, standard error and coefficient of variation were estimated. The character associations were estimated through correlation coefficients using Analysis of Covariance (ANACOVA) technique (Panse and Sukhatme, 1985).

The methodology employed in the estimation of the parameters is given below. For the two characters x and y measured on 'g' genotypes in

completely randomized design with 'r' replications, the variance/ co-variance analysis (ANACOVA) is as follows.

3.1.6.1 Analysis of variance /co-variance

Source	df	Mean square		
		X	**Y**	**XY**
Between genotypes	(g-1)	Gxx	Gyy	Gxy
Error	(r-1) (g –1)	Exx	Eyy	Exy

3.1.6.2 Estimates of components of variance and co-variance

	Genotypes	Environment	Phenotype
X	$\sigma^2_{gx} = \frac{G_{xx} - E_{xx}}{r}$	$\sigma^2_{ex} = E_{xx}$	$\sigma^2_{px} = \sigma^2_{gx} + \sigma^2_{ex}$
Y	$\sigma^2_{gy} = \frac{G_{yy} - E_{yy}}{r}$	$\sigma^2_{ey} = E_{yy}$	$\sigma^2_{py} = \sigma^2_{py} + \sigma^2_{ey}$
XY	$\sigma_{gxy} = \frac{G_{xy} - E_{xy}}{r}$	$\sigma_{exy} = E_{xy}$	$\sigma_{pxy} = \sigma_{gxy} + \sigma_{exy}$

3.1.6.3 Coefficient of variation

Phenotypic and genotypic coefficient of variation (PCV and GCV) for a trait x were estimated as

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

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

Where,

σ_{gx}: genotypic standard deviation

σ_{px}: Phenotypic standard deviation

$\bar{x}$: Mean of the character under study

3.1.6.4 Correlation analysis

The correlation coefficients (phenotypic, genotypic and environmental) between two characters denoted as X and Y were worked out as follows

$$\text{Genotypic correlation } (\gamma_{gxy}) = \frac{\sigma_{gxy}}{\sigma_{gx} \times \sigma_{gy}}$$

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

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

3.1.6.5 Heritability and Genetic Advance

Heritability (H^2) in broad sense was estimated as the proportion of heritable components of variation (Jain, 1982).

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

$$\text{Genetic advance as percentage of mean (GA)} = \frac{kH^2\sigma_{px}}{\bar{x}} \times 100$$

Where k is the selection differential whose value = 2.06 if five per cent selection is to be practiced (Miller *et al.*, 1958).

Genetic advance was categorized according to Robinson *et al.* (1949) as follows :

Definition		Category
Less than 20 per cent	:	Low
Greater than 20 per cent	:	High

3.1.7 Economics of cultivation

The economics of cultivation of crops was worked out considering all aspects of cost of cultivation and the income derived from the plant. Then the net income and the cost benefit ratio was calculated as follows

Net income (Rs/ha) : Gross income-Cost of cultivation

$$BCR = \frac{\text{Gross income}}{\text{Cost of cultivation}}$$

3.2 EXPERIMENT 2 : MOLECULAR CHARACTERIZATION OF *HELICONIA*

Investigation was carried out at the Department of Plant Biotechnology, College of agriculture, Vellayani and CPCRI substation, Kayamkulam.

3.2.1 Materials

The 12 species and varieties of *Heliconia* used in the evaluation were selected for molecular characterization.

3.2.2 Method

3.2.2.1 Standardization of DNA isolation

Quality of DNA is an important factor, which influence the PCR reaction. For isolation of genomic DNA, the tissues from the emerging leaves of all the 12 species and varieties of *Heliconia* before they fully unfurl were used. The methods tried for isolation are described below.

3.2.2.2 Protocol I

The genomic DNA was isolated based on modified Murray and Thompson (1980) method after making necessary changes. About 0.5 g of tender emerging leaf material was collected in the morning hours between

8 and 9 am. It was washed in running tap water followed by one per cent polyvinyl pyrollidone (PVP) solution and in distilled water for four to five times after chopping the leaves coarsely. Then the chopped leaf bits were wiped off completely with tissue paper and transferred to a sterilized pre-chilled porcelain mortar and were ground well to a fine powder in liquid nitrogen. The powder was then transferred to a 15 ml polypropylene centrifuge tube containing 5 ml of pre-warmed CTAB extraction buffer with the help of sterile spatula (extraction buffer: 2 per cent (2w/v) CTAB (Hexa decyl Trimethyl Ammonium Bromide), 100 mM Tris.HCL, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 0.2 per cent β mercaptoethanol). One ml of one per cent PVP solution was also added along with the sample. Then the samples were incubated at 60°C for 30 minutes in water bath with occasional mixing by gentle swirling. After 30 minutes the samples were taken and kept at room temperature for 10 minutes. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed by inversion and centrifuged at 15,000 rpm for 10 minutes at 5°C temperature. The aqueous phase was taken with a wide-bore pipette and transferred to a clean centrifuge tube and to this equal volume of phenol chloroform isoamyl alcohol was added and centrifuged for 10 minutes at 5°C in 15000 rpm. The upper phase was collected and extracted with chloroform: isoamylalcohol (24:1). Then to the aqueous phase, 0.1 volume of 3 M sodium acetate (pH 4.8) and 2/3 volume of isopropanol were added. It was mixed by quick gentle inversion to precipitate the DNA. Then the precipitated DNA was pelleted by centrifugation at 10, 000 rpm for 5 minutes at a temperature of 5°C. The supernatant was decanted carefully and the DNA pellet was washed with 70 per cent cold ethanol and air dried. Then it was dissolved in 100 µl TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and stored at 4°C.

All the materials used in the preparation and storage of reagents including reagent bottles, conical flasks, centrifuge tubes, spatula, glass

rods, funnels and tips of micro pipettes were washed with Labolin solution and rinsed with distilled water and autoclaved for 45 minutes before use. Phenol used was saturated and equilibrated using Tris buffer and pH adjusted to 8.0.

3.2.2.3 Protocol 2

The leaf sample was placed in a pre-chilled mortar and pestle and added 2.5 ml 2 per cent CTAB and 300 µl of one per cent PVP and the leaf tissues were powdered in it. It was poured into 15 ml polypropylene centrifuge tube containing 5 ml of pre-warmed CTAB extraction buffer with the help of sterile spatula (extraction buffer: 2 per cent (2w/v) CTAB (Hexa decyl Trimethyl Ammonium Bromide), 100 mM Tris. HCL, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 0.2 per cent mercaptoethanol) and the remaining procedure was same as that of protocol 1.

3.2.2.4 Protocol 3

Protocol 3 was same as that of protocol 1 except that no liquid nitrogen was used for grinding the leaf tissue. The remaining procedure was same as that of protocol 1.

3.2.2.5 Protocol 4

The method suggested by Mondal *et al.* (2000) was slightly modified without the use of CTAB and tried for DNA isolation in *Heliconia*. Briefly 0.5 g of leaf material was pulverized in liquid nitrogen with 20.0 µl β-mercaptaethanol in pre-cooled mortar by rapid grinding to a fine powder. Then 7.5 ml of hot (65°C) extraction buffer (100 mM Tris-HCl, 20 mM EDTA, 2 M NaCl, 2 per cent (w/v) SDS, pH 8) and a pinch of polyvinyl pyrrollidone (PVP) were added. The fine slurry of grounded plant material was transferred to a 50 ml centrifuge tube and incubated in water bath at 65°C for 20 minutes with occasional gentle shaking. The sample was centrifuged at 4°C for 10 minutes at 10,000 rpm. The supernatant was transferred to another sterile eppendorf tube using a

sterile pipetted tip. To this added equal volume of phenol : chloroform : iso amyl alcohol (25:24:1) and centrifuged as in previous step after gentle mixing. To the supernatant added equal volume of chloroform : iso amyl alcohol (24:1) and centrifuged as said above. This step was repeated. Then, to the supernatant 1/10th volume of 3 M sodium acetate followed by double volume of isopropyl alcohol was added. It was mixed gently and kept in freezer for 30 minutes for better precipitation of DNA. Then centrifuged at 10,000 rpm for 10 minutes at 4°C to pellet the DNA. The supernatant was discarded and the pellet was washed in 70 per cent ethanol. The pellet was air dried and then dissolved in 0.1 ml of 1 x Tris EDTA buffer (10 mM Tris HCl, 1 mM EDTA, pH 8) and stored at –20°C.

3.2.3 Purification of DNA

Major contaminants in crude DNA preparation are RNA, protein and polysaccharides. The following procedure was used for DNA purification.

1. Equal volume of DNA and phenol: chloroform isoamyl alcohol (25: 24: 1) were taken and mixed properly.
2. Centrifuged at 10000 rpm for ten minutes at room temperature. The aqueous phase was removed and transferred to a fresh microfuge tube.
3. Extracted twice with equal volume of chloroform: isoamylalcohol (24: 1). After centrifugation, the aqueous phase was transferred to fresh microfuge tube.
4. 0.1 volume of 3 M sodium acetate p^{H} 5.2 was added to it.
5. 2.5 volume of absolute alcohol was added and mixed by quick gentle inversion to precipitate the DNA.
6. Kept in the freezer for 1 hour.
7. Centrifuged at 10,000 rpm for 10 minutes to pellet the DNA

3.2.4 Quantification of DNA

DNA quantification was carried out with the help of UV- Vis spectrophotometer (Spectronic Genesis 5).

The spectrophotometer was calibrated at 260 nm and 280 nm wavelength using TE buffer, in which DNA was dissolved. The absorbance of the DNA sample dissolved in the TE buffer was recorded at both 260 nm and 280 nm.

Since an absorbance 1.0 at 260 nm represent 50 μgml^{-1} of DNA, the quantity of DNA in the sample was estimated by employing the following formula :

$$\text{Amount of DNA } (\mu gml^{-1}) = A_{260} \times 50 \times \text{dilution factor}$$

(where, A_{260} = absorbance at 260nm)

The quality of DNA could be judged from the ratio of the absorbance values recorded at 260 nm and 280 nm. A ratio between 1.5 and 2.0 indicates good quality of DNA.

3.2.5 Agarose gel electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit. Required amount of agarose was weighed out (0.9 per cent for visualizing the genomic DNA and 1.4 per cent for visualizing the amplified products) and melted in 1xTAE buffer (0.04 M Tris acetate, 0.001 M EDTA, pH 8.0) by boiling. After cooling to about 50°C ethidium bromide was added to a final concentration of 0.5 $\mu g\ ml^{-1}$. The mixture was then poured to a pre set template with appropriate comb. After solidification of the agar, the comb and the sealing tapes were removed and the gel was mounted in an electrophoresis tank. The tank was loaded with 1 x TAE buffer, so that it just covered the entire gel. Required volume of DNA

sample and gel loading buffer (6X loading dye with 40 per cent sucrose, 0.25 per cent bromophenol blue) were mixed. Each well was loaded with 20 µl of sample. One of the wells was loaded with 5.0 µl of PCR molecular weight marker along with required volume of the gel-loading buffer. Electrophoresis was performed at 75 volts until the loading dye reached 3/4th length of the gel. The gel was visualized using an ultraviolet visible (UV-Vis) transilluminator.

3.2.6 Random Amplified Polymorphic DNA (RAPD)

DNA amplification was done using forty arbitrarily designed decamer primers (Operon Inc, CA, USA.) adopting the procedure of Lim *et al.* (1999) with required modifications.

Polymerase chain reactions were carried out in a volume of 25 µl containing 2.5 µl 10 X buffer (10 mM Tris HCl pH 9.0, 1.50 mM Mg Cl_2, 50 mM KCl and 0.01 per cent gelatin), 10 pM primer, 250 µM each of deoxynucleotides (dNTPs), 0.2 units of Taq DNA polymerase and 20 ng of genomic DNA. Amplifications was performed in a programmable Thermal Controller (MJ Research, Inc.) for an initial denaturation at 95°C for one minute, followed by 35 cycles of denaturatioin at 95°C for one minute, annealing at 35°C for two minutes and extension at 72°C for two minutes. A final extension at 72°C for 10 minutes was included after the last cycle. Finally the products of amplification were cooled at 4°C. A negative control containing sterile water instead of template was included in each reaction set.

Amplified products along with DNA molecular weight marker supplied by US Biochemicals were separated by electrophoresis using 1.4 per cent agarose gel, stained with ethidium bromide and visualized on a UV transilluminator.

The number of monomorphic bands, number of polymorphic bands and intensity of bands were recorded. Those primers which when used for

amplification produced the maximum number of bands were used to amplify the DNA of all the twelve *Heliconia* species and varieties. The photographs of the amplification profile obtained in all the *Heliconia* species and varieties amplified *in vitro* using eight primers were taken with the help of gel documentation system. The RAPD bands were represented as '+' (for presence) and '-' (for absence) and recorded. The PCR was repeated at least twice in order to check the reproducibility. The amplification products of those primers alone, which could produce amplification for most of the species and varieties, were used for further analysis.

3.2.7 RAPD Data analysis

The reproducible bands were scored for their presence (+) or absence (-) for all the species and varieties studied. A genetic similarity matrix was constructed using the Jaccard's coefficient method (Jaccard, 1908) (formula)

where, $S_j = a / (a+b+c)$

a = number of bands present in both the species and varieties in a pair

b = number of bands present in the first variety but not in the second one

c = number of bands present in the second but not in the first.

Based on the similarity coefficient the association between the species and varieties was computed with the help of a software package NTSYS (version 2.02). Using the similarity coefficient values between the species and varieties, a dendogram was constructed following the Unweighted Pair Group Method with arithmetic Average (UPGMA). Similarity between the 12 species and varieties of *Heliconia* was assessed from the dendrogram.

3.3 EXPERIMENT 3: *IN VITRO* PROPAGATION OF *HELICONIA*

The experiment was conducted in completely randomized design at Department of Plant Biotechnology, college of Agriculture, Vellayani.

3.3.1 Explants

Explants were collected from actively growing adult plants of *Heliconia psittacorum* cv. Deep Orange. Shoot tips, bits of rhizome, root tip and leaf segments were the explants.

3.3.2 Collection and preparation of explants

The explants were excised from the plants using surgical blades. Rhizome was dugged out and the rhizome bits and shoot tips (apical buds) were removed. The scaly portion attached to the bud was carefully removed. Then the rhizome bits and shoot tips were washed with running tap water later washed in water to which a drop of wetting agent labolene was added. The explants were kept in labolene solution for half an hour. They were further washed three to four times with distilled water to remove the traces of labolene.

In the case of leaf and root explants the same procedure was followed. The tip, middle and basal portions of leaf were cultured separately. Each treatment was replicated twenty times. Observations on bud initiation (%), rate of contamination and number of days taken for bud initiation were recorded. Based on preliminary screening shoot tip was selected for further study.

3.3.3 Surface sterilization

Surface sterilization of the shoot tip explants was carried inside a laminar airflow chamber. The explants after surface sterilization were rinsed four to five times with sterile distilled water.

Experiment was carried out for the standardisation of surface sterilization techniques using sterilants given below. All the treatments

were replicated 20 times. The medium was MS. Observations were made on the percentage of contamination and survival rate.

Sterilant	Concentration (per cent)	Time of treatment (minutes)
Mercuric chloride	0.08	5, 10, 15
	0.10	5, 10, 15
	0.15	5, 10, 15
Sodium hypochlorite	5	25,30
	10	25,30
Calcium hypochlorite	10	25
		30
Ethyl alcohol wiping (70%)+Mercuric chloride (0.1%)		5
		10
		15
Absolute Ethyl alcohol (1 minute washing) + mercuric chloride(0.1%)		5
		10
		15

3.3.4 Inoculation and Incubation

The inoculation was carried out under perfect aseptic conditions in a laminar air flow chamber. All the tools (blades, forceps etc.) and glassware used for inoculation were washed thoroughly and further rinsed in double glass distilled water and autoclaved at 121°C and 1.06 kg cm^{-2} pressure for 40 minutes.

The explants after surface sterilization were given a fresh cut just above the initial cut to remove the portions, which had become brown due to sterilization. For shoot apex about 3-8 mm long apical portions was retained. The rhizome bits had minute buds attached to it. The leaves were cut into sections of about 5 mm from the tip, middle and basal

portion of the leaf sheath. In case of roots, 5-10 mm long root tips were used for culturing.

The explants after preparation were used for inoculation. The sterilized explants were then inoculated into the medium using sterilized forceps. The test tube neck was once again flamed and the cotton plug quickly replaced. The inoculated test tubes were immediately transferred to the culture room provided with light / darkness at 26 ± 1°C.

3.3.5 Media

The media used for getting sterile cultures were MS (Murashige and Skoog, 1962), SH (Schenk and Hilderbrandt, 1972) and White (1963). The chemicals used for the preparation of the culture media were of analytical grade from the Sisco Research Laboratory (SRL, Bombay) (Appendix I).

Standard procedure was followed for the preparation of media. Stock solutions of major and minor nutrients were prepared by dissolving the required quantity of chemicals in specific volume of double glass distilled water. Plant growth substances were first dissolved in dilute acid / alcohol and volume made up with double glass distilled water. The stock solutions were stored under refrigerated conditions.

The stock solutions of plant growth substances were prepared fresh every week, whereas other stocks were maintained under refrigerated conditions up to one month.

The glasswares were thoroughly washed with water containing a few drops of labolene and then rinsed with double distilled water. Specific quantities of stock solutions of chemical and pytohormones were pipetted out into a 100 ml beaker. Sucrose and myo-inositol were added fresh and dissolved. Final volume was made up to 1000 ml after adjusting pH.

The medium was then heated and agar is added and allowed to melt. The medium was then poured into pre-sterilized culture vessels, which were rinsed with double distilled water. Corning brand test tubes (25 x 150 mm) were used as containers. The test tubes were filled with 166.67 ml of medium. The test tubes containing the medium were plugged tightly and autoclaved at 121°C and 1.66 kg cm^{-2} pressure for 20 minutes and after cooling, transferred to the culture room to be used for inoculation as and when required.

Each treatment was replicated twenty times. Observations on rate of contamination, bud initiation (%) and number of days taken for bud initiation were recorded. After initial screening MS medium that showed good response was selected for further study.

3.3.6 Culture establishment

The culture establishment were carried out using shoot tips in MS medium supplemented with cytokinins (BA, kinetin, 2 ip each at 0, 5, 10 mg l^{-1}) alone and in combination with auxins [IAA (0, 0.5 mg l^{-1}), NAA (0, 0.5 mg l^{-1})], and GA_3 (0, 2 mg l^{-1}). BA 10 mg l^{-1} and 2,4-D (10, 20, 40 mg l^{-1}) combination was also tried. Treatment details are given in Table 1. Each treatment was replicated six times. Observations on the number of days taken for complete greenness, number of buds responded and rate of survival were recorded.

3.3.7 Shoot proliferation

The cultures showing complete greenness and bud initiation were then transferred to shoot proliferation medium. Trials were conducted in MS medium supplemented with cytokinins (BAP, kinetin, 2 ip each at 0, 1, 2, 3.5, 5 mg l^{-1}) alone and in combination with IAA (0, 0.2 mg l^{-1}) and GA_3 (0, 2.5 mg l^{-1}). The cultures were kept in the dark room. Treatment details are given in Table 2. Each treatment was replicated six times. Observations on the number of days taken for sprouting or callusing, per

Table 1. Different levels of plant growth regulators tried for culture establishment in *H. psittacorum* cv. Deep Orange

Code	Media
T_1	MS
T_2	MS + BA (5.0 mg l^{-1})
T_3	MS + BA (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1})
T_4	MS + BA (5.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_5	MS + BA (5.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_6	MS + BA (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_7	MS + BA (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_8	MS + BA (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_9	MS + BA (5.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{10}	MS + BA (10.0 mg l^{-1})
T_{11}	MS + BA (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1})
T_{12}	MS + BA (10.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{13}	MS + BA (10.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{14}	MS + BA (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{15}	MS + BA (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{16}	MS + BA (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{17}	MS + BA (10.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{18}	MS + Kinetin (5.0 mg l^{-1})
T_{19}	MS + Kinetin (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1})
T_{20}	MS + Kinetin (5.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{21}	MS + Kinetin (5.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{22}	MS + Kinetin (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{23}	MS + Kinetin (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{24}	MS + Kinetin (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + NAA (0.5 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{25}	MS + Kinetin (5.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{26}	MS + Kinetin (10.0 mg l^{-1})
T_{27}	MS + Kinetin (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1})
T_{28}	MS + Kinetin (10.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{29}	MS + Kinetin (10.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{30}	MS + Kinetin (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{31}	MS + Kinetin (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{32}	MS + Kinetin (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{33}	MS + Kinetin (10.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{34}	MS + 2ip (5.0 mg l^{-1})
T_{35}	MS + 2ip (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1})
T_{36}	MS + 2ip (5.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{37}	MS + 2ip (5.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{38}	MS + 2ip (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{39}	MS + 2ip (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{40}	MS + 2ip (5.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + NAA (0.5 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{41}	MS + 2ip (5.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{42}	MS + 2ip (10.0 mg l^{-1})
T_{43}	MS + 2ip (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1})
T_{44}	MS + 2ip (10.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{45}	MS + 2ip (10.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{46}	MS + 2ip (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1})
T_{47}	MS + 2ip (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{48}	MS + 2ip (10.0 mg l^{-1}) + IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{49}	MS + 2ip (10.0 mg l^{-1}) + GA_3 (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1})
T_{50}	MS + BA (10.0 mg l^{-1}) + 2,4-D (10.0 mg l^{-1})
T_{51}	MS + BA (10.0 mg l^{-1}) + 2,4-D (20.0 mg l^{-1})
T_{52}	MS + BA (10.0 mg l^{-1}) + 2,4-D (40.0 mg l^{-1} 4)

Table 2. Different levels of plant growth regulators tried for shoot proliferation and elongation in *H. psittacorum* cv. Deep Orange

Code	Media
SP_1	MS
SP_2	MS + BA (1.0 mg l^{-1})
SP_3	MS + BA (1.0 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_4	MS + BA (1.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_5	MS + BA (1.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_6	MS + BA (2.0 mg l^{-1})
SP_7	MS + BA (2.0 mg l^{-1}) + IAA (2.0 mg l^{-1})
SP_8	MS + BA (2.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_9	MS + BA (2.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{10}	MS + BA (3.5 mg l^{-1})
SP_{11}	MS + BA (3.5 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{12}	MS + BA (3.5 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{13}	MS + BA (3.5 mg l^{-1}) + GA_3 (2.5 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{14}	MS + BA (5.0 mg l^{-1})
SP_{15}	MS + BA (5.0 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{16}	MS + BA (5.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{17}	MS + BA (5.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{18}	MS + Kinetin (1.0 mg l^{-1})
SP_{19}	MS + Kinetin (1.0 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{20}	MS + Kinetin (1.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{21}	MS + Kinetin (1.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{22}	MS + Kinetin (2.0 mg l^{-1})
SP_{23}	MS + Kinetin (2.0 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{24}	MS + Kinetin (2.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{25}	MS + Kinetin (2.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{26}	MS + Kinetin (3.5 mg l^{-1})
SP_{27}	MS + Kinetin (3.5 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{28}	MS + Kinetin (3.5 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{29}	MS + Kinetin (3.5 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{30}	MS + Kinetin (5.0 mg l^{-1})
SP_{31}	MS + Kinetin (5.0 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{32}	MS + Kinetin (5.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{33}	MS + Kinetin (5.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{34}	MS + 2ip (1.0 mg l^{-1})
SP_{35}	MS + 2ip (1.0 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{36}	MS + 2ip (1.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{37}	MS + 2ip (1.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{38}	MS + 2ip (2.0 mg l^{-1})
SP_{39}	MS + 2ip (2.0 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{40}	MS + 2ip (2.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{41}	MS + 2ip (2.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{42}	MS + 2ip (3.5 mg l^{-1})
SP_{43}	MS + 2ip (3.5 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{44}	MS + 2ip (3.5 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{45}	MS +2ip (3.5 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{46}	MS + 2ip (5.0 mg l^{-1})
SP_{47}	MS + 2ip (5.0 mg l^{-1}) + IAA (0.2 mg l^{-1})
SP_{48}	MS + 2ip (5.0 mg l^{-1}) + GA_3 (2.5 mg l^{-1})
SP_{49}	MS + 2ip (5.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})

cent of shoot proliferation, survival rate, number of shoots and number of leaves were recorded.

3.3.8 Standardisation of media supplements

Effect of media supplements, *viz.*, coconut water (0, 5, 10 and 15 %), activated charcoal (0, 0.5, 1 %), ethylene inhibitors (cobalt chloride 5 mg l^{-1} and 10 mg l^{-1}, PVP 5 mg $l^{-1}$and 10 mg l^{-1}), silver nitrate (5 mg $l^{-1}$and 10 mg l^{-1}), copper sulphate (100 and 200 mg l^{-1}), cefotaxime (250 and 500 mg l^{-1}) and carbon sources (sucrose 30 g l^{-1}, 35 g l^{-1}) were tried for shoot proliferation. After the screening of shoot proliferation, the treatment SP_9 was used for the study of standardization of media supplements. Each treatment was replicated six times. Observations on the number of days taken for sprouting or callusing, per cent of shoot proliferation, survival rate, number of shoots and number of leaves were recorded.

3.3.9 *In vitro* rooting

Studies were carried out for *in vitro* rooting of elongated shoots using different auxins (IAA (0.25, 0.5, 1 mg l^{-1}), and NAA (0.25, 0.5 mg l^{-1}) alone and in combination. Treatment details are given in Table 3. Each treatment was replicated six times. Observations on days taken for root initiation, number of roots, per cent of cultures initiating roots and survival rate were recorded. Treatment details are given in Table 3.

3.3.10 Statistical analysis

The experiment design was completely randomized design. Since the replication did not respond uniformly data generated were not subjected to statistical analysis.

Table 3. Different levels of plant growth regulators tried for rooting in *H. psittacorum* cv. Deep Orange

Code	Media
R_1	MS + IAA (0.25 mg l^{-1})
R_2	MS + IAA (0.5 mg l^{-1})
R_3	MS + IAA (1.0 mg l^{-1})
R_4	MS + NAA (0.25 mg l^{-1})
R_5	MS + NAA (0.5 mg l^{-1})
R_6	MS + IAA (0.25 mg l^{-1}) + NAA (0.25 mg l^{-1})
R_7	MS + IAA (0.25 mg l^{-1}) + NAA (0.5 mg l^{-1})
R_8	MS + IAA (0.5 mg l^{-1}) + NAA (0.25 mg l^{-1})
R_9	MS + IAA (0.5 mg l^{-1}) + NAA (0.5 mg l^{-1})
R_{10}	MS + IAA (1.0 mg l^{-1}) + NAA (0.25 mg l^{-1})
R_{11}	MS + IAA (1.0 mg l^{-1}) + NAA (0.5 mg l^{-1})

Results

4. RESULTS

The present study was conducted as three experiments. In Experiment 1: The evaluation of 12 different species and varieties of *Heliconia* for morphological characters, cytological characters, inflorescence characters and duration of the crop was studied and economics of cultivation calculated. In Experiment 2: Molecular characterization of 12 species and varieties were studied. In Experiment 3: Micropropagation of *H. psittacorum* cv. Deep Orange was carried out. The results of the investigation carried out for evaluation, molecular characterization and *in vitro* propagation of Heliconias are presented in this chapter.

4.1 EXPERIMENT 1: EVALUATION OF *HELICONIA*S

4.1.1 Morphological Characters

4.1.1.1 Height of the plant

Data pertaining to the height of the plant is presented in Table 4.

The species and varieties varied significantly with respect to plant height through out the observation period. The species V_3 (*Heliconia latispatha*) registered the highest plant height with a mean height of 195.32 cm, 200.12 cm, 193.98 cm and 183.59 cm, respectively at three months, five months, seven months and nine months after planting which was significantly superior to all other species and varieties. The variety V_8 (Pedro Ortiz) recorded the highest plant height eleven months and one year after planting (191.18 cm and 248.44 cm, respectively).

Among the species and varieties V_4 (De Rooij) recorded lowest plant height during the whole observation period except five months after planting. It recorded a mean height of 62.39 cm, 86.79 cm, 75.26 cm, 75.77 cm and 80.88 cm, respectively at three months, seven months, nine

Table 4. Plant height of 12 *Heliconia* species and varieties

Treatments	Plant height (cm)					
	3 MAP	5 MAP	7 MAP	9 MAP	11 MAP	1 YAP
V_1	107.09	107.45	109.16	94.79	101.83	105.32
V_2	102.76	106.94	129.27	108.99	117.51	131.27
V_3	195.32	200.12	193.98	183.59	182.13	196.83
V_4	62.39	85.60	86.79	75.26	75.77	80.88
V_5	101.55	107.97	109.40	101.94	99.56	104.05
V_6	107.58	107.93	99.25	99.16	96.37	105.01
V_7	91.20	84.35	101.57	103.36	98.63	107.06
V_8	101.45	106.18	123.33	150.98	191.18	248.44
V_9	101.83	122.13	133.33	148.38	160.88	183.37
V_{10}	113.00	122.05	143.68	132.87	142.63	145.98
V_{11}	105.70	116.98	142.48	162.08	188.43	190.31
V_{12}	103.90	103.48	133.17	151.66	158.28	165.95
F	32.01**	128.98**	144.89**	223.49**	173.21**	192.44**
SE	5.37	2.59	2.34	2.21	3.11	3.62
CD	15.476	7.488	6.746	6.393	8.977	10.445

**Significant at one per cent level

Table 5. Number of leaves of 12 *Heliconia* species and varieties

Treatments	Number of leaves					
	3 MAP	5 MAP	7 MAP	9 MAP	11 MAP	1 YAP
V_1	5.00	5.00	5.25	5.13	5.00	4.88
V_2	6.25	6.13	6.00	5.75	5.88	5.75
V_3	5.38	5.25	4.75	5.13	5.00	5.00
V_4	5.00	5.50	5.25	4.63	4.63	4.88
V_5	5.00	5.13	5.00	5.00	5.00	5.00
V_6	5.25	5.25	5.37	5.63	5.37	5.38
V_7	5.25	5.38	4.63	4.50	4.38	4.75
V_8	5.25	5.00	5.00	4.88	5.00	5.13
V_9	5.00	5.00	5.38	4.50	5.63	4.88
V_{10}	4.87	5.13	5.88	6.13	5.50	6.25
V_{11}	4.37	5.00	4.88	4.50	4.38	4.50
V_{12}	4.75	5.12	4.63	5.50	5.38	5.13
F	4.03**	1.56^{NS}	1.85^{NS}	0.937**	1.95^{NS}	1.94^{NS}
SE	0.22	0.25	0.32	0.32	0.34	0.33
CD	0.641	0.742	0.945	2.671	0.984	0.970

**Significant at one per cent level, NS – Not significant

MAP – Months after planting YAP – Year after planting

months, eleven months and one year after planting. The species V_7 (*Heliconia rostrata*) recorded the lowest plant height (84.35 cm) at five months after planting.

4.1.1.2 Number of leaves

The data pertaining to the number of leaves are presented in Table 5.

There was significant difference in the number of leaves per shoot three months and nine months after planting. The variety V_2 (Lady Di) recorded highest number of leaves at three months after planting (6.25) and it was found significantly superior to all other species and varieties. The variety V_{10} (Guyana) recorded the highest number of leaves (6.13), nine months after planting.

4.1.1.3 Leaf area

The data pertaining to leaf area is presented in Table 6.

The species and varieties showed significant difference in leaf area throughout the observation period. The variety V_8 (Pedro Ortiz) recorded the highest leaf area with mean values of 2905.40 cm^2, 2716.20 cm^2, 2734.39 cm^2, 2986.12 cm^2, 2944.54 cm^2, 2993.18 cm^2, respectively at three months, five months, seven months, nine months, eleven months and one year after planting.

The variety V_2 (Lady Di) recorded the lowest leaf area three months and eleven months after planting (671.94 cm^2, 759.78 cm^2, respectively). The variety V_4 (De Rooij) recorded the lowest leaf area (699.54 cm^2) at five months after planting. The variety V_6 (Deep Orange) recorded the lowest leaf area seven months, nine months and one year after planting (747.75 cm^2, 724.73 $cm^2$and 782.62 cm^2, respectively).

4.1.1.4 Number of shoots

The data pertaining to the number of shoots presented in Table 7.

Table 6. Leaf area (cm^2) of 12 *Heliconia* species and varieties

Treatments	Leaf area (cm^2)					
	3 MAP	5 MAP	7 MAP	9 MAP	11 MAP	1 YAP
V_1	742.45	746.62	837.25	834.71	850.97	868.96
V_2	671.94	708.50	757.85	791.60	759.78	800.77
V_3	1305.05	1338.10	1314.90	1335.87	1293.76	1327.87
V_4	731.60	699.54	758.20	874.26	761.87	848.61
V_5	845.70	776.40	795.10	804.30	855.20	839.94
V_6	756.88	794.62	747.75	724.73	804.8	782.62
V_7	834.35	840.97	789.98	781.56	949.53	873.71
V_8	2905.40	2716.20	2734.39	2986.12	2944.54	2993.18
V_9	2637.47	2705.65	2710.62	2839.37	2868.52	2937.72
V_{10}	835.57	844.57	1064.58	983.15	972.51	1014.16
V_{11}	1649.03	1638.12	1824.69	1899.12	2024.09	2017.64
V_{12}	1574.45	1587.47	1731.87	1814.34	1904.57	1877.29
F	591.81**	110.58**	950.22**	754.89**	1011.98**	585.29**
SE	31.65	22.48	24.29	29.73	25.69	34.29
CD	91.244	64.793	70.014	85.713	74.057	98.689

**Significant at one per cent level

Table 7. Number of shoots of 12 *Heliconia* species and varieties

Treatments	Number of shoots					
	3 MAP	5 MAP	7 MAP	9 MAP	11 MAP	1 YAP
V_1	10.88	14.625	18.88	20.25	22.88	36.88
V_2	15.00	19.38	22.38	25.13	30.50	42.25
V_3	7.38	11.13	13.00	16.13	19.00	26.75
V_4	8.88	11.38	14.25	24.63	32.25	56.37
V_5	12.25	13.80	17.50	26.70	34.25	56.60
V_6	16.00	17.25	20.88	23.63	31.88	52.00
V_7	9.76	12.00	13.88	19.38	23.25	34.00
V_8	7.63	8.38	9.25	9.88	11.25	20.25
V_9	5.63	6.63	8.13	12.13	15.00	26.50
V_{10}	8.88	13.38	18.00	21.00	25.25	38.00
V_{11}	6.88	8.63	10.50	12.38	14.75	26.25
V_{12}	6.88	7.25	8.38	9.50	12.75	16.25
F	44.50**	46.38**	48.18**	72.55**	84.27**	61.40**
SE	0.49	0.57	0.71	0.72	0.88	1.74
CD	1.4227	1.6704	2.0439	2.1029	2.5523	5.0216

**Significant at one per cent level

MAP – Months after planting YAP – Year after planting

There was significant difference in the number of shoots during the whole observation period. The variety V_6 (Deep Orange) yielded the highest number of shoots (16) at three months after planting which was significantly superior to all other species and varieties. The highest number of shoots (19.38) was recorded in variety V_2 (Lady Di) at five months after planting which was significantly superior to all other species and varieties. At seven months after planting, variety V_2 (Lady Di) recorded highest number of shoots (22.38) that was on par with the variety V_6 (Deep Orange, 20.88). At nine months and eleven months after planting, the variety V_5 (Petra Orange) yielded the highest number of shoots (26.70 and 34.25, respectively). The variety V_5 (Petra Orange) recorded the highest number of shoots (56.60) one year after planting and it was on par with the varieties V_4 (De Rooij, 56.37) and V_6 (Deep Orange, 52.00).

Among the species and varieties, V_9 (*Heliconia humilis*) yielded the lowest number of shoots with mean value (5.63, 6.63, 8.13, respectively) at three months, five months and seven months after planting. The variety V_{12} (Wagneriana Yellow) recorded the lowest number of shoots at nine months and one year after planting (9.50 and 16.25, respectively). The variety V_8 (Pedro Ortiz) recorded the lowest number of shoots (11.25), eleven months after planting.

4.1.1.5 Chromosome number

Acetocarmine staining procedure was followed to detect the chromosome number of all twelve selected *Heliconia* species and varieties. Flower buds of one cm length were selected for pollen mother cell isolation and it was observed under 1000x magnification. The exact count was not obtained.

4.1.2 Inflorescence Characters

4.1.2.1 Length of spike

The data pertaining to the length of spike is depicted in Table 8 and Fig. 1.

The species and varieties differed significantly in spike length. Among the species and varieties V_8 (Pedro Ortiz) recorded the highest spike length eleven months and one year after planting (42.83 cm and 43.53 cm, respectively). The variety V_6 (Deep Orange) recorded the lowest spike length eleven months and one year after planting (10.28 cm and 9.63 cm, respectively) which was significantly lower than that of other species and varieties.

4.1.2.2 Length of petiole

The data pertaining to length of petiole is presented in Table 8 and Fig. 2.

There was significant difference recorded in length of petiole during the period of study. The variety V_5 (Petra Orange) yielded the highest petiole length (42 cm) eleven months after planting. The species V_3 (*H. latispatha*) recorded the highest petiole length 41.13 cm, one year after planting. The lowest value was recorded in V_9 (*H. humilis*) with mean value of 1.75cm and 1.88 cm, respectively, eleven months and one year after planting.

4.1.2.3 Number of bracts

The data pertaining to number of bracts is presented in Table 8 and Fig. 3.

The species and varieties differed significantly in number of bracts. The species V_7 (*H. rostrata*) yielded the highest number of bracts (11.88), eleven months after planting. The variety V_8 (Pedro Ortiz) recorded the highest number of bracts 13.50, one year after planting, which was

Table 8. Inflorescence characters of 12 *Heliconia* species and varieties

Treatments	Spike length (cm)		Petiole length (cm)		Number of bracts		Size of bracts (cm^2)	
	11 MAP	1 YAP	11 MAP	1 YAP	11 MAP	1 YAP	11 MAP	1 YAP
V_1	15.75	21.15	32.50	31.88	5.38	5.63	27.98	29.07
V_2	13.00	13.13	35.75	35.75	4.88	4.75	28.07	27.99
V_3	33.90	36.68	39.75	41.13	7.63	9.13	35.89	36.91
V_4	23.45	21.68	13.20	13.43	5.88	5.25	30.91	34.69
V_5	16.83	17.10	42.00	40.00	5.13	5.88	36.56	38.76
V_6	10.28	9.63	26.33	30.00	3.00	3.00	43.44	42.52
V_7	21.29	22.03	13.25	17.60	11.88	11.12	39.36	38.52
V_8	42.83	43.53	4.63	5.38	11.00	13.50	115.71	115.99
V_9	26.08	24.93	1.75	1.88	10.50	9.75	77.20	76.35
V_{10}	22.75	17.88	16.35	15.13	5.03	4.75	26.93	28.33
V_{11}	25.13	32.55	2.38	4.38	9.25	9.50	98.96	96.92
V_{12}	29.99	31.60	2.13	3.63	9.75	9.50	91.00	90.40
F	211.04**	116.46**	143.45**	82.31**	47.96**	42.32**	136.86**	133.12**
SE	0.76	1.10	1.06	1.38	0.42	0.48	2.74	2.70
CD	2.2105	3.1764	3.0588	3.9961	1.2209	1.4084	7.81	7.80

**Significant at one per cent level

MAP – Months after planting YAP – Year after planting

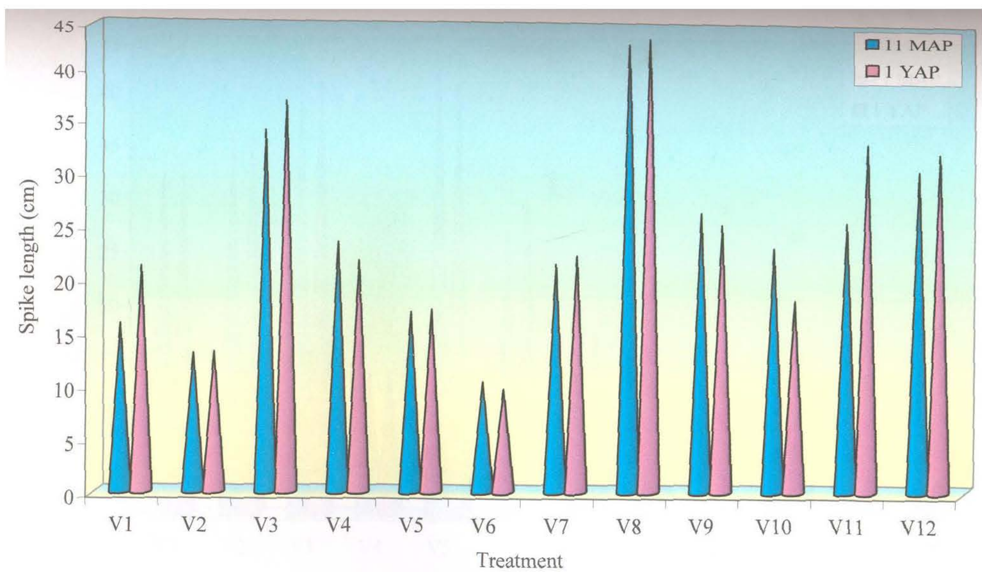


Fig. 1. Spike length (cm) of twelve *Heliconia* species and varieties

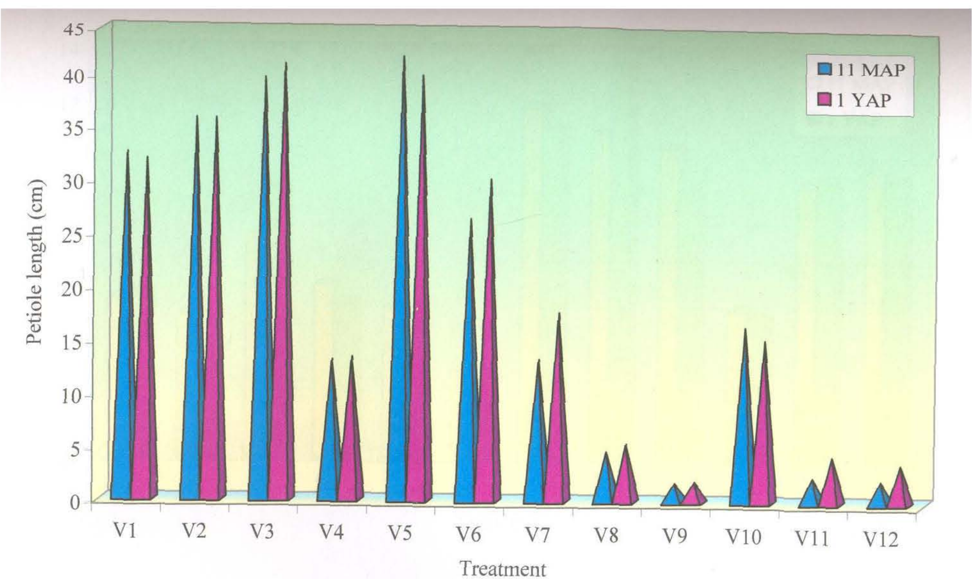


Fig. 2. Petiole length (cm) of twelve *Heliconia* species and varieties

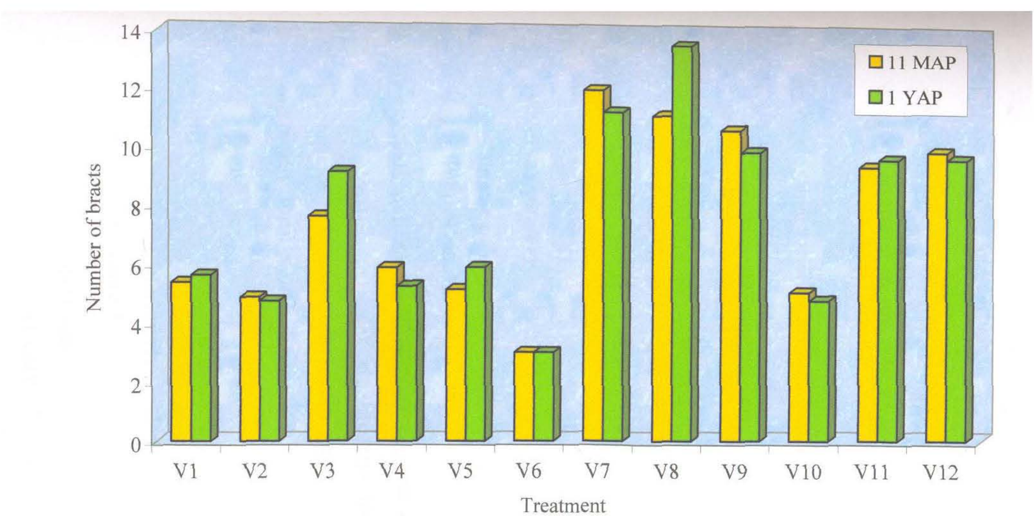


Fig. 3. Number of bracts of the twelve *Heliconia* species and varieties

significantly superior to all other species and varieties. The lowest number of bracts was observed in the variety V_6 (Deep Orange), eleven months and one year after planting (3.00 each), which was significantly lower than that of other species and varieties.

4.1.2.4 Size of bract

The data pertaining to the size of bract is presented in Table 8 and Fig. 4.

The species and varieties differed significantly with regard to size of bracts. Among the species and varieties, V_8 (Pedro Ortiz) recorded the highest bract size eleven months and one year after planting (115.71 cm^2 and 115.99 cm^2, respectively). The variety V_{10} (Guyana) recorded the lowest size of bract (26.93 cm^2) eleven months after planting. The variety V_2 (Lady Di) recorded the lowest size of bract (27.99 cm^2) one year after planting.

4.1.2.5 Pollen morphology

Pollen diameter of twelve selected *Heliconia* species and varieties are presented in Table 9 and Plate 3.

The largest pollen size (360.00μ) was recorded in variety V_{12} (Wagneriana Yellow), which was significantly higher than all other species and varieties. Comparatively smaller pollen size was recorded in V_3 (*H. latispatha,* 238.50μ), V_4 (De Rooij, 249.75μ) and V_1 (Golden Torch, 263.25 μ).

Pollen fertility

Pollen fertility of the twelve selected *Heliconia* species and varieties are presented in Table 9.

Higher fertility per cent was recorded in V_3 (*H. latispatha*, 90.81 per cent) V_2 (Lady Di, 82.71 per cent) and V_6 (Deep Orange, 80.71 per cent). Lower per cent of pollen fertility was recorded in varieties V_{12}

Table 9. Difference in pollen morphology of 12 *Heliconia* species and varieties

Treatments	Pollen size (μ)	Pollen fertility (%)
V_1	263.25	29.00
V_2	301.50	82.71
V_3	238.50	90.81
V_4	249.75	40.83
V_5	312.75	30.31
V_6	288.00	80.71
V_7	283.00	39.50
V_8	294.75	27.08
V_9	279.00	26.72
V_{10}	285.75	26.72
V_{11}	317.20	16.25
V_{12}	360.00	13.75
F	57.86**	4.82**
SE	5.01	20.81
CD (0.05)	10.202	42.345

** Significant at one per cent level

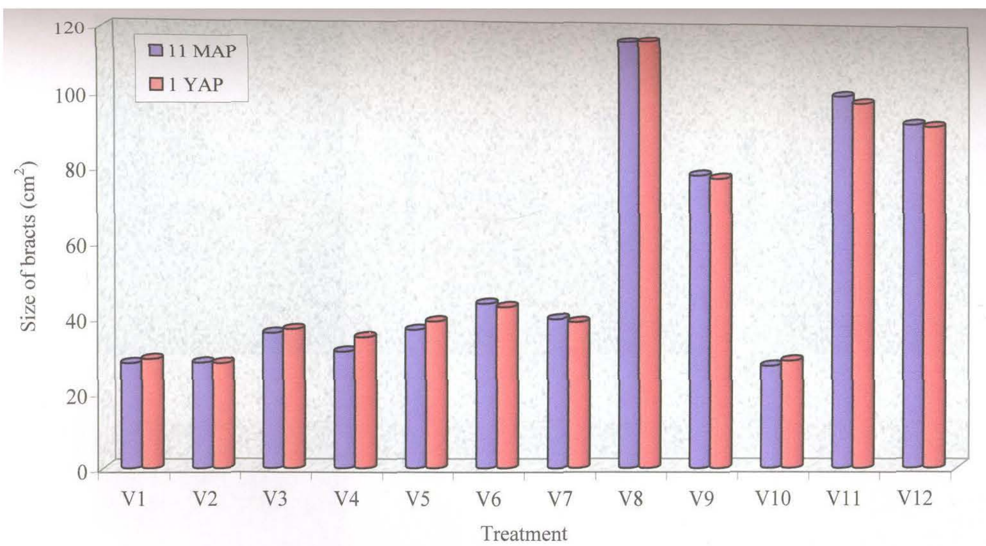
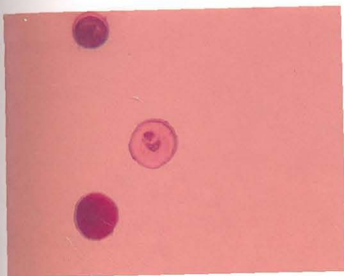
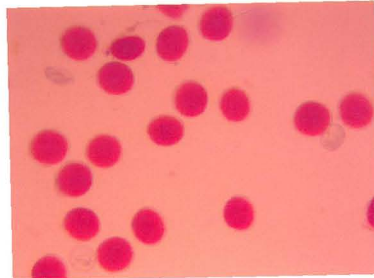


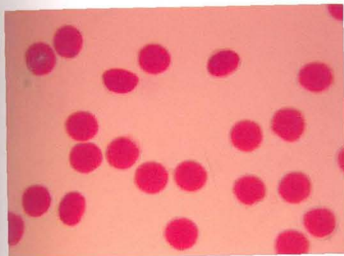
Fig. 4. Bract size of twelve *Heliconia* species and varieties



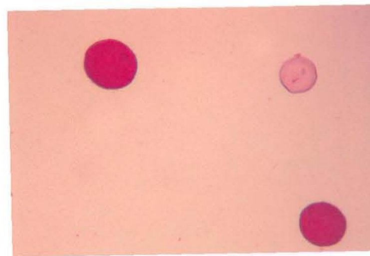
Golden torch



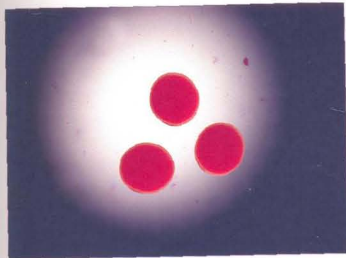
Lady di



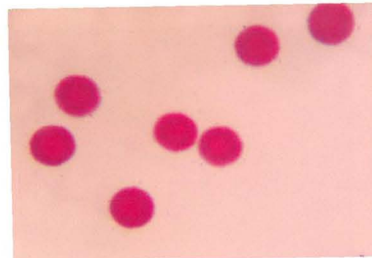
H. latispatha



De rooij

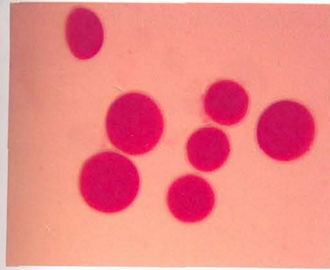


Petra orange

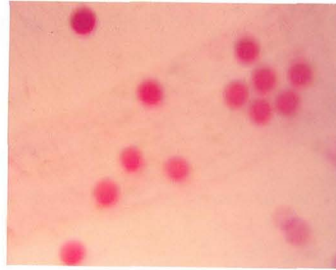


Deep orange

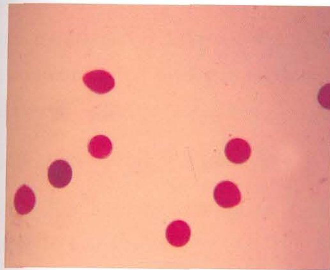
Plate 3. Pollen characteristics of 12 *Heliconia* species and varieties



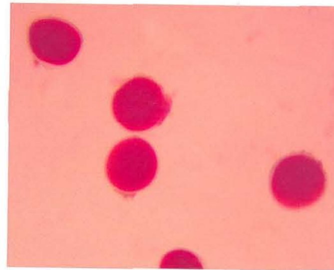
H. rostrata



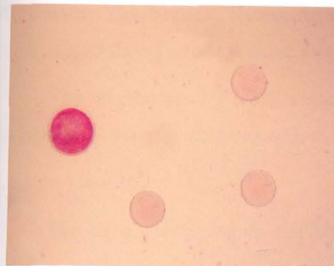
Pedro ortiz



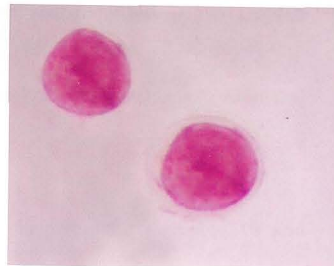
H. humilis



Guyana



Wagneriana red



Wagneriana yellow

Plate 3. Continued

(Wagneriana Yellow, 16.25 per cent) and V_{11} (Wagneriana Red, 13.75 per cent).

4.1.3 Duration of the crops

4.1.3.1 Days taken from planting to flowering

The data pertaining to days taken the flowering is presented in Table 10 and Fig. 5.

There was significant difference among the species and varieties for the days taken for flowering. The variety V_{II} (Wagneriana Red) recorded the highest number of days for flowering (300.50 days), which was significantly different from all other species and varieties. The lowest number of days was taken by V_1 (Golden Torch, 93.50 days) and it was on par with V_6 (Deep Orange, 98.25 days).

4.1.3.2 Flowering duration (days)

The data pertaining to the flowering duration is presented in Table 10 and Fig. 5.

There was significant difference recorded in flowering duration during the period under study. Flowering duration ranged from 23.75 to 40.50 days. The highest flowering duration was recorded in V_1 (Golden Torch, 40.50 days) and it was on par with V_7 (*H. rostrata,* 40.00 days).

The treatments V_3 (*H. latispatha*) and V_5 (Petra Orange) recorded the lowest flowering duration (23.75 each).

4.1.3.3 Vase life

The data pertaining to the vase life is presented in Table 10 and Fig. 5.

The species and varieties differed significantly with regard to vase life. The treatments V_2 (Lady Di, 4.63) and V_{11} (Wagneriana Red, 19.75) recorded the lowest and highest vase life, which significantly differed from all other species and varieties.

Table 10. Crop duration of 12 *Heliconia* species and varieties

Treatments	Days for flowering	Flowering duration (days)	Vase life (days)
V_1	93.50	40.50	10.25
V_2	103.25	25.50	4.63
V_3	107.75	23.75	6.38
V_4	125.50	24.50	6.25
V_5	130.25	23.75	12.00
V_6	98.25	25.50	8.13
V_7	163.00	40.00	8.63
V_8	228.25	24.00	13.25
V_9	287.50	34.25	12.88
V_{10}	255.25	24.50	9.38
V_{11}	300.50	34.00	19.75
V_{12}	292.50	38.75	17.50
F	1119.70**	17.87**	130.27**
SE	2.51	1.65	0.40
CD	7.2381	4.758	1.1530

**Significant at one per cent level

Table 11. Number of flowering shoots of 12 *Heliconia* species and varieties

Treatments	Number of flowering shoots			
	3 MAP	5 MAP	11 MAP	1 YAP
V_1	8.38	16.13	21.75	31.75
V_2	12.75	20.88	28.50	40.25
V_3	6.00	10.13	17.75	24.25
V_4	-	12.00	28.00	53.50
V_5	-	14.38	30.50	55.13
V_6	-	19.00	31.38	48.13
V_7	-	11.13	20.63	32.50
V_8	-	-	4.13	11.25
V_9	-	-	5.50	20.38
V_{10}	-	11.38	20.63	33.25
V_{11}	-	-	2.25	4.25
V_{12}	-	-	2.00	4.00
F	191.90**	191.91**	230.12**	768.47**
SE	0.21	0.55	0.75	0.63
CD	0.6176	1.61	2.25	1.84

**Significant at one per cent level

MAP – Months after planting YAP – Year after planting

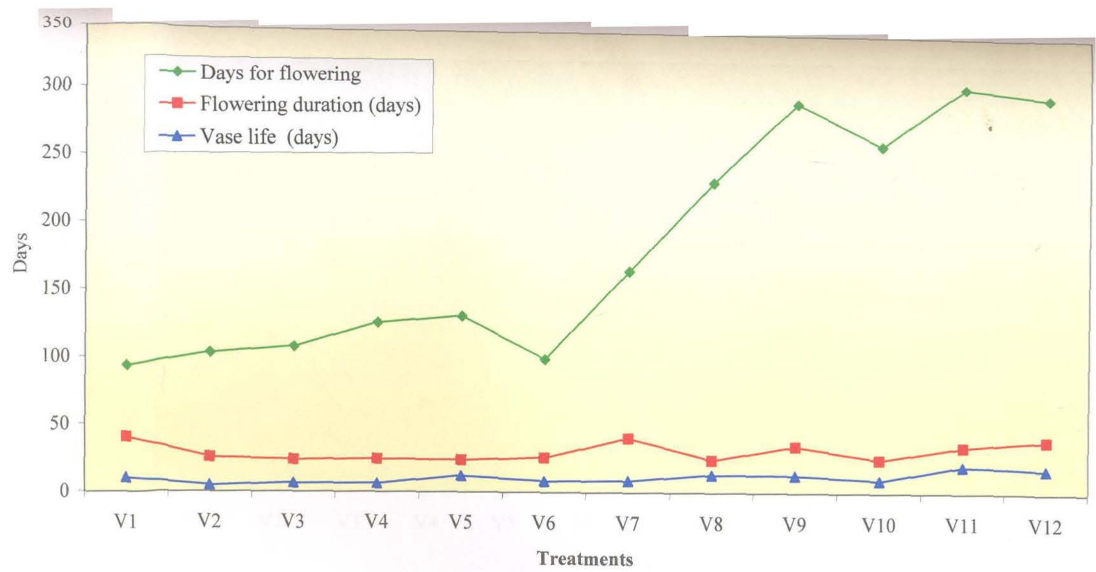


Fig. 5. Crop duration of twelve *Heliconia* species and varieties

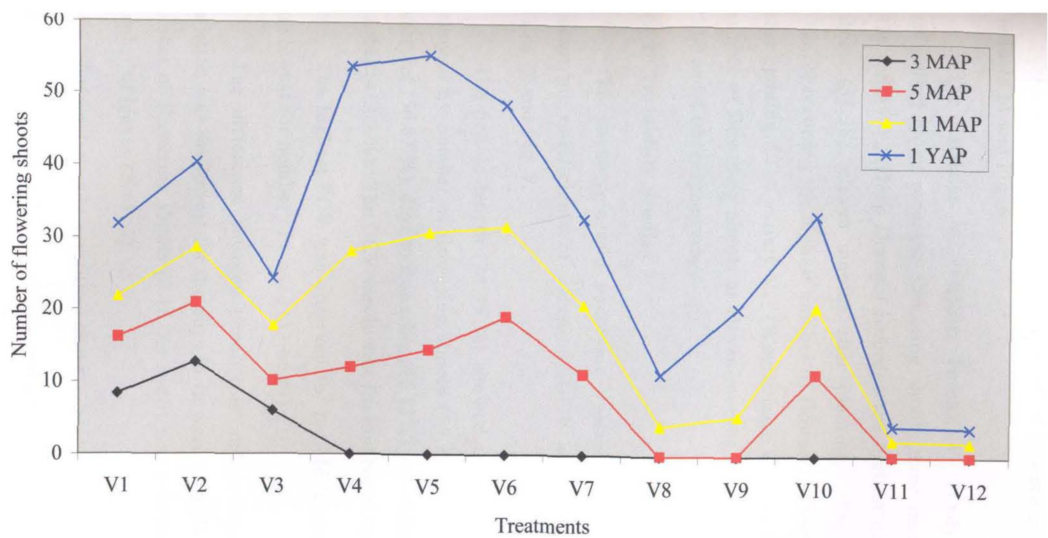


Fig. 6. Number of flowering shoots of twelve *Heliconia* species and varieties

4.1.3.4 Number of flowering shoots

The data pertaining to the number of flowering shoots are presented in Table 11 and Fig. 6.

The varieties V_1 (Golden Torch), V_2 (Lady Di) and V_3 (*H. latispatha*) alone recorded flowering shoots three months after planting. The variety V_6 (Deep Orange) recorded the highest number of flowering shoots (31.38), eleven months after planting. The variety V_5 (Petra Orange) recorded the highest number of flowering shoots (55.13) one year after planting. The variety V_{12} (Wagneriana Yellow) recorded the lowest number of flowering shoots at eleven months and one year after planting (2.00 and 4.00, respectively).

4.1.4 Variability Studies

The phenotypic and genotypic variance and the genotypic and phenotypic coefficients of variation (GCV and PCV) are presented in Table 12 and Fig. 7.

The petiole length (74.45 %) showed the highest value for GCV, followed by number of flowering shoots (59.24 %), leaf area (57.89 %), bract size (56.87 %), days to flowering (46.12%), vase life (42.31%) and bract number (41.09 %). The GCV was lowest for number of leaves (6.50 %).

The highest PCV was observed for petiole length (75.72 %) and it was lowest for number of leaves (14.66 %).

The difference between phenotypic and genotypic coefficient of variation was the lowest for the days to flowering (0.082 %) succeeded by number of flowering shoots (0.15 %) and it was relatively the highest for number of leaves (8.15 %).

4.1.5 Estimation of Heritability and Genetic Advance

The estimates of heritability and genetic advance are presented in Table 13 and Fig. 8.

Table 12. Genotypic and phenotypic coefficient of variation for the characters of 12 *Heliconia* species and varieties

Sl. No.	Characters	GCV (%)	PCV (%)
1	Plant height	34.16	34.51
2	Number of leaves	6.50	14.66
3	Leaf area	57.89	58.09
4	Number of shoots	37.53	38.75
5	Bract number	41.09	43.03
6	Bract size	56.87	57.73
7	Spike length	40.86	41.86
8	Petiole length	74.45	75.72
9	Days to flowering	46.12	46.20
10	Flowering duration	22.67	25.21
11	Vaselife	42.31	42.96
12	Number of flowering shoots	59.24	59.40

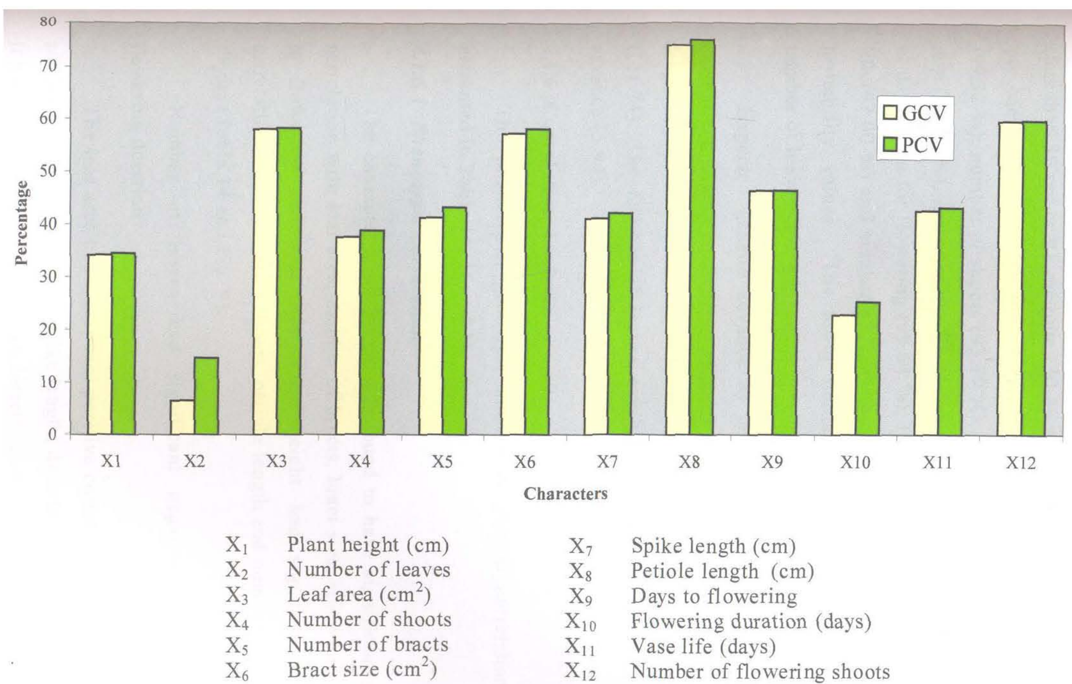


Fig. 7. Coefficient of variation of characters of 12 *Heliconia* species and varieties

Heritability values were classified as per Robinson (1965) as low (less than 30 per cent), medium (30-60 per cent) and high (greater than 60 per cent). Accordingly the characters plant height (97.95), leaf area (99.32 %), number of shoots (93.79 %), number of bracts (91.18 %), bract size (97.06 %), spike length (95.31 %), petiole length, (96.65 %), number of days taken for flowering (99.64 %), flowering duration (80.84 %), vase life (97.00 %) and number of flowering shoots (99.48 %) recorded higher heritability values. The lower heritability values were recorded for number of leaves (19.68 %).

Expected genetic advance as percentage of mean was used for comparison among characters. Highest genetic advance was observed for length of petiole (150.77) followed by number of flowering shoots (121.74). The number of leaves exhibited the lowest value of genetic advance (5.94).

4.1.6 Correlation Analysis

The phenotypic, genotypic and environmental correlations were presented in Table 14, 15 and 16.

4.1.6.1 Phenotypic correlation

The character plant height was found to have significant positive correlation with leaf area, number of bracts, bract size, spike length, days to flowering and vase life. Plant height had significant negative correlation with number of shoots, petiole length and number of flowering shoots (Table 14 and Fig. 9).

Number of leaves had significant negative correlation with flowering duration.

The leaf area had significant positive correlation with plant height, number of bracts, bract size, spike length, days to flowering and vase life. It had shown significant negative correlation with number of shoot, petiole length and the number of flowering shoots.

Table 13. Heritability and genetic advance for the characters of 12 *Heliconia* species and varieties

	Heritability (%)	Genetic advance at 5 %	Genetic advance (as percentage of mean)
X_1	97.95	102.40	69.64
X_2	19.69	0.30	5.94
X_3	99.32	1701.74	118.85
X_4	93.79	27.02	74.87
X_5	91.18	6.18	80.82
X_6	97.06	63.14	115.42
X_7	95.31	19.99	87.18
X_8	96.65	30.17	150.77
X_9	99.64	172.72	94.84
X_{10}	80.84	12.56	41.99
X_{11}	97.00	9.23	85.85
X_{12}	99.48	36.38	121.74

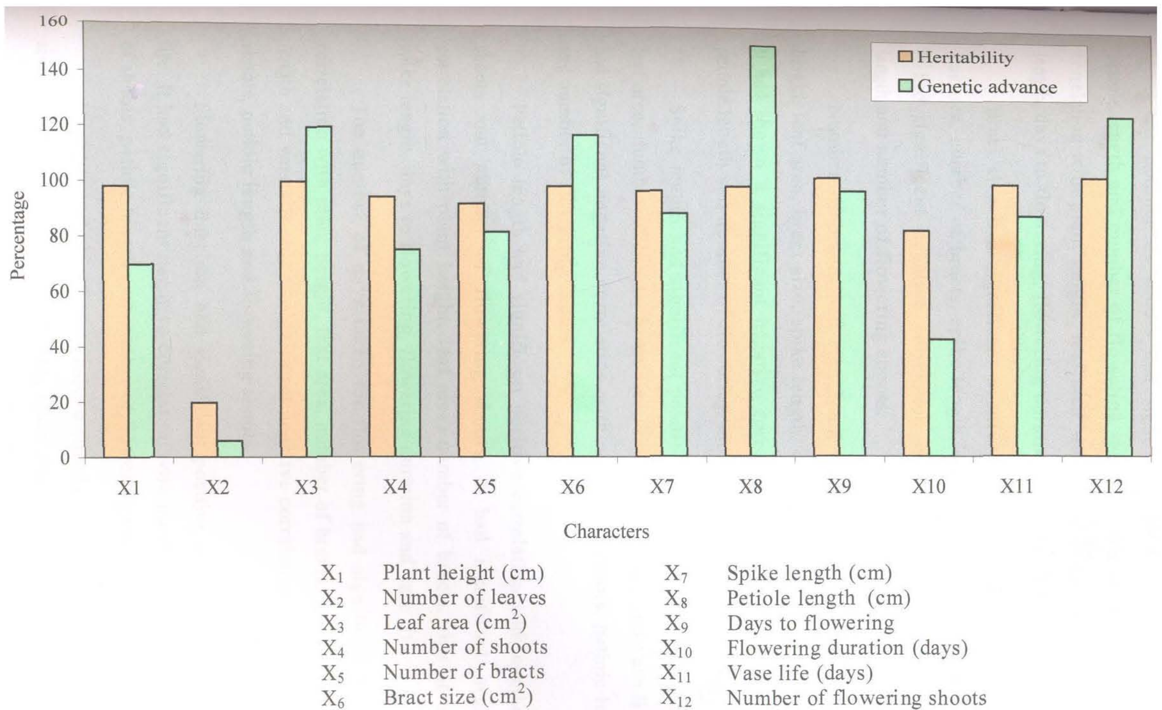


Fig. 8. Heritability and genetic advance of characters of 12 *Heliconia* species and varieites

The number of shoots had significant positive correlation with petiole length and number of flowering shoots. It had significant negative correlation with plant height, leaf area, number of bract, bract size, spike length, days to flowering, flowering duration and vase life.

Bract size had significant positive correlation with plant height, leaf area, number of bracts, spike length, days to flowering and vase life. It had significant negative correlation with number of shoots, petiole length and number of flowering shoots.

Number of bracts had significant positive correlation with plant height, leaf area, bract size, spike length, days to flowering and vase life. It had shown a significant negative correlation with number of shoots, petiole length and number of flowering shoots.

Spike length had significant positive correlation with plant height, leaf area, number of bracts, bract size, days to flowering and vase life. It had significant negative correlation with number of shoots, petiole length and number of flowering shoots.

Petiole length had significant positive correlation with number of shoots and number of flowering shoots. It had significant negative correlation with plant height, leaf area, number of bracts, size of bract, spike length, days to flowering, flowering duration and vase life.

The number of days taken for flowering had significant positive correlation with plant height, leaf area, number of bracts, bract size, spike length and vase life. It had significant negative correlation with number of shoots, petiole length and flowering shoots.

Flowering duration had significant positive correlation with vase life. It had significant negative correlation with number of leaves, number of shoots,, petiole length and number of flowering shots.

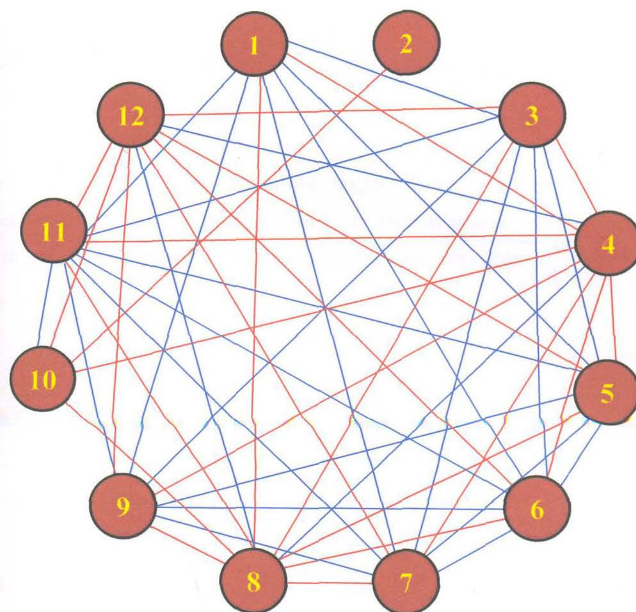
Vase life had significant positive correlation with plant height, leaf area, number of bracts, bract size, spike length, days to flowering and

Table 14. Phenotypic correlation coefficient for the characters of 12 *Heliconia* species and varieties

	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_1	1											
X_2	-0.0380	1										
X_3	0.8399**	-0.1745	1									
X_4	-0.7911**	0.0918	-0.7152**	1								
X_5	0.6959**	-0.2958	0.7252**	-0.7407**	1							
X_6	0.7430**	-0.2497	0.8652**	-0.6806**	0.7150**	1						
X_7	0.8010**	-0.2451	0.7046**	-0.7612**	0.8147**	0.7187**	1					
X_8	-0.4181**	0.1992	-0.6958**	0.5182**	-0.5172**	-0.7207**	-0.4260**	1				
X_9	0.5782**	-0.0280	0.7362**	-0.6430**	0.5047**	0.7113**	0.4460**	-0.8506**	1			
X_{10}	-0.0756	-0.3589*	0.1055	-0.3991*	0.2892	0.1562	0.0638	-0.3246*	0.2732	1		
X_{11}	0.4581**	-0.2844	0.5986**	-0.5563**	0.4764**	0.7855**	0.4833**	-0.6279**	0.7636**	0.4105**	1	
X_{12}	-0.7980**	0.1677	-0.7426**	0.9392**	-0.7090**	-0.7792**	-0.7527**	0.6217**	-0.7356**	-0.4172**	-0.7164**	1

5 % significance : 0.32 *
10 % significance : 0.41 **

X_1	Plant height (cm)	X_7	Spike length (cm)
X_2	Number of leaves	X_8	Petiole length (cm)
X_3	Leaf area (cm^2)	X_9	Days to flowering
X_4	Number of shoots	X_{10}	Flowering duration (days)
X_5	Number of bracts	X_{11}	Vase life (days)
X_6	Bract size (cm^2)	X_{12}	Number of flowering shoots



Positive

Negative

X_1 Plant height (cm)
X_2 Number of leaves
X_3 Leaf area (cm^2)
X_4 Number of shoots
X_5 Number of bracts
X_6 Bract size (cm^2)
X_7 Spike length (cm)
X_8 Petiole length (cm)
X_9 Days to flowering
X_{10} Flowering duration (days)
X_{11} Vase life (days)
X_{12} Number of flowering shoots

Fig. 9. Phenotypic correlation coefficient of characters in 12 *Heliconia* species and cultivars

flowering duration. It had significant negative correlation with number of shoots, petiole length and number of flowering shoots.

Number of flowering shoots had significant positive correlation with number of shoots and petiole length. It had significant negative correlation with plant height, leaf area, number of bracts, bract size, spike length, days to flowering, flowering duration and vase life.

4.1.6.2 Genotypic correlation

The character plant height was found to have significant positive correlation with leaf area, number of bracts, bract size, spike length, days to flowering and vase life. Plant height had significant negative correlation with number of shoots, petiole length and number of flowering shoots (Table 15 and Fig. 10).

Number of leaves had significant positive correlation with number of flowering shoots. It had significant negative correlation with leaf area, number of bracts, bract size, spike length, flowering duration and vase life.

Leaf area had significant positive correlation with plant height, number of bracts, bract size, spike length, days to flowering and vase life.

Number of shoots had significant positive correlation with petiole length and number of flowering shoots. It had significant negative correlation with plant height, leaf area, number of bract, bract size, spike length, days to flowering, flowering duration and vase life.

Number of bracts had significant positive correlation with plant height, leaf area, bract size, spike length, days to flowering and vase life. It had significant negative correlation with number of leaves, number of shoots, petiole length and number of flowering shoots.

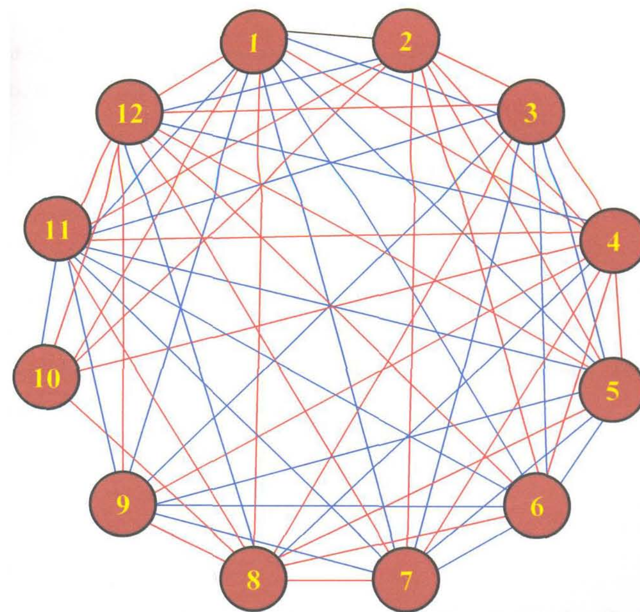
Bract size had significant positive correlation with plant height, leaf area, number of bracts, spike length, days to flowering and vase life.

Table 15. Genotypic correlation coefficient for the characters of 12 *Heliconia* species and varieties

	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_1	1											
X_2	-0.0911	1										
X_3	0.8492**	-0.3889*	1									
X_4	-0.8294**	0.2594	-0.7418**	1								
X_5	0.7401**	-0.7024**	0.7590**	-0.7867**	1							
X_6	0.7780**	-0.5051**	0.8865**	-0.7054**	0.7444**	1						
X_7	0.8380**	-0.5997**	0.7276**	-0.7832**	-0.5642**	0.7357**	1					
X_8	-0.4340**	0.2283	-0.7106**	0.5535**	0.5462**	-0.7392**	-0.4494**	1				
X_9	0.5822**	-0.0837	0.7402**	-0.6669**	0.5380**	0.7256**	0.4577**	-0.8690**	1			
X_{10}	0.1019	-0.6183**	0.1203	-0.4464**	0.3195	0.1799	0.0875	-0.3626*	0.3076	1		
X_{11}	0.4738**	-0.5625**	0.6112**	-0.5674**	0.4923**	0.7984**	0.4862**	-0.6440**	0.7764**	0.4499**	1	
X_{12}	-0.8043**	0.3547*	-0.7452**	0.9705**	-0.7399**	-0.7933**	-0.7722**	0.6364**	-0.7389**	-0.4622**	-0.7273**	1

5 % significance : 0.32 *
10 % significance : 0.41 **

X_1	Plant height (cm)	X_7	Spike length (cm)
X_2	Number of leaves	X_8	Petiole length (cm)
X_3	Leaf area (cm^2)	X_9	Days to flowering
X_4	Number of shoots	X_{10}	Flowering duration (days)
X_5	Number of bracts	X_{11}	Vase life (days)
X_6	Bract size (cm^2)	X_{12}	Number of flowering shoots



Positive

Negative

X_1	Plant height (cm)	X_7	Spike length (cm)
X_2	Number of leaves	X_8	Petiole length (cm)
X_3	Leaf area (cm^2)	X_9	Days to flowering
X_4	Number of shoots	X_{10}	Flowering duration (days)
X_5	Number of bracts	X_{11}	Vase life (days)
X_6	Bract size (cm^2)	X_{12}	Number of flowering shoots

Fig. 10. Genotypic correlation coefficient of characters in 12 *Heliconia* species and cultivars

It had significant negative correlation with number of leaves, number of shoots, petiole length and number of flowering shoots.

Spike length had significant positive correlation with plant height, leaf area, number of bracts, bract size, days to flowering and vase life. Spike length had significant negative correlation with number of leaves, number of shoots, petiole length and number of flowering shoots.

Petiole length had significant positive correlation with number of shoots and flowering shoots. It had significant negative correlation with plant height, leaf area, number of bract, bract size, spike length, days to flowering, flowering duration and vase life.

Days taken for flowering had significant positive correlation with plant height, leaf area, number of bracts, bract size, spike length and vase life. It had significant negative correlation with number of shoots, petiole length and number of flowering shoots.

Flowering duration had significant positive correlation with vase life. It had significant negative correlation with number of leaves, number of shoots, petiole length and number of flowering shoots.

Vase life had significant positive correlation with plant height, leaf area, number of bract, bract size, spike length, days to flowering and flowering duration. It had significant negative correlation with number of leaves, number of shoots, petiole length and number of flowering shoots.

Number of flowering shoots had significant positive correlation with number of leaves number of shoots and petiole length. It had significant negative correlation with plant height, leaf area, number of bract, bract size, spike length, days to flowering, flowering duration and vase life.

4.1.6.3 Environmental correlations

Low values of correlation coefficients due to environmental effect were obtained for most of the characters studied (Table 16).

172549

Table 16. Environmental correlation coefficient for the characters of 12 *Heliconia* species and varieties

	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_1	1											
X_2	0.0158	1										
X_3	0.1980	-0.0342	1									
X_4	0.1084	-0.0883	0.0330	1								
X_5	-0.0831	0.0067	0.1220	-0.1781	1							
X_6	-0.6380**	-0.1878	-0.3649*	-0.1770	0.2890	1						
X_7	-0.2819	0.0757	-0.1883	-0.3845*	0.1400	02991	1					
X_8	0.1591	0.6071**	0.0314	-0.1922	-0.0825	-0.1504	0.1337	1				
X_9	0.3550*	0.1695	-0.0465	0.1181	-0.4591**	-0.2267	-0.0022	0.1970	1			
X_{10}	0.2405	-0.2862	-0.0634	-0.0955	0.1142	-0.0421	-0.1371	-0.0518	-0.1090	1		
X_{11}	-0.1479	-0.2485	-0.0894	-0.3480*	0.2616	0.3644*	0.4237**	-0.1362	0.2330	0.1603	1	
X_{12}	-0.3931*	0.1665	-0.3173	0.0958	-0.2024	0.0302	-0.0500	-0.1769	-0.0013	-0.0844	-0.1593	1

5% significance : 0.32 *
10 % significance : 0.41 **

X_1 Plant height (cm)
X_2 Number of leaves
X_3 Leaf area (cm^2)
X_4 Number of shoots
X_5 Number of bracts
X_6 Bract size (cm^2)
X_7 Spike length (cm)
X_8 Petiole length (cm)
X_9 Days to flowering
X_{10} Flowering duration (days)
X_{11} Vase life (days)
X_{12} Number of flowering shoots

Plant height had shown significant positive correlation with bract size, days to flowering and number of flowering shoots.

The number of leaves of had high significant positive correlation with peduncle length.

The leaf area had significant positive correlation with bract size.

The number of shoots had significant positive correlation with spike length.

Bract number had significant positive correlation with days to flowering.

The size of bract has significant positive correlation with vase life.

4.1.7 Economics of cultivation

The data pertaining to the economics of cultivation is depicted in Table 17.

Among the *Heliconia* species and varieties, V_5 (Petra Orange) recorded highest net returns (Rs. 6,28,900). It was followed by variety V_7 (Pedro Ortiz, Rs. 5,12,500). Lower net returns were observed in V_{11} (Wagneriana Red, Rs. 50,000) and V_{12} (Wagneriana Yellow, Rs. 25,000).

Highest cost benefit ratio was recorded in V_5 (Petra Orange, 1.54) followed by V_7 (Pedro Ortiz, 1.40). Lower ratio was recorded in V_{11} (Wagneriana red, 1.04) and V_{12} (Wagneriana yellow, 1.02).

4.2 EXPERIMENT 2 : MOLECULAR CHARACTERIZATION OF *HELICONIA*

4.2.1 Genomic DNA Isolation

Two different procedures reported earlier and their modifications were tried for standardizing the extraction of genomic DNA from *Heliconia*. Standardisation studies were carried out using *Heliconia psittacorum* cultivars *viz.* Deep Orange, Lady Di, and Petra Orange. Results of experiments are presented in the Table 18. There was no

Table 17. Economics of cultivation of 12 *Heliconia* species and varieties

Treatments	Net returns (Rs. ha^{-1})	Benefit : Cost ratio
V_1	270000.00	1.35
V_2	250622.00	1.29
V_3	115400.00	1.17
V_4	430000.00	1.30
V_5	628900.00	1.54
V_6	121950.00	1.18
V_7	300000.00	1.40
V_8	512500.00	1.41
V_9	480000.00	1.38
V_{10}	380000.00	1.36
V_{11}	50000.00	1.04
V_{12}	25000.00	1.02

Table 18. Quantity and yield of DNA from *Heliconia psittacorum* var. Deep Orange, Lady Di and Petra Orange

Cultivars	A_{260}				A_{280}				A_{260}/A_{280}				Yield of DNA ($\mu g\ ml^{-1}$)			
	P1	P2	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4
Deep Orange	0.038	0.039	0.017	0.004	0.028	0.021	0.009	0.002	1.5	1.8	1.85	1.7	1140	1170	510	1200
Lady Di	0.016	0.017	0.017	0.017	0.009	0.009	0.009	0.010	1.7	1.8	1.85	1.6	480	510	510	510
Petra Orange	0.029	0.030	0.039	0.031	0.016	0.017	0.020	0.019	1.8	1.7	1.95	1.6	870	900	1170	930

difference among the treatments for the yield of DNA. Hence for the further study protocol 2 was followed which does not require liquid nitrogen and the leaf tissues were ground in CTAB.

Study was conducted to know the effect of storage on yield and quality of DNA using *H. psittacorum* var. Deep Orange. Emerging leaves before fully unfurled were collected. The leaves collected were either used fresh for extraction or stored in an ultra freezer at -85°C for one week (Sanyo Ultra low). It was shown that this storage did not interfere with the yield of DNA (Table 19). When fresh leaves were used the yield of DNA was 500 $\mu g\ ml^{-1}$. The leaves after storage in the ultra freezer for one week yielded 540 $\mu g\ ml^{-1}$. Purity ratio was also not affected by storage. The purity ratio of the sample used fresh was 1.80 while that of sample used after storage was 1.86.

The purity and yield of the selected 12 *Heliconia* species and varieties are depicted in Table 20. The purity value of A_{260}/A_{280} ranged from 1.50 to 1.95 with the mean of 1.81. The average genomic DNA yields of 12 species and varieties of *Heliconia* was 707.50 $\mu g\ ml^{-1}$ and it ranged from 360 to 1170 $\mu g\ ml^{-1}$. The highest DNA yield (1170 $\mu g\ ml^{-1}$) was recorded by V_6 (Deep Orange) and the lowest yield of 360 $\mu g\ ml^{-1}$ was recorded by V_{12} (Wagneriana Yellow).

The electrophoretic assay of DNA samples using agarose gel electrophoresis (1.4 per cent) revealed that the integrity of the DNA samples was good, without any smearing. The buffer used (1 x TAE buffer 0.04 M Tris acetate, 0.001 M EDTA, pH 6.0) was found to be good for the separation of the bands.

4.2.2 Polymerase Chain Reaction (PCR)

Out of the 40 primers used, all the primers except OPA-6, OPA-09, OPA-10, OPA-12, OPB-09 and OPB-13 yielded amplified products with the DNA from the *Heliconia* variety Deep Orange. The total number of

Table 19. Effect of storage of leaves on the quantity and quality of the DNA of *Heliconia psittacorum* var Deep Orange

	A_{260}	A_{280}	A_{260}/A_{280}	DNA yield ($\mu g\ ml^{-1}$)
Fresh leaves	0.017	0.009	1.80	500
Leaves stored at -85°C	0.018	0.009	1.86	540

Table 20 Quantity and yield of DNA from 12 *Heliconia* species and varieties

Treatments	A_{260}	A_{280}	A_{260}/A_{280}	Yield of DNA $\mu g\ ml^{-1}$
V_1	0.037	0.619	1.94	1110
V_2	0.060	0.016	1.85	900
V_3	0.021	0.11	1.90	630
V_4	0.018	0.010	1.80	540
V_5	0.017	0.009	1.88	510
V_6	0.039	0.200	1.95	1170
V_7	0.018	0.010	1.87	540
V_8	0.019	0.011	1.72	570
V_9	0.028	0.017	1.60	840
V_{10}	0.031	0.016	1.93	930
V_{11}	0.013	0.007	1.85	390
V_{12}	0.012	0.008	1.50	360

bands, number of faint band and the number of intense bands produced by the primers are given in Table 21 and Fig. 11, 12 and 13. The primers amplified 80 RAPD marker bands (average of 1.9 bands per primer). Of these 75 (93.75 %) were polymorphic and five were monomorphic. The primers OPA-02, OPA-03, OPA-18, OPB-15 and OPB-20 produced monomorphic bands.

The highest number of RAPDs was produced by the primers OPA-17 and OPA-18 (6 each). Among them, OPA-18 produced, the highest number of intense bands (5 bands). OPA-17 produced four intense bands. OPA-14 also produced four intense bands. The primers OPA-01, OPB-12, OPB-18 and OPB-20 produced three intense bands each.

The eight primers, which produced the highest number of bands as well as the highest number of intense bands, were selected for DNA amplification from the 12 *Heliconia* species and varieties. The PCR reaction was repeated at least twice in order to confirm the reproducibility.

The data obtained from eight primers that gave reproducible bands were used for statistical analysis. They are OPA-01, OPA-14, OPA-17, OPA-18, OPB-07, OPB-12, OPB-18 and OPB-20. The primers amplified 57 scorable RAPD marker bands with an average of 7.10 bands per primer. The primer OPA 18 yielded one polymophic band for each species and varieties, except in V_9 (*H. humilis*). Similarly the primer OPA 14 yielded one monomorphic band for all the species and varieties except for V_7 (*H. rostrata*). The number of bands resolved per amplification was primer dependent and varied from a lowest of eight to a highest of eleven. The nucleotide sequence of these primers and the number of informative RAPD markers amplified by each primer are given in Table 22 and Fig. 14.

The primer OPA-01 yielded seven scorable bands. The varieties V_4 (De Rooij), V_5 (Petra Orange) and V_6 (Deep Orange) yielded four bands each (Fig. 15 and Plate 4.). Six bands were obtained on amplification using the primer, OPA-14 (Fig. 16 and Plate 5). With OPA-14 one band

Table 21. Primer associated banding patterns with the DNA of *Heliconia* using 40 primers belonging to kit A and kit B of Operon Inc, CA, USA

Sl. No.	Primers	Number of faint bands	Number of intense bands	Total number of bands
1	OPA-01	1	3	4
2	OPA-02	2	0	2
3	OPA-03	2	0	2
4	OPA-04	0	1	1
5	OPA-05	1	0	1
6	OPA-06	0	0	0
7	OPA-07	0	2	2
8	OPA-08	0	1	1
9	OPA-09	0	0	0
10	OPA-10	0	0	0
11	OPA-11	0	1	1
12	OPA-12	0	0	0
13	OPA-13	1	0	1
14	OPA-14	1	4	5
15	OPA-15	1	0	1
16	OPA-16	1	1	2
17	OPA-17	2	4	6
18	OPA-18	1	5	6
19	OPA-19	2	1	3
20	OPA-20	1	0	1
21	OPB-01	0	1	1
22	OPB-02	0	2	2
23	OPB-03	1	0	1
24	OPB-04	2	0	2
25	OPB-05	1	1	2
26	OPB-06	1	0	1
27	OPB-07	4	1	5
28	OPB-08	3	0	3
29	OPB-09	0	0	0
30	OPB-10	2	0	2
31	OPB-11	0	1	1
32	OPB-12	1	3	4
33	OPB-13	0	0	0
34	OPB-14	0	2	2
35	OPB-15	1	0	1
36	OPB-16	2	0	2
37	OPB-17	1	1	2
38	OPB-18	1	3	4
39	OPB-19	2	0	2
40	OPB-20	1	3	4

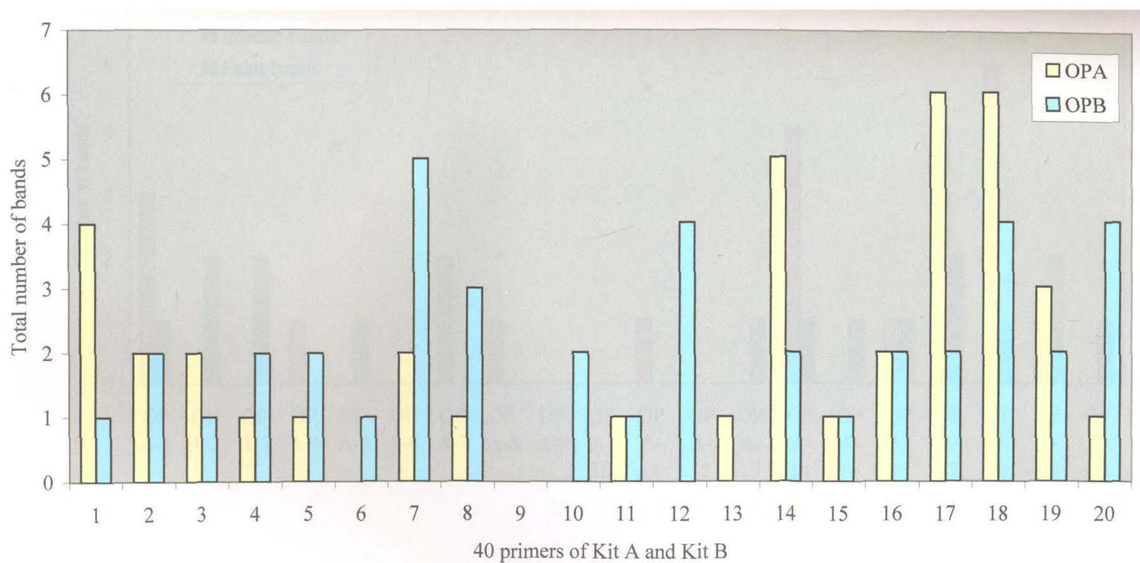


Fig. 11. Amplification profiles (total bands) of the DNA of *Heliconia psittacorum* cv. Deep Orange using 40 primers of Kit A and Kit B

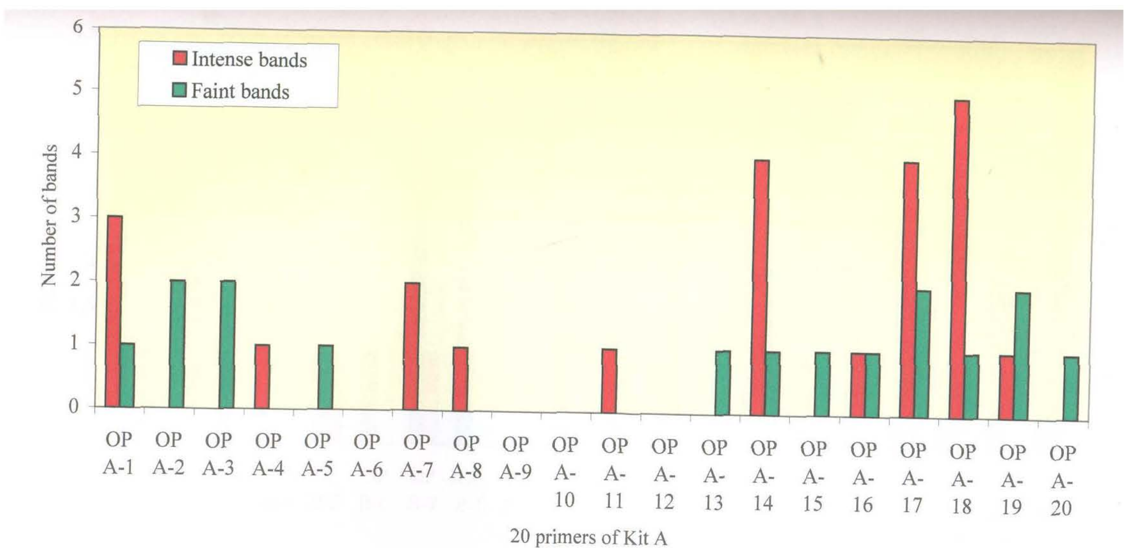


Fig. 12. Amplification profiles (intense and faint bands) of the DNA of *Heliconia psittacorum* cv. Deep Orange using 20 primers of Kit A

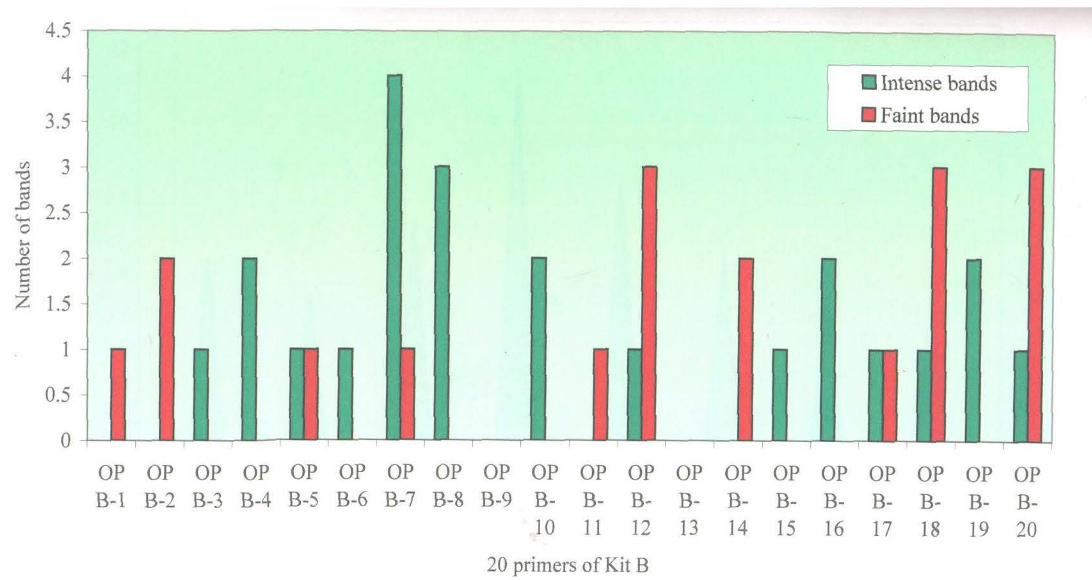


Fig. 13. Amplification profiles (intense and faint bands) of the DNA of *Heliconia psittacorum* cv. Deep Orange using 20 primers of Kit B

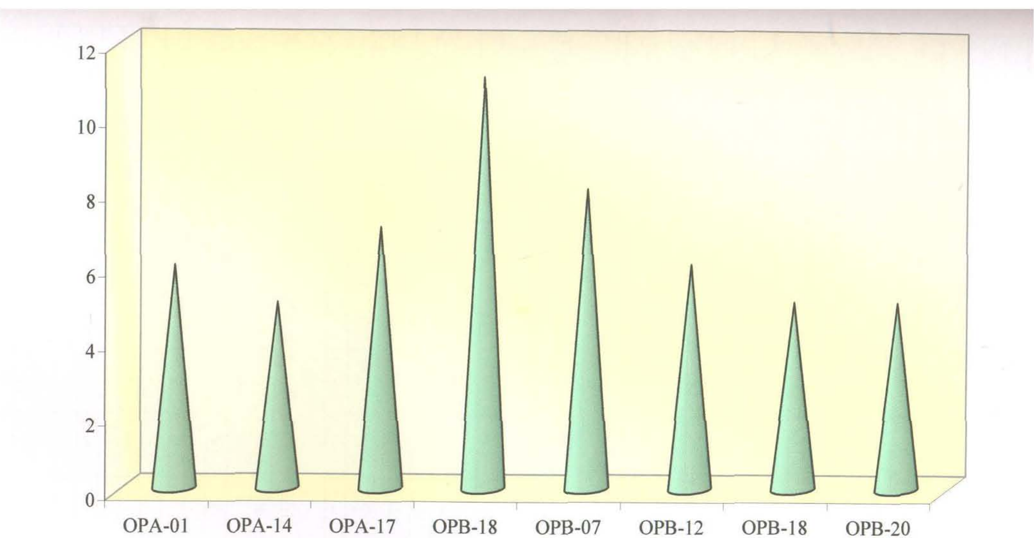


Fig. 14. Total number of informative RAPD markers amplified by the eight primers in 12 *Heliconia* species and cultivars

Fig. 15. Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPA-01

V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
-	+	+	+	+	+	-	-	-	-	+	-
-	-	-	+	-	-	-	-	-	-	+	-
+	-	-	+	+	+	+	-	-	-	-	+
-	+	-	-	+	+	+	+	+	+	-	-
+	-	+	+	+	+	-	-	+	+	-	-
-	-	+	-	-	-	+	+	-	-	+	+

Fig. 16. Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPA-14

V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
-	-	-	+	-	-	-	-	-	-	-	-
-	-	+	+	-	-	-	-	-	-	+	-
+	-	+	+	-	-	-	+	-	+	+	-
+	+	+	+	+	+	+	-	+	+	+	+
-	+	+	+	+	+	-	+	+	-	+	+
-	-	+	+	+	+	+	-	-	+	-	-

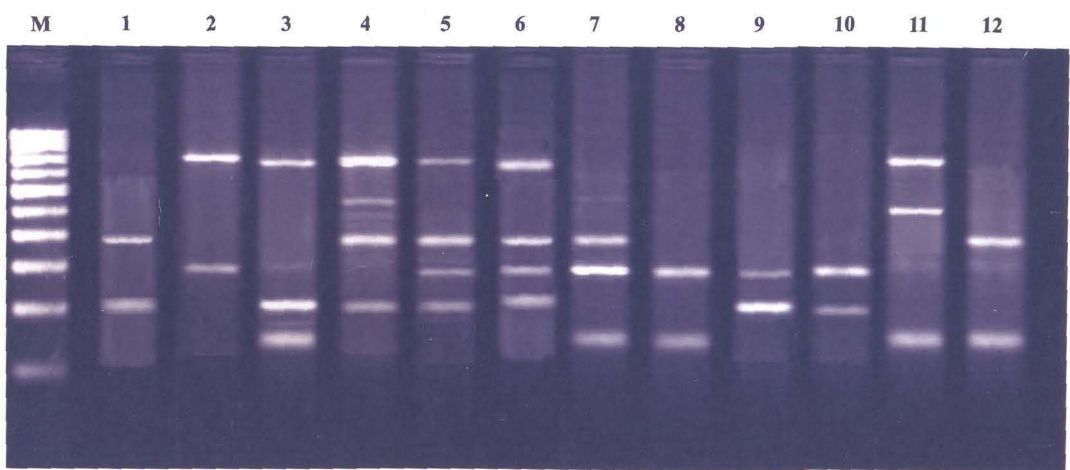
Fig. 17. Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPA-17

V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
-	-	-	-	+	-	-	-	-	+	-	+
-	-	+	-	+	-	-	+	+	+	+	+
+	+	-	+	+	-	+	-	-	-	+	-
+	+	-	-	+	-	-	+	-	+	-	+
-	-	-	+	+	+	-	-	-	-	+	+
+	-	-	-	+	+	+	-	-	+	+	+
+	-	-	-	-	-	-	-	-	+	-	+

Fig. 18. Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPA-18

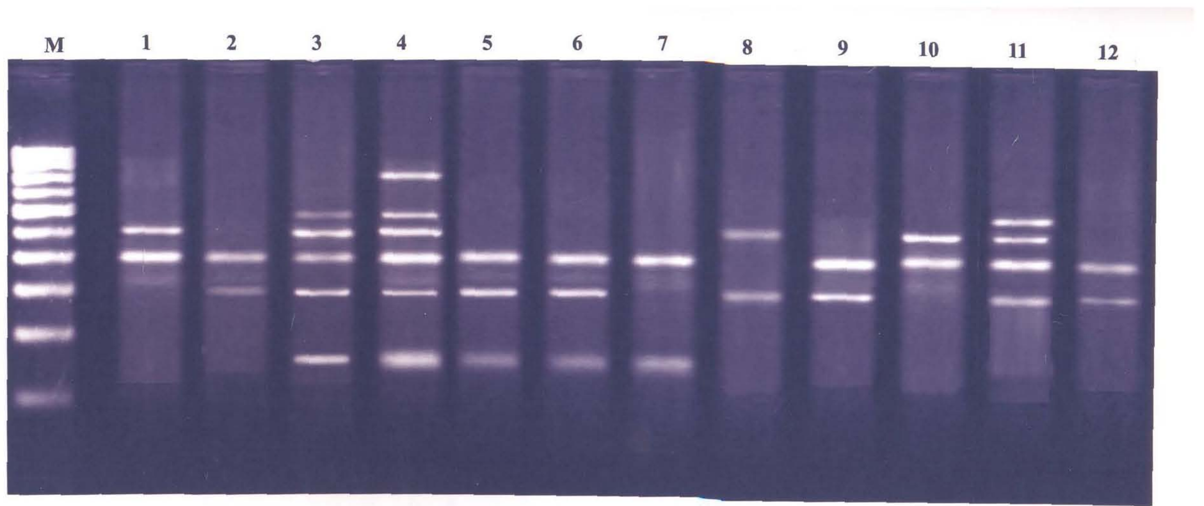
V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
-	+	-	-	+	-	-	-	-	-	+	-
-	-	-	-	+	+	-	-	-	-	-	+
+	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-	-
+	-	-	-	-	-	-	-	-	-	-	-
+	+	+	+	+	+	+	-	-	+	+	+
+	-	-	-	+	+	-	-	-	-	-	-
-	-	-	-	+	-	-	-	+	-	-	-
+	+	+	+	+	+	+	+	-	+	+	+
+	-	-	-	-	+	-	-	-	-	-	-
-	+	-	+	+	+	+	+	-	+	-	-

+ : Presence of band
- : Absence of band



M - Molecular marker

Plate 4. Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPA-01



M - Molecular marker

Plate 5. Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPA-14

was monomorphic for all species and varieties except for V_8 (Pedro Ortiz). The highest number of bands (6) was given by V_4 (De Rooij).

Eight scorable bands were obtained on amplification with the primer OPA-17 (Fig. 17 and Plate 6). The varieties V_{12} (Wagneriana Yellow) and V_5 (Petra Orange) yielded six bands each. The variety V_{10} (Guyana) yielded five bands. The variety V_{11} (Wagneriana Red) yielded four bands. The species V_3 (*H. latispatha*) gave only one band.

The highest number of scorable bands (11) was given by the primer OPA-18 (Fig. 18 and Plate 7). It produced a monomorphic band for all the species and varieties except for V_9 (*H. humilis*). The varieties V_5 (Petra Orange) and V_1 (Golden Torch) gave seven bands each when OPA-18 was used for amplification. The variety V_6 (Deep Orange) yielded six bands. The species V_9 (*H. humilis*) produced only one band.

A total of nine scorable bands were obtained on *in vitro* amplification of the *Heliconia* species and varieties using the primer OPB-07 (Fig. 19 and Plate 8). The species V_7 (*H. rostrata*) yielded five bands. The treatments V_{11} (Wagneriana Red), V_4 (D Rooij), V_9 (*H. humilis*), V_2 (Lady Di) and V_6 (Deep Orange) yielded three bands each. The varieties V_8 (Pedro Ortiz) and V_1 (Golden Torch) yielded only one band.

Six bands were obtained on amplification using the primer, OPB-12 (Fig. 20 and Plate 9). With the primer OPB-12, V_9 (*H. humilis*) yielded the highest number of scorable bands (4).

The primers OPB-18 (Fig. 21 and Plate 10) and OPB-20 (Fig. 22 and Plate 11) yielded a total of five scorable bands when used for amplification. With OPB-18 the varieties, V_{12} (Wagneriana Yellow) and V_5 (Petra Orange) gave three bands each. The treatments V_8 (Pedro Ortiz), V_3 (*H. latispatha*) and V_4 (De Rooij) gave only one band each. With the primers OPB-20, V_5 (Petra Orange) gave four bands. The species V_7 (*H. rostrata*) and V_3 (*H. latispatha*) yielded only one band.

Table 22. Nucleotide sequence of primers and total number of informative RAPD markers amplified by them in the 12 *Heliconia* species and varieties

Primer	Nucleotide sequence	Number of informative RAPD markers
OPA-01	CAGGCCCTTC	7
OPA-14	TCTGTGCTTGT	6
OPA-17	GACCGCTTGT	8
OPB-18	AGGTGACCGT	11
OPB-07	GGTGACGCAG	9
OPB-12	CCTTGACGCA	6
OPB-18	CCACAGCAGA	5
OPB-20	GGACCCTTAC	5

Fig. 19. Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPB-07

V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
-	-	-	+	-	+	+	-	+	-	-	-
-	+	-	-	+	+	+	-	+	-	-	+
-	-	+	-	-	-	+	-	-	+	+	-
-	+	-	+	-	-	-	-	+	-	-	-
-	-	-	-	-	-	-	+	-	-	+	+
-	+	-	-	+	+	-	-	-	-	+	-
+	-	-	-	-	-	+	-	-	+	-	-
-	-	+	+	-	-	+	-	-	-	-	-

Fig. 20. Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPB-12

V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
-	-	+	-	-	-	+	-	+	-	+	+
-	-	+	+	+	-	+	+	+	+	+	-
-	+	-	-	+	-	-	-	+	-	-	+
-	-	+	-	+	-	-	+	+	+	-	-
+	-	-	+	-	+	+	+	-	+	-	+
+	+	-	-	-	+	-	-	-	-	-	-

Fig. 21. Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPB-18

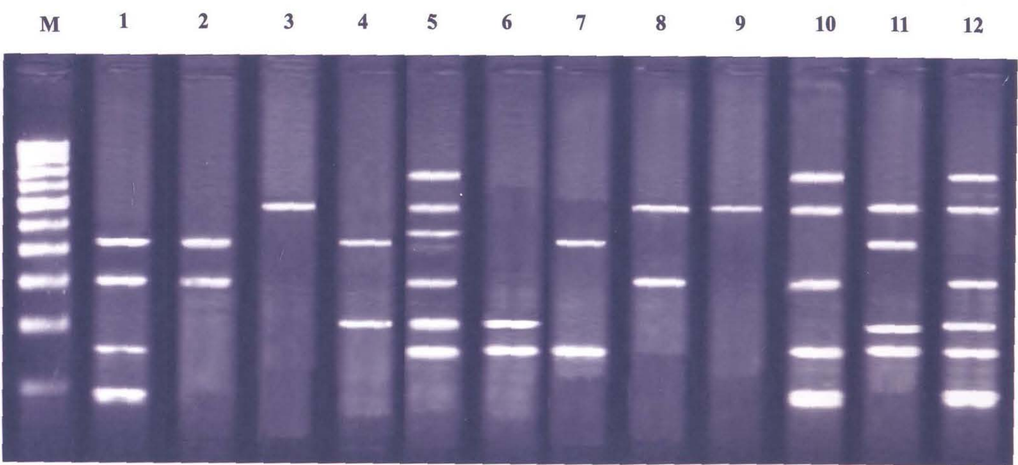
V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
-	-	-	-	-	-	+	-	+	-	+	+
-	+	+	+	+	-	-	-	+	-	+	+
+	+	-	-	+	+	+	-	-	-	-	+
+	+	-	-	+	+	-	-	+	+	+	+
+	-	-	-	+	+	-	+	-	+	-	-

Fig. 22. Representation of the amplification profile of the DNA of 12 *Heliconia* species and varieties using the primer OPB-20

V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
-	-	-	-	-	-	-	+	+	-	+	+
-	-	-	-	+	-	+	-	-	-	+	+
-	+	-	+	+	-	-	+	+	-	+	+
+	-	+	+	+	+	-	-	-	+	-	-
+	+	-	+	+	+	-	-	-	+	-	-

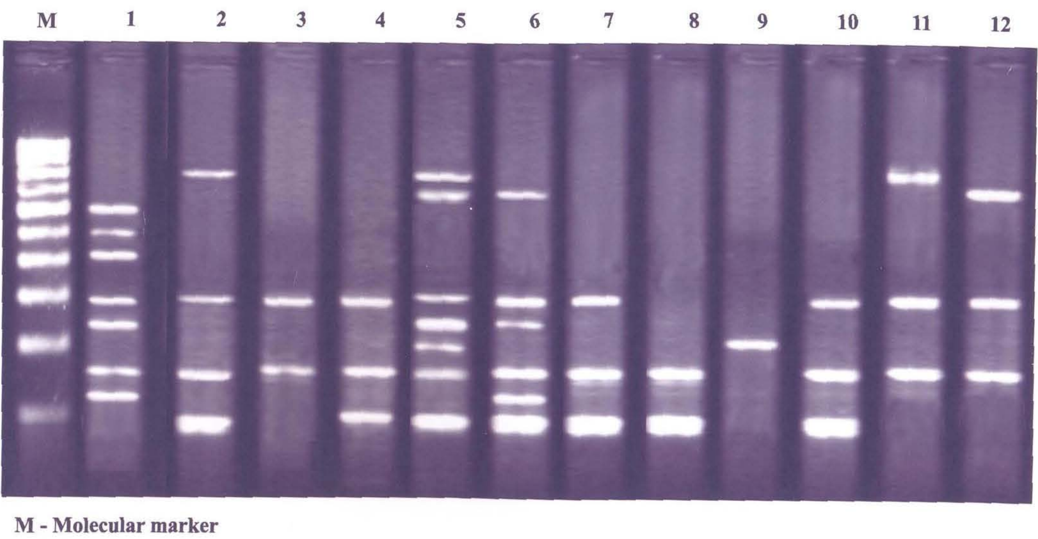
+ : Presence of band

- : Absence of band



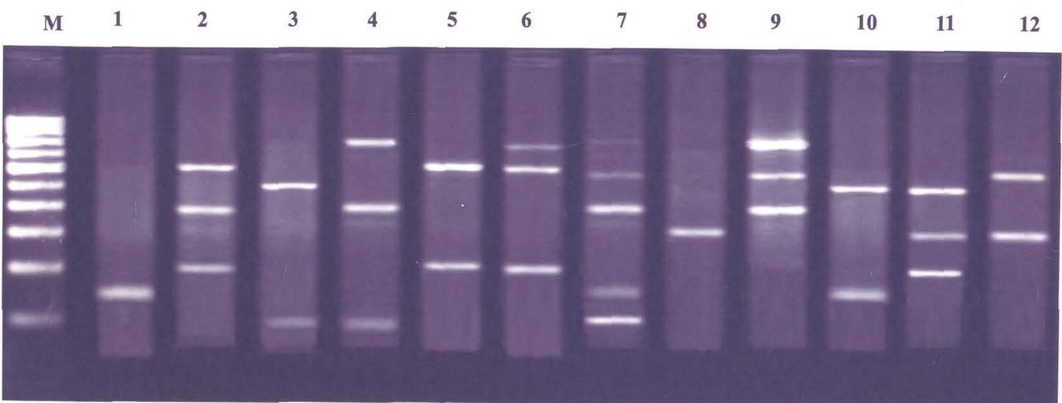
M - Molecular marker

Plate 6. Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPA-17



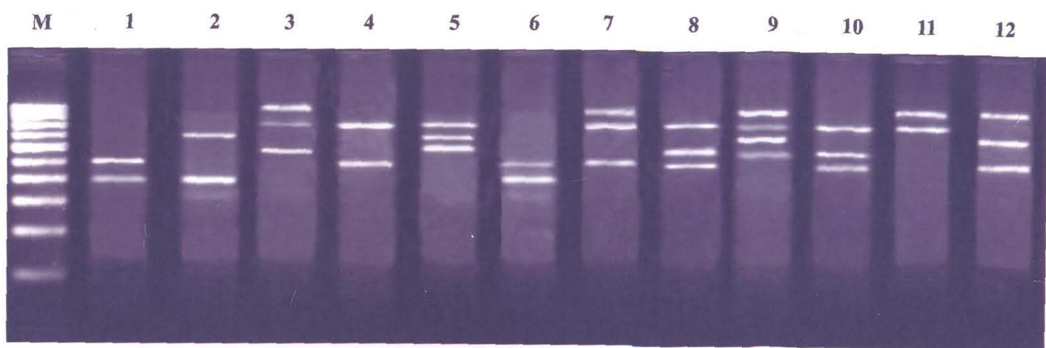
M - Molecular marker

Plate 7. Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPA-18



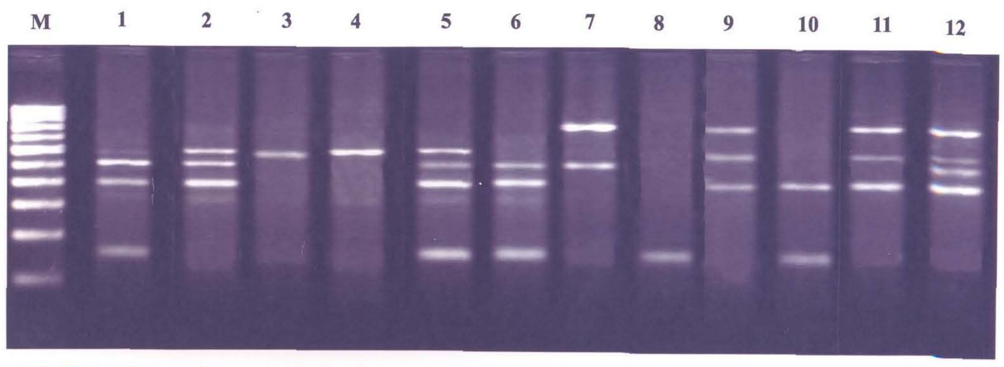
M - Molecular marker

Plate 8. Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPB-07



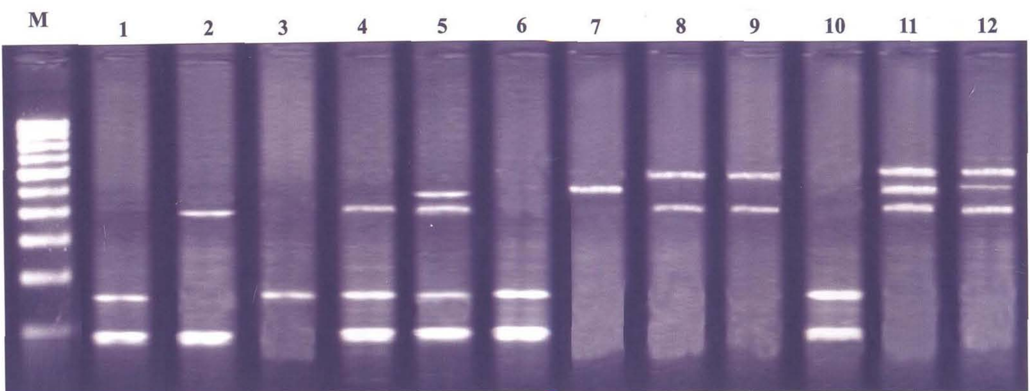
M - Molecular marker

Plate 9. Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPB-12



M - Molecular marker

Plate 10. Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPB-18



M - Molecular marker

Plate 11. Amplification profile of the DNA of 12 *Heliconia* species and varieties with the primer OPB-20

4.2.3 RAPD data analysis

The banding pattern for each primer was scored by visual observation. Based on the presence or absence of RAPD band data, score value was given as one for presence of band and zero for absence of band. Genetic similarity matrix was constructed using the Jaccard's coefficient method (Table 23) with the score values of RAPD marker bands.

The least similarity coefficient (0.083) was recorded by V_1 (Golden Torch) and V_9 (*H. humilis*) followed by 0.125 (V_1, Golden Torch) and V_{11} (Wagneriana Red).

The highest value for similarity index (0.556) was obtained for V_5 (Deep Orange) and V_6 (Petra Orange), followed by (0.470) V_2 (Lady Di) and V_5 (Petra Orange).

On drawing a vertical line (Fig.23) corresponding to the similarity coefficient value of 0.32, the 12 species and varieties of *Heliconia* were grouped into five clusters, while at a coefficient value of 0.40, the 12 species and varieties of *Heliconia* were got divided into seven clusters.

While considering the five clusters, Deep Orange (V_6), Petra Orange (V_5), Golden Torch (V_1), Lady Di (V_2) and Guyana (V_{10}) formed the biggest cluster. Within this cluster, Petra Orange and Deep Orange were more close to each other. De Rooij (V_4) and *H. latispatha* (V_3) formed another cluster. Wagneriana Red (V_{11}), Wagneriana Yellow (V_{12}) and *H. humilis* (V_9) formed another cluster. *Heliconia rostrata* (V_7) and Pedro Ortiz (V_8) formed two separate clusters.

4.3 EXPERIMENT 3 : *IN VITRO* PROPAGATION OF HELICONIAS

4.3.1 Standardisation of explants :

Shoot apex, bits of rhizome, leaf segments and root segments were used for standardising the explant (Table 24). Standard procedure was followed for surface sterilization (Mercuric chloride 0.08 per cent for 10

Table 23. Similarity matrix of twelve *Heliconia* species and varieties obtained by RAPD analysis using three primer combinations

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
V_1	1.000											
V_2	0.258	1.000										
V_3	0.181	0.161	1.000									
V_4	0.257	0.281	0.464	1.000								
V_5	0.292	0.470	0.315	0.375	1.000							
V_6	0.406	0.354	0.235	0.382	0.556	1.000						
V_7	0.193	0.214	0.259	0.258	0.263	0.250	1.000					
V_8	0.129	0.142	0.230	0.193	0.243	0.151	0.200	1.000				
V_9	0.083	0.241	0.285	0.291	0.315	0.167	0.133	0.230	1.000			
V_{10}	0.400	0.278	0.392	0.294	0.433	0.363	0.321	0.346	0.181	1.000		
V_{11}	0.125	0.272	0.400	0.343	0.365	0.200	0.290	0.267	0.313	0.216	1.000	
V_{12}	0.216	0.272	0.200	0.205	0.435	0.297	0.290	0.267	0.354	0.250	0.454	1.000

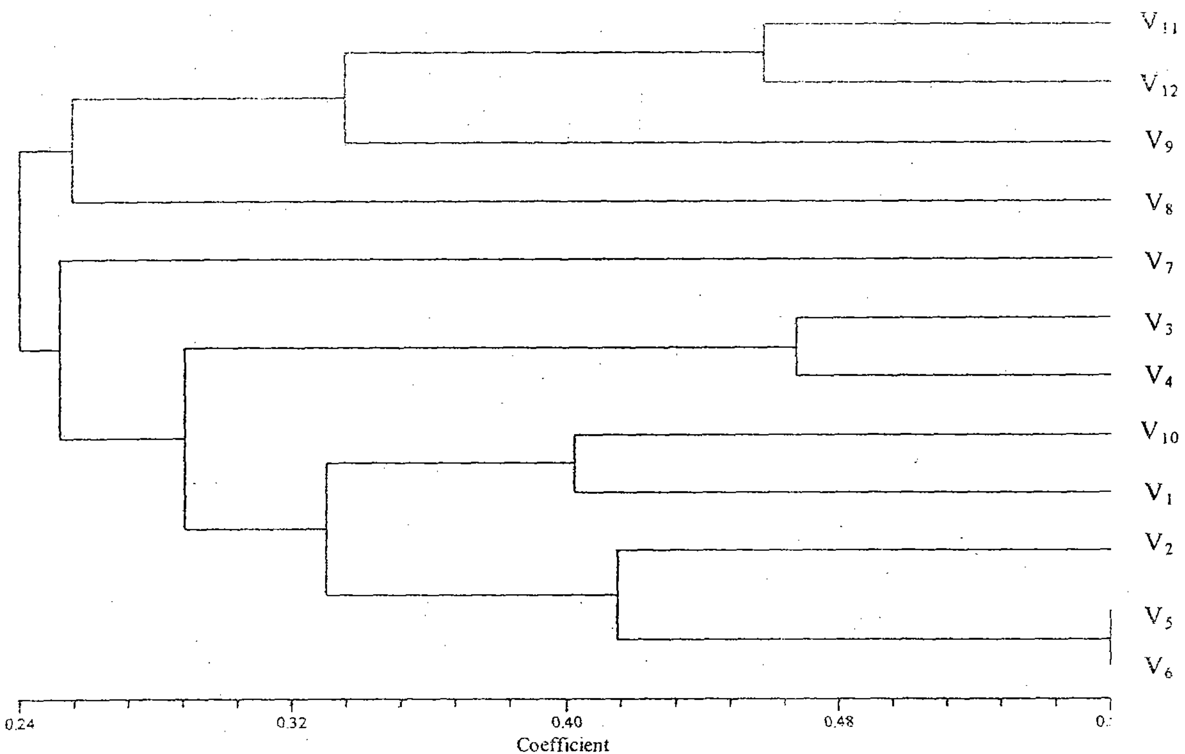


Fig. 23. Dendrogram obtained from RAPD analysis using UPGMA method

minutes). Shoot apex was found to be the most effective. Hence shoot apex was selected for subsequent studies.

4.3.2 Surface sterilization

Highest survival percentage (45.00) was observed in the treatment with absolute alcohol one minute wash + mercuric chloride (0.10 %) for 10 minutes (Table 25). It was followed by the treatment with mercuric chloride 0.1 per cent for 10 minutes (41.00 %). The lowest percentage of survival (10.00) was recorded in treatment with mercuric chloride (0.08 %) for five minutes.

4.3.3 Season of explant collection

Data pertaining to the influence of season of explant collection and establishment are presented in Table 26.

The lowest rate of microbial contamination was observed from January to May, compared to the period from June to December. The shoot apex collection during March resulted in the lowest rate of contamination (50.00 %).

4.3.4 The Routes

4.3.4.1 Enhanced release of axillary buds

4.3.4.1.1 Culture establishment

The culture establishment trials were carried out using shoot tips in MS medium supplemented with auxins (IAA 0.05 mg l^{-1}, NAA 0.5 mg l^{-1}), cytokinins (BA, kinetin and 2-ip each at 5 and 10 mg l^{-1}) and gibberellins (GA_3 2.5 mg l^{-1}). The responses observed were number of days for bud emergence, survival rate and per cent of bud initiation. The data pertaining to this are presented in Tables 27, 28 and 29.

Effect of BA alone and in combination with auxins and gibberellins

Data pertaining to the culture establishment responses of shoot tip explants as influenced by BA (5 and 10 mg $l^{-1)}$ alone and in combination

Table 24. Effect of explants on days taken for bud initiation and survival rate in MS medium

Explants	Days for bud initiation	Survival % after 1 month
Shoot tips	30.00	45.00
Bits of rhizome	60.00	30.00
Leaves	–	0.00
Roots	–	0.00

The data represent mean value of twenty replications.

Table 25. Effect of surface sterilization on contamination in shoot tip explants of *Heliconia psittacorum* cv. Deep Orange

Sterilant	Concentration (%)	Time (Minute)	Contamination (%)		Cell death (%)	Survival rate (%)
			Fungal	Bacterial		
T_1	0.08	5	55.00	35.00	0.00	10.00
		10	45.00	35.00	0.00	20.00
		15	25.00	35.00	10.00	30.00
	0.10	5	50.00	30.00	0.00	20.00
		10	30.00	25.00	6.00	41.00
		15	30.00	20.00	10.00	40.00
	0.15	5	40.00	35.00	10.00	25.00
		10	20.00	20.00	25.00	35.00
		15	15.00	10.00	40.00	35.00
T_2	5.00	25	40.00	25.00	15.00	20.00
		30	35.00	25.00	18.00	22.00
	10.00	25	25.00	25.00	18.00	32.00
		30	20.00	20.00	26.00	34.00
T_3	10.00	25	30.00	30.00	12.00	28.00
		30	25.00	25.00	18.00	32.00
T_4	-	5	45.00	30.00	0.00	20.00
		10	25.00	25.00	15.00	35.00
		15	20.00	20.00	22.50	37.50
T_5	-	5	25.00	25.00	0.00	30.00
		10	30.00	15.00	10.00	45.00
		15	20.00	20.00	20.00	40.00

The data represent mean value of 20 replication.

T_1 : Mercuric chloride
T_2 : Sodium hypochlorite
T_3 : Calcium hypochlorite
T_4 : Ethyl alcohol (70%)+Mercuric chloride (0.1 %)
T_5 : Absolute alcohol 1 minute wash + Mercuric chloride (0.1%)

Table 26. Effect of season on explants collection on rate of microbial contamination

Month	Contamination (%)
January	60.00
February	58.00
March	50.00
April	58.00
May	60.00
June	65.00
July	85.00
August	85.00
September	80.00
October	75.00
November	70.00
December	70.00

with auxins (IAA 0.05 mg l^{-1}, NAA 0.5 mg l^{-1}) and gibberellins (GA_3 2.5 mg l^{-1}) are presented in Table 27.

Number of days for bud initiation

The average number of days taken for bud initiation ranged from 16.00 to 28.00 days. The lowest period (16.00) was taken by the treatment T_{16}. Among the treatments T_{12} recorded highest number (28.00) of days for bud emergence.

Per cent of bud initiation

Highest per cent of bud initiation was 33.33 (T_8, T_{14}, T_{15} and T_{16}). Treatments T_7, T_9, T_{10}, T_{11}, T_{12}, T_{13} and T_{17} recorded 16.66% bud initiation. No bud initiation was observed in treatments T_2, T_3, T_4, T_5 and T_6.

Survival rate

The percentage of survival ranged from 0.00 to 100.00. The treatments T_9 and T_{17} recorded 100.00% survival. The treatments T_8, T_{14}, T_{15} and T_{16} recorded 50.00 % survival.

Effect of kinetin alone and in combination with auxins and gibberellins

Data pertaining to the culture establishment responses of shoot tip explants as influenced by Kinetin (5 and 10 mg l^{-1}) alone and in combination with auxins (IAA 0.05 mg l^{-1}, NAA 0.5 mg l^{-1}) and gibberellins (GA_3 2.5 mg l^{-1}) are presented in Table 28.

Number of days for bud initiation

Number of days for bud initiation ranged from 23.00 to 35.00 days. The lowest period (23.00) was taken by the treatment T_{30}. The treatment T_{21} recorded highest number of days (35.00) for bud emergence.

Per cent of bud initiation

Per cent of bud initiation ranged from 0.00 to 16.66. The treatments T_{21}, T_{22}, T_{23}, T_{24}, T_{25}, T_{30}, T_{31}, T_{32} and T_{33} recorded 16.66 per cent bud initiation.

Table 27. Effect of BA, auxins and gibberellins alone and in combination on culture establishment of *Heliconia psittacorum* cv. Deep Orange

Treatments	Days for bud initiation	Bud initiation (%)	Survival rate (%)
T_1	–	0.00	0.00
T_2	–	0.00	0.00
T_3	–	0.00	0.00
T_4	–	0.00	0.00
T_5	–	0.00	0.00
T_6	–	0.00	0.00
T_7	19.00	16.66	0.00
T_8	17.50	33.33	50.00
T_9	18.00	16.66	100.00
T_{10}	24.00	16.66	0.00
T_{11}	25.00	16.66	0.00
T_{12}	28.00	16.66	0.00
T_{13}	22.00	16.66	0.00
T_{14}	17.00	33.33	50.00
T_{15}	16.50	33.33	50.00
T_{16}	16.00	33.33	50.00
T_{17}	17.00	16.66	100.00

The data represent mean value of six replications. Details of treatments are presented in chapter III.

Table 28. Effect of Kinetin, auxins and gibberellins alone and in combination on culture establishment of *Heliconia psittacorum* cv. Deep Orange

Treatments	Days for bud initiation	Bud initiation (%)	Survival rate (%)
T_{18}	–	0.00	0.00
T_{19}	–	0.00	0.00
T_{20}	–	0.00	0.00
T_{21}	35.00	16.66	0.00
T_{22}	32.00	16.66	0.00
T_{23}	28.00	16.66	0.00
T_{24}	29.00	16.66	0.00
T_{25}	28.00	16.66	0.00
T_{26}	–	0.0	0.00
T_{27}	–	0.00	0.00
T_{28}	–	0.00	0.00
T_{29}	–	0.00	0.00
T_{30}	23.00	16.66	100.00
T_{31}	25.00	16.66	100.00
T_{32}	25.00	16.66	0.00
T_{33}	25.00	16.66	0.00

The data represent mean value of six replications. Details of treatments are presented in chapter III.

Survival rate

All treatments, except T_{30} and T_{31} recorded zero per cent survival. Treatments T_{30} and T_{31} recorded hundred per cent survival.

Effect of 2-ip alone and in combination with auxins and gibberellins

Data pertaining to the culture establishment responses of shoot tip explants as influenced by 2-ip (5 and 10 mg $l^{-1)}$ alone and in combination with auxins (IAA 0.05 mg l^{-1}, NAA 0.05 mg l^{-1}) and gibberellins (GA_3) are presented in Table 29.

Number of days for bud initiation

Number of days taken for bud initiation ranged from 25.00 to 35.00. The treatment T_{49} recorded the lowest number of days for bud emergence (25.00). The treatment T_{44} recorded highest number of days for bud emergence (35.00).

Per cent of bud initiation

Highest per cent of bud initiation was 16.66. The treatments T_{38}, T_{39}, T_{44}, T_{45}, T_{46}, T_{47}, T_{48} and T_{49} recorded 16.66 per cent bud initiation. The other treatments did not yield any bud initiation.

Survival rate

The buds yielded zero per cent survival when 2-ip at 5 mg l^{-1} and 10 mg l^{-1} was used alone and in combinations with auxins and gibberellins (From treatment T_{34} to T_{49}).

Effect of BA and 2,4-D

Trials were conducted with BA at 10 mg $l^{-1}$and 2,4-D at different levels (10, 20 and 40 mg l^{-1}) on culture establishment of shoot tip explants (Table 30). 2,4-D at 40 mg l^{-1} yielded good callus 30.00 days after inoculation. Highest per cent of bud initiation was 33.33. Percentage of survival was 50.00. The treatments using 2,4-D at 10 mg $l^{-1}$and 20 mg l^{-1} yielded no callusing.

Table 29. Effect of 2-ip, auxins and gibberellins alone and in combination on culture establishment of *Heliconia psittacorum* cv. Deep Orange

Treatments	Days for bud initiation	Bud initiation (%)	Survival rate (%)
T_{34}	–	0.00	0.00
T_{35}	–	0.00	0.00
T_{36}	–	0.00	0.00
T_{37}	–	0.00	0.00
T_{38}	30.00	16.66	0.00
T_{39}	28.00	16.66	0.00
T_{40}	–	0.00	0.00
T_{41}	–	0.00	0.00
T_{42}	–	0.00	0.00
T_{43}	–	0.00	0.00
T_{44}	35.00	16.66	0.00
T_{45}	32.00	16.66	0.00
T_{46}	30.00	16.66	0.00
T_{47}	28.00	16.66	0.00
T_{48}	28.00	16.66	0.00
T_{49}	25.00	16.66	0.00

The data represent mean value of six replications. Details of treatments are presented in chapter III.

Table 30. Effect of BA and 2,4-D on culture establishment of *Heliconia psittacorum* cv. Deep Orange

Treatments	Days for bud initiation	Bud initiation (%)	Survival rate (%)
T_{50}	–	0.00	0.00
T_{51}	–	0.00	0.00
T_{52}	30.00	33.33	50.00

The data represent mean value of six replications. Details of treatments are presented in chapter III.

Effect of media

Data on effect of different media on the culture establishment are given in Table 31.

Days taken for bud initiation

Lowest number of days for culture establishment was noticed in full strength MS medium (40.00 days). It was followed by half strength MS medium (45.00 days). Highest days for culture establishment were recorded in White Medium (65.00 days).

Percentage of bud initiation

SH medium and White medium recorded lowest percentage of bud initiation (20.00). Half strength MS medium recorded 30.00 per cent of bud initiation. Highest bud initiation was observed in full strength MS media (40.00).

Survival rate

Highest survival rate was observed in full strength MS media (50.00 %), followed by half strength MS media (30.00 %). The lowest rate of survival was observed in SH media and White media (20.00 %).

4.3.4.1.2 Shoot proliferation and elongation

The elongated buds derived from the shoot tip explants were cultured in MS medium containing auxins (IAA), cytokinins (BA, KIN and 2-ip) and gibberellins. Data pertaining to the results are presented in Table 32, 33 and 34

Effect of BA alone and in combination with IAA and GA_3

Data on organogenesis responses of elongated shoots in MS medium as influenced by BA (1, 2, 3.5 and 5 mg l^{-1}) alone and in combination with IAA (0.2 mg l^{-1}) and GA_3 (2.5 mg l^{-1}) are given in Table 32.

Table 31. Effect of Basal media on on culture establishment of *Heliconia psittacorum* cv. Deep Orange

Media	Days for bud initiation	Bud initiation (%)	Survival rate (%)
MS(Full strength)	40.00	40.00	50.00
MS(half strength)	45.00	30.00	30.00
SH	60.00	20.00	20.00
Whites	65.00	20.00	20.00

The data represent mean value of twenty replications.

Table 32. Effect of BA auxins and gibberellins alone and in combination on shoot proliferation and elongation of *H. psittacorum* cv. Deep Orange

Treatments	Days for shoot proliferation	Shoot proliferation (%)	Survival (%)	Number of Shoots	Number of leaves
SP_1	–	–	–	–	–
SP_2	–	–	–	–	–
SP_3	–	–	–	–	–
SP_4	–	–	–	–	–
SP_5	–	–	–	–	–
SP_6		0.00	0.00	0.00	–
SP_7	52.00	16.66	0.00	4.00	–
SP_8	65.00	16.66	100.00	3.00	–
SP_9	42.00	33.33	50.00	5.00	–
SP_{10}	–	0.00	0.00	0.00	–
SP_{11}	68.00	16.66	0.00	3.00	–
SP_{12}	60.00	16.66	100.00	2.00	–
SP_{13}	65.00	16.66	0.00	2.00	–
SP_{14}	–	–	–	–	–
SP_{15}	–	–	–	–	–
SP_{16}	–	–	–	–	–
SP_{17}	–	–	–	–	–

The data represent mean value of six replications. Details of treatments are presented in chapter III.

Days taken for shoot proliferation and elongation

Days taken for axillary bud production ranged from 42.00 to 68.00. The treatment SP_9 recorded lowest number of days (42.00) for shoot proliferation. The treatment SP_{11} recorded highest number of days (68.00) for shoot proliferation and elongation.

Percentage of culture responded

The percentage of cultures proliferating multiple shoots ranged from 0.00 to 33.33. The treatment SP_9 recorded highest percentage of multiple shoot proliferation (33.33). Treatments from SP_2 to SP_6 and SP_{14} to SP_{17} did not show any shoot proliferation and elongation response.

Survival rate

The percentage of survival ranged from zero to hundred. The treatments SP_8 and SP_{12} recorded highest survival per cent (100.00).

Number of shoots

The number of shoots produced by various treatments ranged from 2.00 to 5.00. The treatment SP_9 recorded highest number of shoot (5.00). It was followed by treatment SP_7 that recorded 4.00 shoots per culture.

Number of leaves

There was no leaf formation throughout the experiment.

Effect of Kinetin alone and in combination with IAA and GA_3

Organogenic responses of elongated shoots were studied using KIN (1, 2, 3.5 and 5 mg l^{-1}) alone and in combination with IAA (0.2 mg l^{-1}) and GA_3 (2.5 mg l^{-1}). Kinetins alone and in combinations do not yield any response (From treatment SP_{18} to SP_{33}). Data pertaining to the result are presented in Table 33.

Table 33. Effect of kinetin, auxins and gibberellins alone and in combination on shoot proliferation and elongation of *H. psittacorum* cv. Deep Orange

Treatments	Days for shoot proliferation	Shoot proliferation (%)	Survival (%)	Number of Shoots	Number of leaves
SP_{18}	–	–	–	–	–
SP_{19}	–	–	–	–	–
SP_{20}	–	–	–	–	–
SP_{21}	–	–	–	–	–
SP_{22}	–	–	–	–	–
SP_{23}	–	–	–	–	–
SP_{24}	–	–	–	–	–
SP_{25}	–	–	–	–	–
SP_{26}	–	–	–	–	–
SP_{27}	–	–	–	–	–
SP_{28}	–	–	–	–	–
SP_{29}	–	–	–	–	–
SP_{30}	–	–	–	–	–
SP_{31}	–	–	–	–	–
SP_{32}	–	–	–	–	–
SP_{33}	–	–	–	–	–

The data represent mean value of six replications. Details of treatments are presented in chapter III.

Effect of 2-ip alone and in combination with IAA and GA_3

Data on the effect of 2-ip (1, 2, 3.5 and 5.0 mg l^{-1}) alone and in combination with IAA (0.2 mg l^{-1}) and GA_3 (2.5 mg l^{-1}) with respect to shoot proliferation and elongation are given in Table 34.

Days taken for shoot proliferation and elongation

Days taken for shoot proliferation and elongation ranged from 65.00 to 72.00. Lowest number of days (65.00) for multiple bud production and elongation was noticed in treatment SP_{41}. The treatment SP_{35} recorded highest number of days (72.00) for shoot proliferation and elongation.

Percentage of culture responded

Percentage of culture responded ranged from 0.00 to 33.33. Treatments from SP_{46} to SP_{49} that used 2-ip at higher concentration (5 mg l^{-1}) alone and in combination with auxins and gibberellins did not yield any shoot proliferation response.

Survival rate

Percentage of survival ranged from 0 to 100. Highest survival per cent was noticed in treatment SP_{41}.

Number of shoots

A very high rate of shoot proliferation (4.00) was observed in treatments SP_{41} and SP_{45}. The treatment SP_{39} recorded 3.00 shoots.

Number of leaves

There was no leaf formation throughout the experiment.

4.3.4.1.3 *In vitro* Rooting

The proliferated buds were cultured in MS medium containing various levels of IAA (0.25, 0.5 and 1.0 mg l^{-1}) and NAA (0.25 and 0.5 mg l^{-1}) alone and in combination. The responses observed were days to

Table 34. Effect of 2-ip, auxins and gibberellins alone and in combination on shoot proliferation and elongation of *H. psittacorum* cv. Deep Orange

Treatments	Days for shoot proliferation	Shoot proliferation (%)	Survival (%)	Number of Shoots	Number of leaves
SP_{34}	-	-	-	-	-
SP_{35}	72.00	16.66	-	2.00	-
SP_{36}	-	-	-	-	-
SP_{37}	70.00	16.66	50.00	2.00	-
SP_{38}	-	-	-	-	-
SP_{39}	67.00	33.33	50.00	3.00	-
SP_{40}	66.00	33.33	50.00	2.00	-
SP_{41}	65.00	33.33	100.00	4.00	-
SP_{42}	-	-	-	-	-
SP_{43}	70.00	33.33	-	2.00	-
SP_{44}	70.00	33.33	50.00	2.00	-
SP_{45}	68.00	33.33	50.00	4.00	-
SP_{46}	–	–	–	–	–
SP_{47}	–	–	–	–	–
SP_{48}	–	–	–	–	–
SP_{49}	–	–	–	–	–

The data represent mean value of six replications. Details of treatments are presented in chapter III.

Table 35. Effect of plant growth regulators on rooting of *Heliconia psittacorum* cv. Deep Orange

Treatments	Days to rooting	Root initiation (%)	Survival (%)	Number of roots
R_1	–	0.00	0.00	0.00
R_2	–	0.00	0.00	0.00
R_3	35.00	16.66	0.00	2.00
R_4	–	0.00	0.00	0.00
R_5	30.00	16.66	0.00	3.00
R_6	–	0.00	0.00	0.00
R_7	–	0.00	0.00	0.00
R_8	38.00	16.66	100	6.00
R_9	35.00	33.33	50	8.00
R_{10}	30.00	16.66	100	10.00
R_{11}	25.00	33.33	50	12.00

The data represent mean value of six replications. Details of treatments are presented in chapter III.

rooting, percentage of root initiation, percentage of survival and number of roots. Data pertaining to this are presented in Table 35. Various stages of *in vitro* propagation of *Heliconia psittacorum* cv. Deep Orange are given in Plate 12.

Days to rooting

Number of days taken for rooting ranged from 25.00 to 38.00. Lowest number of days (25.00) was observed in treatment R_{11} where MS medium was supplemented with IAA (1.0 mg l^{-1}) + NAA (0.5 mg l^{-1}). Highest number of days (38.00) was recorded in treatment R_8.

Percentage of root initiation

Percentage of root initiation ranged from 0.00 to 33.33. The treatments R_9 and R_{11} recorded highest percentage of root initiation (33.33). The treatments R_3, R_5, R_8 and R_{10} recorded 16.66 per cent root initiation. The other treatments did not show any root initiation.

Survival rate

Percentage of survival ranged from 0.00 to 100.00. Highest survival percentage was observed in treatments R_8 and R_{10} (100.00). The treatments R_9 and R_{11} recorded 50.00 per cent survival. The other treatments recorded zero per cent survival.

Number of roots

Number of roots ranged from 2.00 to 12.00. Highest number of roots (12.00) was observed in treatment R_{11} where MS medium was supplemented with IAA (1.0 mg l^{-1}) and NAA (0.5 mg l^{-1}). The treatment R_3 recorded the lowest number of roots (2.00).

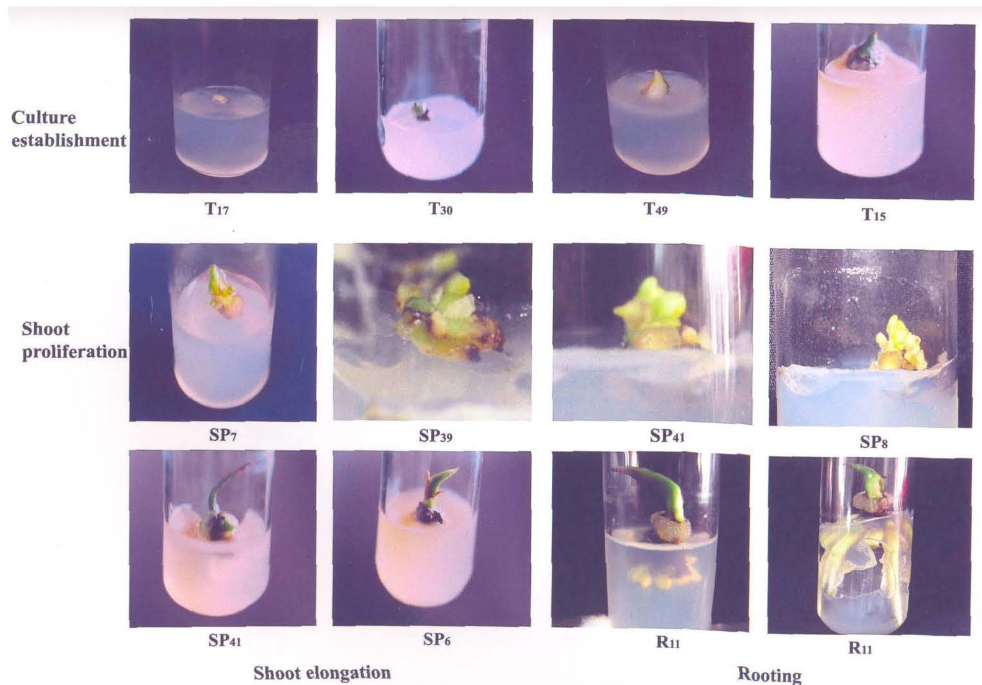


Plate 12. Stages of *in vitro* propagation of *H. psittacorum* var. Deep Orange

Discussion

5. DISCUSSION

Heliconias are excellent commercial cut flowers due to its exotic shape, attractive colour, excellent vase life and prolific flower producing capacity. It became favorite quickly among the florists and plant lovers almost round the world. The present study was conducted as three experiments. In Experiment 1: The evaluation of 12 different species and varieties of *Heliconia* for morphological characters, cytological characters, inflorescence characters and duration of the crop was studied and economics of cultivation calculated. In Experiment 2: Molecular characterisation of 12 species and varieties were studied. In Experiment 3: Protocol for micropropagation of *H. psittacorum cv.* Deep Orange was carried out.

5.1 EXPERIMENT 1 : EVALUATION OF HELICONIA

Evaluation of 12 *Heliconia* species and varieties were carried out for morphological characters such as height of plant, number of leaves, leaf area and number of shoots; inflorescence characters such as length of spike, length of petiole, number of bracts, bract size and pollen morphology; cytological characters such as chromosome number and crop duration characters such as number of days taken for flowering, flowering duration, number of flowering shoots and vase life. Cost of cultivation was also calculated.

The 12 *Heliconia* species and varieties studied comes under five different species *viz; H. psittacorum, H. latispatha, H. humilis, H. wagneriana* and *H. rostrata* and four of them were hybrids. There is significant difference in the morphological, cytological and inflorescence characters between different species and varieties of *Heliconia* belonging to different species and parentage. In general, species and varieties belonging to *H. psittacorum* were smaller in stature and produced inflorescence of lesser dimensions compared to other

species. *H. rostrata* was different from the other species and varieties studied producing pendent flowers.

5.1.1 Morphological and Cytological Characters

5.1.1.1 Height of the plant

Reduced plant height is a desirable character in a cut flower like *Heliconia*. As the plant height increases the weight of inflorescence also increases, thereby increasing the transportation charges and freight charges. For acceptance as garden plant also, reduced plant height is desirable. Increase in plant height will incur more cost of cultivation due to additional operations such as staking. Decrease in height also increases the suitability of the plant for container planting.

Goel (2004) reported that *Heliconia rostrata* grew up to a height of 3.0 m tall and *Heliconia jacquinii* grew up to a height of 1.5 to 2 m tall. According to him, Golden torch is ideal choice for containers. Lalrinawmi and Talukdar (2000a) reported that *Heliconia psittacorum* grew up to a height of 116.80 cm when rhizome of 40 g was used and 91.77cm when rhizome of 10 g was used, at the planting time. Variation in stem length of *H. stricta* and *H. rauliniana* was reported by Ibiapaba *et al.* (1997).

In the present investigation the species *Heliconia latispatha* recorded highest height at three months, five months, seven months and nine months after planting. The cultivar De Rooij recorded the lowest plant height during the whole observation period except five months after planting. The species *Heliconia rostrata* recorded the lowest plant height, five months after planting. These results confirmed that there is variation in the height among different species and varieties of *Heliconia*.

5.1.1.2 Number of leaves

The number of leaves per shoot showed significant difference three months after planting and five months after planting. The cultivar Lady Di recorded the highest number of leaves per shoot at three months after

planting (6.25). The cultivar Guyana recorded the highest leaf number (6.13), 9 months after planting. Lalrinawmi and Talukdar (2000b) reported 4-6 lance shaped leaves in *Heliconia psittacorum*. High variability in number of leaves per m^2 and number of leaves per clump was recorded by Lalrinawmi and Talukdar (2000a). Present investigation also confirms that there is variation in number of leaves among different species and varieties of Heliconia.

5.1.1.3 Leaf area

Leaf area had recorded significant variation during the period under study. In the present study leaf area was correlated both at genotypic and phenotypic levels with plant height, number of bract, bract size, spike length, days to flowering and vase life. This highlights the importance of leaf area in this flowering plant.

Lalrinawmi and Talukdar (2000a) reported the leaf area of *Heliconia psittacorum* varied from 700 to 870 cm^2 per leaf. Variability in leaf area among *Heliconia* cultivars was also reported by Lopez *et al.* (2001).

Leaf area had recorded significant variation during the period under study. The cultivar Pedro Ortiz recorded highest leaf area during the period under study. The varieties Golden Torch, Lady Di and De Rooij recorded comparatively lower leaf area.

5.1.1.4 Number of shoots

All the shoots in *Heliconia* terminate in an inflorescence. The total number of shoots produced in unit time by a plant is therefore a critical factor in determining the yield potential of a cultivar. Variability in number of shoots per plant was recorded by Lalrinawmi and Talukdar (2000b).

The *H. psittacorum* varieties generally showed a tendency for earlier flowering followed by production of successive shoots. The cultivar Deep Orange yielded the highest number of shoots (16), three months after planting. The

cultivar Lady Di recorded highest number of shoots at five months and seven months after planting (19.38 and 22.38), respectively. The cultivar Petra Orange yielded the highest number of shoots at nine months, eleven months and one year after planting (26.70, 34.25 and 56.60, respectively).

However, the stout varieties (varieties having robust nature for each shoot) *H. humilis*, Wageneriana Red, Wagneriana Yellow and Pedro Ortiz produced less number of shoots per plant.

5.1.1.5 Chromosome number

Acetocarmine staining procedure was followed for detection of chromosome number. Lee *et al.* (1994) reported that cultivars namely Tay, Andromeda and Lady Di are diploids with 2n = 24 chromosomes and the cultivars Petra, Sassy and Iris are triploids (2n = 3x = 36).

The slides were observed under 1000x magnification. However, the greater number and relatively smaller size of chromosome precluded the taking of exact counts accurately and hence the size variation of the chromosome could not be accounted accurately.

5.1.2 Duration of the crop

5.1.2.1 Days taken for flowering

The *H. psittacorum* varieties recorded lower number of days for flowering. The lower the number of days taken for flowering, the earlier the flowers are ready for market. Comparing this factor the varieties Golden torch, Lady Di, Deep Orange and Petra Orange were ready for the market at earlier dates compared to the robust varieties like Pedro Ortiz, Wagneriana Red and Wagneriana Yellow which recorded more number of days for flowering. Catley and Brooking (1996) reported that *H. psittacorum* cv. Golden Torch initiated flowering 140-146 days after planting. Ibiapaba *et al.* (2000) reported that *H. psittacorum* cv. Sassy and Andromeda initiated flowering after 120 days.

In this study, it was observed that seasonal variation in flowering was observed in *H. latispatha,* Wagneriana Red and Wagneriana Yellow

which is in agreement with the findings of Criley *et al.* (1999) and Criley, 2000 who reported seasonal variation of flowering in *Heliconia*.

5.1.2.2 Flowering duration

Flowering duration plays an important role in home gardens, avenues and roadside gardens. Flowering duration had shown significant positive correlation with both at genotypic level and phenotypic level with vase life. The more the flower duration, the more will be the vase life.

In the present investigation the highest flowering duration was recorded in Golden torch (40.50 days). *H. latispatha* and Petra Orange recorded the lowest flowering duration.

5.1.2.3 Vase life

Heliconia have long vase life as a result of which its demand for cut flower trade is increasing day by day. It is observed that the thickness and texture of bracts is critical in increasing the vase life of spike. Zimmer and Carow (1977) suggested 4 weeks of vase life when *Heliconia* flowers were kept at temperature between 0 and 12 °C. Bredmose (1987) reported 2 to 4 weeks of vase life in water. Goel (2004) reported variation of vase life from 3 to 4 days in *H. psittacorum* cv. Rubra and 10 to 14 days in large erect varieties and 28 days in *H. humilis*.

Lady Di which recorded the lowest vase life (4.63) had rather thin and fragile bracts compared to the bracts of Wagneriana Yellow and Wagneriana Red, which recorded vase life of 19.75 and 17.5 days, respectively. The present investigation confirms these findings that there is variability in vase life between different species and varieties.

5.1.2.4 Number of flowering shoots

Number of flowering shoots is a critical factor contributing to the yield potential of the cut flowers. In general, it was observed that the *H. psittacorum* varieties, which came to flowering earlier and also, produced higher number of flowering shoots per year. The cultivar Petra Orange stood out distinctly

producing remarkably higher number of flowering shoots (55.13) than other species and varieties. This in combination with other desirable characters exhibited (petiole length and vase life) and also the attractive coloring of the bract makes it an ideal choice for cut flower. The cultivar De Rooij recorded second highest number of flowering shoots (53.50). The flowers though attractive, had lower petiole length and low vase life thereby diminishing its value as a cut flower.

The varieties Wagneriana Red and Wagneriana Yellow showed seasonality in flowering and also produced less number of flowering shoots per year indicating lower potential for production of total number of flowers per year. In these species and varieties, this is a critical factor in the selection of cut flower.

5.1.3 Inflorescence characters

5.1.3.1 Length of spike

The length of spike is an important character of display value. It had shown significant positive correlation both at genotypic and phenotypic levels with plant height, leaf area, bract size, spike length, days to flowering and vase life.

Goel (2004) reported variation in spike length of *Heliconia rostrata* (20-45 cm). In the present investigation the species *Heliconia rostrata* showed 21.29 cm and 22.03 cm of spike length 11 months after planting and one year after planting, respectively. Variation in spike length of *Heliconia psittacorum* was reported by Lalrinawmi and Talukdar (2000a) Ibiapaba *et al.* (1997) reported such variations in *H. stricta* and *H. rauliniana.*

Among the species and varieties Pedro Ortiz recorded the highest spike length eleven months and one year after planting (42.83 cm and 43.53 cm, respectively). The cultivar Deep Orange recorded the lowest spike length eleven months and one year after planting.

5.1.3.2 Length of petiole

Higher petiole length enables easy handling of the inflorescence, making it suitable for flower arrangements and bouquet making. Higher petiole length also increases the vase life enabling the cutting of the basal end of inflorescence on alternate days.

Lalrinawmi and Talukdar (2000 b) reported variation in petiole length of *Heliconia psittacorum* (60-80 cm.). According to them the petioles of *H. psittacorum* are long and slender yet remarkably strong. Similar variations were also reported by Ibiapaba *et al.* (2000).

Highest petiole length was observed in cultivars *H. latispatha* and Petra Orange. The cultivar Petra Orange yielded inflorescence of convenient size for flower arrangement, at the same time the extra long petiole length possessed by this cultivar increases the cut flower value of this cultivar. *H. latispatha* though had a high petiole length, the haphazard arrangement of bracts in varied directions make packing and transportation of this inflorescence difficult, diminishing its cut flower value.

H. humilis, Pedro Ortiz, Wagneriana Red, Wageneriana Yellow produced large showy inflorescence of higher dimension, however the lower length of petiole distinctly diminishes the convenience of arranging these spikes in vases or use in bouquet, thereby lowering the cut flower value drastically.

5.1.3.3 Number of bracts

As the number of bracts increases, the size of inflorescence increases. In a cut flower like *Heliconia* the economic value depends on the attractive nature of inflorescence. The display value of *Heliconia* increases with increase in number of bracts. In the present study number of bracts, had shown significant positive correlation both at genotypic and phenotypic level with plant height, leaf area, bract size, spike length, days to flowering and vase life.

Variation in bract number of *Heliconia rostrata* (6-20 per inflorescence) was reported by Goel (2004). Lalrinawmi and Talukdar

(2000b) reported 4 to 5 Orange-Yellow boat shaped bracts in *Heliconia psittacorum*.

More number of bracts was recorded in Pedro Ortiz, *H. humilis* and *H. rostrata* and these varieties took more number of days for flowering (287.50, 255.25 and 163 days, respectively) and recorded more spike length (42.83, 26.08 and 21.29 cm, respectively).

The lowest number of bracts was recorded in Deep Orange (3). In general *H. psittacorum* varieties recorded lower number of bracts and lower spike length and showed early initiation of flowering.

5.1.3.4 Size of bract

Higher bract size contributes to greater attractiveness in *Heliconia* varieties. *H. psittacorum* yielded inflorescence of smaller dimension and correspondingly smaller bracts. In contrast robust varieties Pedro Ortiz, Wagneriana Red, Golden torch, *Heliconia latispatha*, and Guyana recorded highest bract size.

5.1.3.5 Pollen morphology

Pollen morphology is useful tool for classifying plants (Erdtman, 1952; Nair, 1970). In *Heliconia* thread like structures connecting the pollen grains are described by Rose and Barthlot (1995).

Pollen grains of all the species and varieties studied were more or less round in shape. Largest (360.00μ) pollen grains was observed in the cultivar Wagneriana Yellow and the smallest in *H. latispatha* (238.50 μ).

The most effective and most suitable test for pollen viability is staining with acetocarmine. In the present study acetocarmine staining method was used to find the pollen fertility of the selected species and varieties.

Self and cross compatibility problems has been reported in *Heliconia*, which hinders the seed production of various varieties by selfing / crossing (Le *et al.*, 1997). In the present study pollen fertility range from 13.75 per cent

(Wagneriana Red) to 90.81 per cent (*H. latispatha*). High pollen fertility was observed in *H. latispatha*, Lady Di and Deep Orange, and very low rate of fertility was observed in Wagneriana Red and Wagneriana Yellow. This shows that the sterility of pollen might be the reason for poor seed set in some varieties whereas in some others these might be reasons other than fertility of pollen leading to the incompatibility problems and poor seed set.

5.1.4 Heritability, Coefficient of Variation and Genetic Advance

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

In the present study except for number of leaves (19.68%), all other characters under study recorded high heritability. The highest heritability was exhibited by the number of days taken for flowering (99.64%) followed by number of flowering shoots (99.48%) number of leaves (99.32%), plant height (97.95%) and bract size (97.06%).

High heritability along with high genetic gain was observed for petiole length (heritability 96.65%, GA 150.77%), number of flowering shoots (heritability 99.48, GA 121.73%), leaf area (heritability 93.78%, GA : 118.85%) and bract size (heritability 97.06%, GA 115.42%). These characters are genetically controlled by additive gene action and hence amenable to genetic improvement through selection (Panse, 1957).

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

High phenotypic (75.72%) and genotypic (59.25%), coefficients of variations were found for petiole length, followed by number of flowering shoots (PCV- 59.40%, GCV- 59.24%). This indicate a greater extend of variability for these characters thereby suggesting scope for improvement of these characters through selection. The difference between phenotypic and genotypic coefficient of variation was the lowest for the days to flowering (0.08238%) followed by number of flowering shoots (0.1542%). These small differences point out that the environmental influence on these characters is less.

5.1.5 Correlation studies

The degree and direction of association between different characters could be better understood based on correlations. In a cut flower like *Heliconia*, the critical characters determining the economic value are length of spike, length of petiole, number of bracts, size of bracts, number of flowering shoots and vase life. They are generally complex in nature and influenced by many factors. Character genetically related to each other tends to move in the same direction under selection. Such a correlated response to selection is the basic property of qualitative traits under the control of polygenic system. The genotypic correlation between characters provides a reliable measure of genetic association between them and helps to differentiate the vital association useful in breeding (Falconer, 1981).

In the present study, the plant height had significant positive correlation at phenotypic and genotypic levels with the leaf area, number of shoots, number of bracts, size of bract, spike length, petiole length, days to flowering, vase life and number of flowering shoots.

The estimates of genotypic coefficient of correlation were much higher in magnitude than the corresponding estimates at phenotypic level. It indicates that though there was strong inherent association between the various characters studied, the phenotypic expression of correlation was lessened under the influence of environment.

The number of flowering shoots had significant and positive genotypic correlation with number of leaves, number of shoots and petiole length. This is in accordance with the findings of Bruna *et al.* (2002).

A positive genotypic correlation between pairs of characters indicated that an improvement in one character would improve the other character also, thus enabling the breeder to select characters responsive to selection. The length of petiole is influenced by number of shoots and flowering shoots. The selection for more number of shoots and flowering shoots can thus result in higher petiole length.

Number of bracts per spike is an important cut flower attribute. This character showed high positive and significant correlation both at phenotypic and genotypic levels with plant height, leaf area, bract size, spike length, days to flowering and vase life.

Environment correlations are present only for a few pairs of characters. Plant height had shown significant negative correlation with bract size and number of flowering shoots.

5.1.6 Economics of cultivation

Economics of cultivation of all the selected 12 *Heliconia* species and varieties was calculated. Economics of cultivation enables farmer in making decisions on production planning.

Ngo and Hoult (2000) studied the cost : benefit ratio of *Heliconia* in Australia. He recorded a profitability of 9.30 to 14.70 per cent and recommended *Heliconia* cultivation as a profitable crop for Australia.

In the present study the cultivar Petra Orange recorded highest net returns and cost : benefit ratio (Rs. 6,28,900 and 1.54, respectively). Pedro Ortiz stood second with net return of Rs. 5,12,500 and cost : benefit ratio of 1.40. Lower net returns and cost : benefit ratio was recorded in Wagneriana Red (Rs. 25,000 and 1.02, respectively).

The cultivar Petra Orange recorded highest number of flowering shoots and highest length of petiole during the period under study. It had other desirable characters such as lower number of days for flowering (130.25 days), more vase life (13.25 days) and good length of petiole (40-42 cm). These desirable characters along with highest cost : benefit ratio will boost the demand of Petra Orange.

The cultivar Pedro Ortiz recorded highest plant height, more leaf area, more number of days for flowering, more number of bracts, bract size and highest spike length. But it had lower number of shoots and flowering shoots. But price of inflorescence is high in comparison to *H. psittacorum* varieties (Rs. 15 for inflorescence for Pedro Ortiz and Rs. 1.50 - 5.00 for *H. psittacorum*). In order to get returns from Pedro Ortiz farmers have to wait for long duration (228.25 days). But due to higher market demand and high cost : benefit ratio this cultivar will be also gaining popularity in the market.

H. wagneriana being highly seasonal and not profitable under Kerala climate. It had lower number of flowering shoots and longer duration for flowering. Eventhough the price of inflorescence is highest (Rs. 20.00) it produced lower number of inflorescence. Seasonality in flowering behaviour of *H. wagneriana* was reported by Criley *et al.* (1999) and Criley (2000).

5.2 EXPERIMENT 2: MOLECULAR CHARACTERISATION OF *HELICONIA*

RAPD is one of the reliable methods of identifying varieties at the genotypic level and is fast, easy, comparatively cheap and free from environmental influences.

The present study was undertaken to characterize 12 species and varieties of *Heliconia* using RAPD markers. Isolation of genomic DNA was carried out using four protocols. The modified Murray and Thompson (1980) protocol and the modified procedure of Mondal *et al.* (2000) were used for standardizing the DNA isolation techniques in *Heliconia. H. psittacorum* varieties Lady Di, Deep Orange and Petra Orange were used for standardization. There was no difference in yield and purity of yield and purity of DNA. Hence for the further studies protocol 2, which did not require liquid nitrogen and where the leaves were ground to fine powder using CTAB, was used for DNA isolation.

A protocol was developed to extract DNA from *Heliconia* leaves and to analyse genetic variation using RAPD by Kumar *et al.* (1998).

Usually tender leaves of *Heliconia* were rich in phenolics and this resulted in the yield of poor quality DNA that was brown in colour. Addition of 1 ml of 1.0 per cent PVP in extraction buffer along with the other reagents reduced browning and yielded good quality DNA. Similar observations were made by Nayar (2001) and Simi (2001) in red banana and it was over come by addition of 0.1 per cent PVP in extraction buffer.

Fresh unfurled tender leaves were found to yield good quality DNA. This may be due to the easy disruption of the leaves during grinding.Young tender leaves were reported to be used for the isolation of genomic DNA in chrysanthemum (Scot *et al.*, 1996), roses (Vainstein and Ben-meir, 1994), gladiolus (Pathania and Mishra, 2001), dendrobium (Pillai, 2003) and banana (Nayar, 2001, Simi 2001, Rajamanickam, 2003).

Moreover tender leaves contain actively dividing cells with lesser intensity of extra nuclear materials like proteins, carbohydrates and other metabolities that interfere with isolation of nucleic acids which in turn improve the quality of DNA (Mondal *et al.*, 2000).

The leaves collected for DNA isolation were used either fresh or after storing in an ultra freezer for one week at -85°C. The storage did not

interfere with the yield and purity of DNA. When fresh leaves were used, the yield of DNA was 500 $\mu g\ ml^{-1}$. The leaves after storage in the ultra freezer for one week yielded 540 $\mu g\ ml^{-1}$. The purity ratio of the sample used fresh was 1.80 while that of sample used after storage was 1.86. This observation has an implication that the unfurled leaves once collected can be stored without any damage to DNA for a few days in ultra freezer in cases where there is dearth of samples. Ram and Sreenath (1999) have made a similar observation in coffee where stored samples permitted batch processing. Simi (2001) reported similar observation in plantain.

The yield and quality of the DNA varied with the species and varieties. The DNA yield varied from 360 to 1170 $\mu g\ ml^{-1}$. The absorbance value (260/280) varied between 1.50 and 1.95 indicating the DNA of good quality. The variation in DNA yield and quality may be due to genetic factor or due to the chemical reaction that taking place during the extraction of DNA.

5.2.1 Purification of DNA

The DNA isolated was brown in colour. The inclusion of the antioxidant mercaptoethanol along with PVP during extraction improved the quality of DNA as observed by the reduction of the browning. Mondel *et al.* (2000) and Simi (2001) reported similar observation in tea and plantain, respectively. Weining *et al.* (1995) reported that high phenolic oxidation in coffee tissues to brown coloured quinonic compounds damaged DNA and proteins. They suggested that this could be effectively counteracted by the use of certain ingredients in the extraction medium. Reduction of browning could be due to the binding of PVP to phenolic compounds and its co-precipitation as well as inhibition of the action of polyphenol oxidase.

5.2.2 RAPD analysis

The PCR amplification of the DNA sample from Deep Orange was carried out using forty decamer primers of Kit A and Kit B (operon inc.

CA, USA). Thirty four primers out of the 40 used yielded amplification products. The total number of bands ranged from 1.0 to 6.0. The primers OPA-06, OPA- 09,OPA-10, OPA-12, OPB-06 and OPB-13 did not yield any bands. This indicates that there is no sequence complementary to the sequence of these primers in the DNA of the *Heliconia* cultivar Deep Orange.

A Total of 80 (average of 1.9 bands per primer) were generated by the 34 primers of which 93.75% (75 bands) were polymorphic. This accounts to an average of 1.88 bands per primer. Bhat and Jarret (1995) suggested that the number of polymorphism is more important than the number of primers for the generation of stable phenogram. They also suggested that the number of polymorphism required to generate a stable phenetic analysis would vary with the plant material under investigation and the sequences that are amplified. In the present study 8 primers were identified based on the highest number of bands and highest number of intensive bands. They were OPA-01, OPA-14, OPA-17, OPA-18, OPB-07, OPB-12, OPB-18 and OPB-20. These Primers amplified 53 scorable RAPD marker bands.

RAPD analysis was used to detect genetic variations and similarities among *Heliconia* species, cultivars and hybrids by Goh *et al.* (1995). Significant differences in RAPD profile occurred amongst different *Heliconia* species and some distantly related plants. *H. rostrata* had three prominent bands that were absent in *H. psittacorum* cultivars.

Many *Heliconia* sp. are polymorphic with a large number of cultivars. Cultivar identification has been primarily based on morphological differences of the flower and inflorescence (Kumar *et al.*, 1998).

According to Li and Midmore (1999) the number of RAPD markers needed to differentiate the genotypes of a gene pool may vary with the test material. When the variation between cultivars are high, few primers will be sufficient to detect the diversity. For instance, 11 navy bean genotypes were satisfactorily distinguished by only two primers (Graham *et al.*, 1994). In the

present study also, eight primers *viz.*, OPA-01, OPA-14, OPA-17, OPA-18, OPB-07, OPB-12, OPB-18 and OPB-20 revealed satisfactory polymorphism among the twelve species and varieties of *Heliconia*.

5.2.3 RAPD data analysis

Jaccards similarity coefficient values ranged from 0.12 (Golden torch and Wagneriana Red) to 0.556 (Petra Orange and Deep Orange). The estimation of similarity coefficients and construction of dendrogram revealed the presence and extend of genetic relationship among the twelve *Heliconia* species and varieties examined.

Using the similarity matrix data of RAPD profiles, UPGMA (Unweighted Pair Group Method with Arithmetic Average) based dendrogram was constructed to represent the inter and intra specific relationships among the species and varieties. On drawing a vertical line in the dendrogram along the point corresponding to the similarity coefficient value of 0.32, the 12 species and varieties got divided into five clusters. The varieties Deep Orange, Petra Orange, Golden torch, Lady Di and Guyana formed the largest cluster. At a similarity coefficient value of 0.40, the varieties *viz.*, Deep Orange, Petra Orange and Lady Di formed a single cluster and these varieties belong to *Heliconia psittacorum* species. The varieties Golden torch and Guyana formed another cluster and these varieties are crosses between *H. psittacorum* and *H. spathocircinata*.

On drawing a vertical line in the dendrogram along the point corresponding to the similarity coefficient value of 0.32 *Heliconia rostrata* and Pedro Ortiz formed two separate clusters. It is clearly evident that *Heliconia rostrata* is different from all other species and varieties. *Heliconia rostrata* produce hanging type of inflorescence. The other species and varieties under study produce erect inflorescence (Goel, 2004). Similarly Pedro Ortiz is a cross between the pendent *H. collinsiana* and the erect *H. bourgena* and it produce erect inflorescence having the tendency to twist and hang down like a pendant (David, 1985).

At similarity coefficient value of 0.32, Wagneriana Yellow, Wagneriana Red and *H. humilis* were grouped together. Timothy, (1996) reported that the species *H. wagneriana* is similar to species *H. humilis*. The varieties Wagneriana Red and Wagneriana Yellow belong to *Heliconia Wagneriana* species. The present investigation confirmed this report, since *H. wagneriana* and *H. humilis* formed a single cluster.

In the present study, De Rooij and *Heliconia latispatha* did not cluster as expected. These two were grouped together. De Rooij is a cross between *Heliconia psittacorum* x *Heliconia marginata*. However, further studies are necessary for confirmation.

Not much literature is available pertaining to the molecular characterization of *Heliconia*. So proper comparison of the results of this study with those earlier studies is not possible. The polymorphism obtained in the present study will be useful in fingerprinting and is determining the genetic diversity among the *Heliconia* species and varieties. Knowledge of the degree of genetic relationship between these species and varieties will be important for the development of new accessions and to establish a core collection as part of the germplasm collection and management.

5.3 EXPERIMENT 3: *IN VITRO* PROPAGATION OF *HELICONIA*

Micropropagation has become one of the important methods for propagation of cut flowers for domestic as well as export market. This is an alternative method of propagation which help in bye-passing season barrier, building up of new cultivars of disease free stocks and production of sufficient materials in a short period for field planting (Bharathi, 2000).

5.3.1 Standardisation of explants

Selection of proper explant is an important aspect of commercial tissue culture production as it amounts to the cost of production. Selection

of a suitable explant, which will yield true to type plant and at the same time will not increase the cost considerably, is considered ideal.

Of the various explants tried, shoot tips were found to be ideal. This was in confirmity with the results of the studies by Nathan *et al.* (1992), Shiau *et al.* (1999), Pederson and Brandt (1992), Shiau et al. (1998), Lalrinawmi and Talukdar (2000b), Talukdar *et al.* (2002) and Bora and Paswan (2003).

5.3.2 Standardisation of surface sterilization methods

The explants collected from the field harbour a variety of microorganisms which have to be removed before inoculation into the culture medium. Though general sterilization procedure have been outlined by various workers (Dodds and Roberts, (1982) and George and Sherrington, (1984) specific sterilization procedures have been evolved based on the tissues being handled. Hence, in the present study, the sterilization procedure with respect to the sterilant, its concentration and duration of exposure was standardized.

Among the different treatments tried, mercuric chloride (0.1%) was found to be ideal. Though in literature use of sodium hypochlorite is recommended in surface sterilization of *Heliconia* (Bora and Paswan, 2003), in the present investigation it was found to be less efficient than mercuric chloride at the various levels tried. The best treatment for shoot tip explant was dipping in absolute alcohol for one minute followed by a dip in 0.1% mercuric chloride for 10 minutes. After trimming and before culturing the buds were dipped in 0.05% mercuric chloride for 10 minutes followed by washing with sterile distilled water 2-3 times. Mercuric chloride 0.1% has been effectively used as a surface sterilant in cut flowers by various workers (Kuriakose, 1997) in *Aranthera* and *Dendrobium* and Krishnamurthy *et al.* 2001 in *Polyanthes tuberosa*) . The results obtained in the present study agree with these reports in respect of the effectiveness of mercuric chloride as surface sterilant. It is advantageous cost wise also.

5.3.3 Seasonal Influence on the *in vitro* in Establishment of Explants

The percentage of contamination observed for the explants collected in the different months showed variation. The explants collected from January to March showed lower contamination as compared to those in other months. In Kerala, the relative humidity is usually high, which provides a congenial condition for the growth and development of microorganisms.

5.3.4 The Routes

5.3.4.1 Enhanced Release of axillary buds

5.3.4.1.1 Culture Establishment

Enhanced release of axillary buds is a common route followed for mass multiplication of several ornamental plants. Establishment of the cultures is the first and the most important *in vitro* step which is influenced by several factors, the most important being the type of basal media and growth substances like cytokinins, auxins and gibberellins.

The type of cytokinin and its combination with auxins and gibberellins is often seen to influence culture establishment. In the present study, the effect of BA, KIN and 2-ip alone and in combination with auxins (IAA and NAA) and gibberellins (GA_3), on culture establishment of shoot tip explant was done. Highest percentage of establishment of shoot tip was observed in MS medium supplemented with BA. Precocity in bud emergence was observed in MS medium supplemented with BA 10 mg l^{-1}. This was in conformity with the results obtained by Nathan *et al.* (1992).

BA was superior with respect to early bud initiation, percentage of initiation, and survival rate compared to KIN and 2-ip. BA was found to be the best for culture establishment in the case of gladiolus (Bhojwani and Dantu, 1986) and Dantu and Bhojwani (1995).

BA in combination with auxins and gibberellins results in good culture establishment. The lowest number of days for culture initiation was recorded

when MS medium was supplemented with BA 10 mg l^{-1} along with IAA (0.05 mg l^{-1}) + GA_3 (2.0 mg l^{-1})+NAA (0.5 mg l^{-1}). The favourable effect of BA + NAA in *Heliconia* has been reported by Shiau *et al.* (1999). Effect of BA + IAA for culture establishment was reported by Lalrinawmi and Talukdar (2000b) and Talukdar *et al.* (2002). Bora and Paswan (2003) reported the favourable effect of BA, IAA and GA_3 for culture establishment.

In the present investigation KIN and 2-ip along with gibberellins and auxins also initiated bud emergence. But the number of days for bud emergence was high and survival rate was low.

Plant multiplication is slowest by enhanced release of axillary buds. Murashige *et al.* (1974). In the present investigation also the days taken for multiplication was high.

Rate of contamination was also very high which resulted in lower survival percentage. Browning of the medium occurred from one week after inoculation. It is due to phenolic substances, which is toxic to the tissue and can inhibit their growth. Continuous subculturing even though reduces this problem, the viability of tissues declines (Razdan, 1993).

The effect of 2,4-D on culture establishment was carried out. 2,4-D at 40 mg l^{-1} initiated callusing. 2,4-D at lower concentration did not show any response. Goh *et al.* (1995) reported that 2,4-D at 40 to 80 µM induced highly morphogenic callus. The present study confirms these findings.

In most of the reports on cut flowers, the medium used was Murashige and Skoog (1962) medium. In the present study also, for culture of shoot tip explants, MS medium was found to be better than SH and Whites medium with respect to early release of buds. MS medium has been reported to be the most suitable medium for *Heliconia* by Nathan *et al.* (1992), Goh *et al.* (1995), Pedersen and Brandt (1992), Shiau *et al.* (1998, 1999), Lalrinawmi and Talukdar (2000b) and Talukdar *et al.* (2002). The advantage of MS medium is reported to be due to the desired level of nutrients, which helped in hastening the release of buds.

5.3.4.1.2 Shoot proliferation and elongation

In the present investigation when the elongated buds were inoculated to MS medium containing different levels of cytokinins (BA, KIN, 2-ip) and their combinations with different levels of IAA and GA_3, production of multiple axillary buds was observed. The treatments with BA alone do not yield any shoot proliferation. BA at 2 mg l^{-1} and 3.5 mg l^{-1} along with different combination of IAA and GA_3 yielded shoot proliferation. But at lower and higher level of BA (1.0 mg l^{-1} and 5.0 mg l^{-1}) alone and in the combinations with IAA, GA_3 did not yield any response. At higher level of BA callus production increased with reduction in the rate of multiple axillary bud production. High levels of cytokinins proved to have deleterious effect on shoot growth (Ludergergran and Janick, 1980).

It was found that the rate of axillary bud production, survival rate and bud initiation was high in the medium containing 2-ip in combination with IAA and GA_3. Higher concentration of 2-ip resulted in low rate of callus growth.

In the present investigation KIN alone and in different levels of combinations with IAA and GA_3 yielded no response. Though shoot proliferation was observed in the treatment formation of leaves were not noticed. The buds elongated and remained green for longer duration ranging from three to six months, but leaves were not formed.

Monocotyledons are difficult to propagate through *in vitro* method than dicotyledons (Razdan, 1993). *Heliconia* being a monocot was difficult to propagate through *in vitro* methods during the present study. The percentage of bud proliferation was very low ranging from 0.00 to 33.33. Survival percentage was also very less. Cent per cent survival was noticed only in treatment SP_8, SP_{12} and SP_{41}.

The desirable effect of BA and NAA for shoot proliferation was reported by Shiau *et al.* (1999). For shoot proliferation of *Heliconia* IAA and BA is recommended by Lalrinawmi and Talukdar (2000a) and Talukdar *et al.* (2002).

Bora and Paswan (2003) recommended various combinations of BA, IAA and GA_3 for *Heliconia* shoot tip proliferation.

Media supplements like coconut water, activated charcoal, cobalt chloride, PVP, silver nitrate and copper sulphate were used for the proliferation of shoots. Activated charcoal adsorbs the toxic substances and residual cytokinin from the medium (Fridbory *et al.,* 1978). Hussain (1995) obtained a reduction in shoot length and shoot number in gladiolus when activated charcoal was used. Priyakumari (2001) reported that shoot proliferation was absent when activated charcoal was used. Addition of activated charcoal to the medium resulted in early induction of roots, but number of shoots and length of shoots reduced drastically in Drosera (Blehova *et al*., 1990) and in Iris (Bach, 1988).

Coconut water delayed shoot elongation and root initiation. Coconut water contains some inhibitory substances (George and Sherrington, 1984). Nathan *et al.* (1992) reported shoot multiplication of *Heliconia* on MS medium without coconut water. In *Anthurium andreanum* cv. Dragons tongue, coconut water inhibited shoot proliferation (Thomas, 1996). Hussain (1995) reported that gladiolus cultures in coconut water supplemented media took more days for shoot elongation and produced lesser number of leaves. Present investigation confirms these findings.

Cefotaxime at 500 mg l^{-1} reduced the contamination rate up to 50 per cent. Shiau *et al.* (1999) also obtained lower rates of contamination of *Heliconia* in liquid MS medium supplemented with 500 mg l^{-1} Cefotaxime.

5.3.4.1.3 *In vitro* Rooting

Rooting of the micro shoots is an important stage in micro propagation. Earliness of induction of roots and morphological characters of the roots are of primary concern in *in vitro* rooting. Various studies conducted earlier on this aspect indicate that relative concentration of auxins and cytokinins, source of auxin, salt level, environmental factors, especially light etc. contributed *in vitro* rooting.

In the present study, experiments were carried out for *in vitro* rooting, using various combinations of IAA and NAA. IAA at lower concentrations (0.25 and 0.5 mg l^{-1}) did not initiate roots. Similarly NAA at lower concentration (0.25 mg l^{-1}) did not initiate roots. IAA and NAA at higher levels, alone and in combinations initiated roots.

Morphological characters of the roots differed among the treatments tried. Roots induced in the MS medium supplemented with IAA were slender and white in colour, while those in the medium supplemented with NAA were thick, short and creamish in colour. Besides, NAA in the medium induced callus formulation at the base before root induction. The presence of callus on plantlets rooted on media with NAA was reported by Kunisaki (1975).

Nathan *et al.* (1992) reported rooting of *Heliconia psittacorum* on MS basal medium without any plant growth substances. Beneficial effects of NAA and IAA for rooting of *Heliconia* were reported by Bora and Paswan (2003). Talukdar *et al.* (2002) and Lalrinawmi and Talukdar (2000b) reported IAA as an ideal hormone for rooting in *Heliconia*.

The present investigation revealed that *H. psittacorum* cultivars are most suited to Kerala's agro climatic conditions and economic conditions. They were earlier bloomers, producing more number of flowering shoots of good dimensions. They also had higher fertility and cost benefit ratio.

Grouping of species and varieties based on RAPD markers (OPA-01, OPA-14, OPA-17, OPA-18, OPB-07, OPB-12, OPB-18 and OPB-20) yielded five clusters. *H. psittacorum* varieties were grouped together. Similarly *H. wagneriana* and *H. humilis* were grouped together.

The micropropagation was difficult in H. *psittacorum* cultivar Deep Orange. BA along with auxins and gibberellins yielded better response than kinetin and 2, ip. The time taken for culture establishment, shoot elongation etc were considerably high. There was no leaf formation.

Summary

6. SUMMARY

Investigation on evaluation, molecular characterization and *in vitro* propagation of Heliconias were carried out at the Department of Pomology and Floriculture and Department of Plant Biotechnology at College of Agriculture, Vellayani and at CPCRI substation, Kayamkulam during 2001-2005. The main objectives were to evaluate *Heliconia* species / varieties as cut flowers, to standardize *in vitro* propagation procedures in *H. psittacorum* and to undertake molecular characterization of *Heliconia* species and varieties using RAPD (Random Amplified Polymorphic DNA) markers.

For the study Heliconias belonging to different species and crosses were selected. It includes species such as *H. latispatha*, *H. rostrata*, *H. humilis*; varieties of *H. psittacorum viz.*, Deep Orange, Lady Di and Petra Orange and varieties of *H. wagneriana viz.*, Wagneriana Red and Wagneriana Yellow. The crosses were Pedro Ortiz (*H. collinsiana* x *H. bourgens*), De Rooij (*H. psittacorum* x *H. martinata*) and Goldern Torch and Guyana (*H. psittacorum* x *H. spathocirinata*).

Evaluation of morphological, cytological and inflorescence characters showed significant variations. *Heliconia psittacorum* varieties were of short stature and produced inflorescence of lesser dimensions. They started flowering earlier than other species and varieties and produced more number of flowers.

The robust varieties like Wagneriana Yellow, Wagneriana Red and Pedro Ortiz recorded more plant height. They were late bloomers and produced less number of flowers. Though the dimension of inflorescence was high, the lower petiole length diminished its cut flower value.

Golden Torch and Guyana though had similar parentage differed each other for the number of days for flowering, flowering duration, spike length and petiole length. Golden Torch produced flowers earlier (93.50)

whereas Guyana recorded late flowering (255.25). Similarly Golden Torch remained fresh even after 40.50 days of emergence. Guyana had flowering duration of only 24.50 days.

H. rostrata was of short stature producing pendent inflorescence. *Heliconia latispatha* recorded more plant height, early flowering and high fertility. *H. humilis* and De Rooij showed moderate growth patterns.

The magnitude of variation was calculated. PCV was slightly higher than GCV, in most of the characters studied, indicating the influence of environment also. The small difference point out that the variation observed is mainly due to genetic reasons and environment influence is less. Among the 12 characters studied length of petiole recorded highest PCV (75.72) and GCV (74.45), suggesting scope for improvement of this character through selection, as increased petiole length is a desirable character in *Heliconia*.

Heritability was high in all the characters except for the number of leaves. High heritability along with high genetic advance was noticed in characters such as petiole length (heritability: 96.65, GA: 105.77) and number of flowering shoots (heritability: 99.48, GA: 121.74). Hence these characters can be improved through selection. The lower heritability and genetic advance were recorded for number of leaves (heritability: 19.68 %, GA: 5.94).

A significant positive association had shown between number of flowering shoots with number of shoots and length of petiole both at genotypic and phenotypic level. Thus selection for higher number of shoots will help in obtaining more number of flowering shoots and increased length of petiole.

The variety Petra Orange recorded highest net returns and cost : benefit ratio (Rs. 6,28,900 and 1.54, respectively). Pedro Ortiz stood second with net return of Rs. 512500 and cost : benefit ratio of 1.40. Lower net returns and cost : benefit ratio was recorded in Wagneriana

yellow (Rs. 25,000 and 1.02, respectively). The variety Petra Orange recorded highest number of flowering shoots and highest length of petiole during the period under study. It had other desirable characters such as lower number of days for flowering (130.25), more vase life (13.25) and good length of petiole (40-42 cm). These desirable characters along with highest cost : benefit ratio will boost the demand of Petra Orange.

The present study was also undertaken to characterize 12 cultivars of Heliconia using RAPD markers. The modified Murray and Thompson (1980) protocol and the modified procedure of Mondal *et al.* (2000) were used for standardizing the DNA isolation techniques in *Heliconia*. Trials were carried out in *H. psittacorum* cultivars Lady Di, Deep Orange and Petra Orange. The yield and purity of DNA was similar in all the protocols. Hence for the further studies protocol 2, which did not require liquid nitrogen and where the leaves were ground to fine powder using CTAB, was used for DNA isolation.

Fresh unfurled tender leaves were found to yield good quality DNA. This may be due to the easy disruption of the leaves during grinding. The storage did not interfere with the yield and purity of DNA. When fresh leaves were used, the yield of DNA was 500 μg ml^{-1}. The leaves after storage in the ultra freezer for one week yielded 540 μg ml^{-1}. The purity ratio of the sample used fresh was 1.80 while that of sample used after storage was 1.86.

The yield and quality of the DNA varied with the species and varieties. The DNA yield varied from 360 to 1170 μg ml^{-1}. The absorbance value (260/280) varied between 1.50 and 1.95 indicating the DNA of good quality.

The PCR amplification was carried out using forty decamer primers using DNA of Deep Orange. Thirty four primers out of the 40 used yielded amplification products. The total number of bands ranged from 1.0 to 6.0. The primers OPA-06, OPA- 09,OPA-10, OPA-12, OPB-06 and OPB-13

did not yield any bands. In the present study 8 primers were identified based on the highest number of bands and highest number of intensive bands. They were OPA-01, OPA-14, OPA-17, OPA-18, OPB-07, OPB-12, OPB-18 and OPB-20. These Primers amplified 53 scorable RAPD marker bands.

Jaccards similarity coefficient values ranged from 0.12 (Golden Torch and Wagneriana Red) to 0.556 (Petra Orange and Deep Orange). The estimation of similarity coefficients and construction of dendrogram revealed the presence and extend of genetic relationship among the twelve *Heliconia* species and varieties examined.

On drawing a vertical line in the dendrogram along the point corresponding to the similarity coefficient value of 0.32, the 12 species and varieties got divided into five clusters. The cultivars Deep Orange, Petra Orange, Golden Torch, Lady Di and Guyana formed the largest cluster. Deep Orange, Petra Orange and Lady Di belong to *Heliconia psittacorum* species. The varieties Golden Torch and Guyana are crosses between *H. psittacorum* and *H. spathocircinata*. *Heliconia rostrata* and Pedro Ortiz formed two separate clusters. Wagneriana Yellow, Wagneriana Red and *H. humilis* were grouped together.

For *in vitro* propagation, surface sterilization with absolute alcohol one minute wash + mercuric chloride (0.10 %) for 10 minutes recorded highest survival percentage (45.00) and hence this treatment was standardized for further studies. Among the various explants tried (shoot tip, bits of rhizomes, root and leaf) after the preliminary screening on culture establishment, shoot tips explant was selected for further studies. Among the various media tried (full strength MS, half strength MS, SH and White), MS media recorded bud initiation earlier and was selected for further studies.

The effect of plant growth substances auxins (IAA 0.05 mg l^{-1}, NAA 0.5 mg l^{-1}), cytokinins (BA, kinetin and 2-ip each at 5 and 10 mg l^{-1}) and gibberellins (GA_3 2.5 mg l^{-1}) on culture establishment was also

172549

studied. The treatment T_{16} (MS + BA 10 mg l^{-1}+ IAA0.05 mg l^{-1} + GA_3 2.0 mg l^{-1}+ NAA 0.5 mg l^{-1}) recorded lowest number of days for bud emergence. BA at 10 mg l^{-1} and its combination with auxins and gibberellins was found to be more effective than kinetin and 2-ip alone and its combinations. The treatment with 2,4-D at 40 mg l^{-1} yielded good callusing 30 days after bud inoculation.

The effect of auxins (IAA), cytokinin (BA, kinetin and 2-ip) and gibberellins (GA_3) on shoot proliferation and elongation was also carried out. The treatment SP_9 (MS + BA (2.5) + IAA (0.2) + GA_3 (2.5)) recorded lowest number of days (42) for shoot proliferation. It was followed by SP_7 (MS + BA (2.5) + IAA (0.2)), which recorded 52 days for shoot proliferation and elongation. The treatment SP_9 recorded highest number of shoots (5). It was followed by SP_7, SP_{41} and SP_{43} that recorded four shoots each. The treatments having kinetin and its combination did not produce shoot proliferation and elongation. Though there was shoot proliferation and elongation, no treatments recorded leaf formation.

Effect of various media supplements *viz.*, coconut water (0, 5, 10 and 15 %), activated charcoal (0, 0.5, 1 %), ethylene inhibitors (cobalt chloride 5 mg l^{-1} and 10 mg l^{-1}, PVP 5 mg $l^{-1}$and 10 mg l^{-1}), silver nitrate (5 mg $l^{-1}$and 10 mg l^{-1}), copper sulphate (100 and 200 mg l^{-1}), cefotaxime (250 and 500 mg l^{-1}) and carbon sources (sucrose 30 g l^{-1}, 35 g l^{-1}) were tried for shoot proliferation. The treatments with cefotaxime 500 mg l^{-1} reduced the contamination per cent up to 50.00. Other treatments did not record bud proliferation and elongation.

The proliferated buds were cultured in MS medium containing various levels of IAA (0.25, 0.5 and 1.0 mg l^{-1}) and NAA (0.25 and 0.5 mg l^{-1}) alone and in combination for *in vitro* rooting. Lowest number of days (25.00) was observed in treatment R_{11} where MS medium was supplemented with IAA (1.0 mg l^{-1}) + NAA (0.5 mg l^{-1}). Highest number of days (38.00) was recorded in treatment R_8.

References

7. REFERENCES

*Aboelwafa, A., Murai, K. and Shimada, T. 1995. Intra and interspecific variations in *Lens esculenta* revealed by RAPD markers. *Theor. Appl. Genet.* 90: 335-340

Alan, M.A. 2004. Online plant information : heliconia. http://www. hortcopia. com/heliconia-psittacorum-spathocircinata

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

Aloisi, S.R., Bollereau, P., Morisot, A. and Ricci, P. 1996. Characterization of polymorphic DNA. *Acta Hort.* 424: 253-259

Al-Zahim, M.A., Ford, L.B.V. and Newburg, H.J. 1999. Detection of somaclonal variation in garlic using RAPD and cytological analysis. *Pl. Cell Rep.* 18: 473-477

Anand, M. and Bir, S.S. 1984. Organogenetic differentiation in tissue culture of *Dalbergia lanceolaria. Curr. Sci.* 53: 1305-1307

Anandhi, S. and Sekar, K. 2000. Studies on *in vitro* callus production of gladiolus cv Friendship. *S. Indian Hort.* 46: 299-302

Angel, F., Barney, V.E., Thome, J. and Roca, W.M. 1996. Stability of cassava plants at the DNA level after retrieval from 10 years of *in vitro* storage. *Euphytica* 90: 307-313

Anolles, C., Bassam, G.B. and Gnesshoff, P.J. 1991. DNA amplification on' fingerprinting using very short arbitrary oligonucleotide primers. *Biotech. News Inf.* 9: 235-242

Anolles, C.G. and Trigiano, R.N. 1997. Nucleic acid markers in agricultural biotechnology. *Ag. Biotech. News Inf.* 9: 235-242

Arafeh, R.M.H., Sapir, Y., Shmida, A., Iraki, N., Fragmen, O. and Comes, H.P. 2002. Patterns of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* along an ecogeographical gradient in Israel and the West Bank. *Mol. Ecol.* 11: 39-53

Arora, J.S. and Grewal, H.S. 1990. *In vitro* propagation of gladiolus. *Abst. Int. Sem. New Frontier Hort.*, Bangalore, p. 38

Astley, D. 1992. Preservation of genetic diversity and accession integrity. *Fld. Crops Res.* 219: 205-224

Babu, H.T.P. 2000. RAPD analysis to assess the genetic stability in tissue culture derived black pepper (*Piper nigrum* L.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, p. 81

Babu, P. and Chawla, H.S. 2000. *In vitro* regeneration and *Agrobacterium* mediated transformation in gladiolus. *J. hort. Sci. Biotech.* 75: 400-404

*Bach, A. 1988. Effect of activated charcoal on *in vitro* propagation of *Iris hollandica. Bull. Polish. Acad. Sci. Biol. Sci.* 36: 4-6

Baird, E., Cooper, B.S., Waugh, R., De Maine, M. and Powel, W. 1992. Molecular characterization of inter and intra-specific somatic hybrids of potato using randomly amplified polymorphic DNA (RAPD) markers. *Mol. Gen. Genet.* 233: 469-475

Baishya, S., Sachdev, A., Jojari, R.P. and Mehta, S.L. 2000. RAPD analysis of aromatic and non aromatic rice. *J. Pl. Biochem. Biotech.* 9: 23-26

Bajaj, Y.P.S., Sindhu, M.M.S. and Gill, A.P.S. 1983. Some factors affecting the *in vitro* propagation of gladiolus. *Scient. Hort.* 18: 265-275

Ballard, R., Rajapakse, S., Abbot, A., Bryne, D.H., Morisot, A. and Ricci, R. 1996. DNA markers in rose and their use for cultivar identification and genome mapping. *Acta Hort.* 424: 265-268

Banerjee, N.S., Manoj, P. and Das, M.R. 1999. Male sex associated RAPD markers in *Piper longum* L. *Curr. Sci.* 77: 693-695

Bano, K., Yifei, L., Ishikawa, H., Nakano, S. and Nobatake, S. 2000. Isozymes and RAPD markers to identify the parenthood of Japanese pear. *J. Jap. Soc. hort. Sci.* 69: 208-213

Beneditti, L.D., Burchi, G., Mercuri, A. and Schiva, T. 2000. Use of RAPD analysis for genotype identification in *Alstroemeria*. *Acta Hort.* 508: 277-279

Bharathi, N. 2000. Problems and prospects of micropropagation in horticulture crops. *Scient. Hort.* 21: 101-103

Bhat, K.V. and Jarret, R.L. 1995. Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. *Genet. Resour. Crop Evaluation* 42: 107-118

Bhat, K.V., Bhat, S.R., Chandel, K.P.S., Lakhanpaul, S. and Ali, S. 1995. DNA fingerprinting of *Musa* cultivars with oligonucleotide probes specific for simple repeat motifs. *Mol. Breed.* 41: 37-39

Bhojwani, S.S. and Dantu, P.K. 1986. *In vitro* propagation and corm formation in gladiolus. *Pl. Cell Tiss. Organ Cult.* 6: 435-436

Blehova, A., Somsakova, V. and Bobak, M. 1990. Anatomical studies of the development of new plants from the leaves of the sundew (*Drosera spathulata* L.) in *in vitro* conditions. *Physiol. Pl.* 26: 33-42

Bora, S. and Paswan, L. 2003. *In vitro* propagation of *Heliconia psittacorum* through axillary bud. *J. Ornam. Hort.* 6: 11-15

Bose, T.K., Jana, B.K. and Moulik, S. 1987. A note on the micropropagation of tuberose from scale-stem section. *Indian J. Hort.* 44: 1-2

Bracaccia, G. 1994. Development, comparability and potential applications of RAPD markers in the genus *Medicago*. *Pl. Mol. Biol. Rep.* 9: 105-111

*Bredmose, N.1987. Keeping quality of some new flowers for cutting. Nogle nye snitblomsters holdbarhed. *Gartner Tidende*103:146-147 (Translated from the Russian)

Bretting, P.K. and Widrlechner, M.P. 1995. Genetic markers and horticultural germplasm management. *HortScience* 30: 1349-1356

Broschat, T.K., Donselman, H. M. and Will, A. A.1984. Golden Torch, an orange heliconia for cut-flower use. *Circular agric. Exp. Stn. Univ. Florida.* 308: 4 -5

Brown, P.T.H., Lang, F.D., Kranz, E. and Lorz, H. 1993. Analysis of single protoplasts and regenerated plants by PCR and RAPD technology. *Mol. Gen. Genet.* 237: 311-317

Bruna, E.M., Nardy, O., Strauss, S.Y. and Harison, G. 2002. Experimental assessment of *H. accuminata* growth in a fragmented Amazonian landscape. *J. Ecol.* 90: 639-649

Cabrera, O., Macaya, G., Villatobos, H., Diaz, M. and Leon, O. 1998. Study of the genetic variability in *Musa* species with the random amplified polymorphic DNA technique (RAPD). *Revista Fitotecnia Mexicana* 21: 61-67

Caetano-Anollees, G., Bassam, B.J. and Greshoff, P.M. 1991. DNA amplification fingerprinting : a strategy for genome analysis. *Pl. Mol. Biol. Rep.* 9: 297-307

Cafasso, O., Pellegrino, G., Musacchio, A., Widmer, A. and Cozzolino, S. 2001. Characterization of a minisatellite repeat locus in the chloroplast genome of *Orchis palustris*. *Curr. Genet.* 39: 394-398

Catley, J. L. and Brooking, I. R. 1996. Temperature and light influence growth and flower production in Heliconia 'Golden Torch'. *HortScience* 31: 213-217

Chaque, V., Mercir, J.S., Guinard, M.A. and Vedel, F. 1996. Identification and mapping on chromosome of RAPD markers linked to SW-5 in tomato by bulk segregant analysis. *Theor. Appl. Genet.* 92: 1045-1051

Charleston,W.V.1997. *Heliconia stricta.* General description. http://www. heliconia- amazon. Com /heliconia - stricta

*Choudhary, P.R., Kohli, S., Srinivasan, K., Mohapatra, T. and Sharma, R.P. 2001. Identification and classification of aromatic rices based on DNA fingerprinting. *Euphytica* 118: 243-251

*Christofani, M., Machado, M.A. and Grattapagha, D. 1999. Genetic linkage mapping of citrus. *Euphytica* 709: 25-32

Collins, G.G. and Symons, R.H. 1993. Polymorphisms in grape vine DNA detected by the RAPD - PCR technique. *Pl. Mol. Biol. Rep.* 11: 105-112

Cooke, R.J. 1994. The characterization and identification of crop cultivars by electrophoresis. *Electrophoresis* 5: 59-72

*Coussirat, J.C. 1994. Genetic diversity and varietal identification in the species *Nicotiana tabaccum* by RAPD markers. *Ann. du. Tabae* 26: 1-7

Criley, R. A.1999. Propagation of tropical cut flowers: Strelitzia, Alpinia and Heliconia. *Acta Hort.* 226: 509-517

Criley, R. A.2000. Seasonal flowering patterns for Heliconia shown by grower records. *Acta Hort.*541: 159-165

Criley, R.A. and Kawabata, O.1986. Evidence for a short-day flowering response in *Heliconia stricta* 'Dwarf Jamaican' *Hortscience* 21: 506-507

Criley, R.A., Sakai,W.S., Lekawatana, S. and Kwon, E.1999. Photoperiodism in the genus Heliconia and its effect upon seasonal flowering. *Acta Hort.* 11:323-327

Crouch, H.K., Crouch, J.H., Maidsen, S., Vuylasteke, D.R. and Oritz, R. 2000. Comparative analysis of phenotypic and genotypic diversity among plantain landraces. *Theor. Appl. Genet.* 101: 1056-1065

Crouch, J.H., Crouch, H.K., Constandt, H. and Bryne, K. 1997. Microsatellite markers for molecular breeding of *Musa*. *Infomusa* 6: 5-6

Crouch, J.H., Crouch, H.K., Constandt, H., Gysel, A. van, Breyne, P., Montagu, M. van, Jarret, R.L. and Oritz, R. 1999. Comparison of PCR-based molecular marker analysis of *Musa* breeding populations. *Mol. Breed.* 5: 233-244

Cruzan, M.B. and Arnold, M.L. 1994. Ecological and genetic associations in an Iris hybrid zone. *Evolution* 47: 1432-1445

Damasco, O.P., Adkins, S.W., Godwin, I.D. and Smith, M.K. 1998. Use of a SCAR-based marker for early detection of dwarf off-types in micropropagated Cavendish bananas. *Acta Hort.* 461: 157-167

Damasco, O.P., Graham, G.C., Henry, R.J., Adkins, S.W., Smith, M.K. and Godwin, I.D. 1996. Random amplified polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish banana. *Pl. Cell Rep.* 16: 118-123

Dantu, P.K. and Bhojwani, S.S. 1995. *In vitro* corm formation and field evaluation of corm derived plants of Gladiolus. *Kasetsart J. Nat. Sci.* 31: 51-53

David, B.Z. 1985. Notes on heliconia. http://www.fairchildtropicalgardens.com/heliconia-latispatha

*De Michele, L., Paynter, K.T. and Powers, P.A. 1991. Evidence of lactate dehydrogenase B allozyme effects in teleost. *Fundulus heteroclitus Sci.* 253: 989-990 (Translated from the Russian)

Debener, T. and Mettiesch, L. 1999. Construction of a genetic linkage map for roses using RAPD and AFLP markers. *Theor. Appl. Genet.* 21: 201-203

Debener, T., Bartels, C. and Mattiesch, L. 1996. RAPD analysis of genetic variation between a group of rose cultivars and selected wild rose species. *Mol. Breed.* 2: 321-327

*Debener, T., Bartles, C. and Spethmann, W. 1997. Parentage analysis in interspecific crosses between rose species with RAPD markers. *Gartenbauwissenschaft* 62: 180-184 (Translated from the French)

Debener, T., Janakiram, T. and Mattiesch, L. 2000. Sports and seedlings of rose varieties analysed with molecular markers. *Pl. Breed.* 119: 71-74

De-Bruyn, M.H. and Ferreira, D.I. 1992. *In vitro* corm production of *Gladiolus dalenii* and *Gladiolus tristis*. *Pl. Cell Tiss. Organ Cult.* 31: 123-128

Demek, T., Adams, R.P. and Chibber, R. 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD) : a case study in *Brassica*. *Theor. Appl. Genet.* 84: 990-994

Demek, T., Lynch, D.R., Kawchut, L.M., Kozur, G.C. and Armstrong, J.D. 1996. Genetic diversity of potato determined by random amplified polymorphic DNA analysis. *Pl. Cell Rep.* 15: 662-667

Deverna, J.W. and Alpert, K.B. 1990. RFLP technology. *J. Chinese Soc. Hort. Sci.* 35: 140-142

Dickens, C.W.S., Kelley, K.M. and Manning, J. 1986. *In vitro* propagation of *Gladiolus flanaganii*. *S. Afr.J. Bot.* 52: 485-487

Dodds, J.H. and Roberts, L.W. 1982. *Experiments in Plant Tissue Culture.* Cambridge University Press, London, 28 p.

Dunemann, F., Kahuan, R. and Schmidt, H. 1994. Genetic relationships in *Malus* evaluated by RAPD finger printing of cultivars and wild species. *Pl. Breed.* 113: 150-159

Duran, Y., Rohde, W., Kullaya, A. and Ritter, E. 1997. Molecular analysis of East African tall coconut genotypes by DNA marker technology. *Jpn. J. Theor. Appl. Genet.* 93: 1185-1192

Egashira, H., Ishihara, H., Takashina, T. and Imanshi, S. 2000. Genetic diversity of the peruvianum-complex (*Lycopersicon peruvianum* L.) and *L. chilense* D. revealed by RAPD analysis. *Euphytica* 116: 23-31

Endre,G. 1996. Heliconias and gingers. http://www. tciendre @ coqui. net / heliconia - types

Erdtman, G. 1952. *An Introduction to Pollen Analysis*. The Chronica Botanica Co., Waltham, 239 p.

Erlich, H.A., Gelfand, D. and Srinsky. 1991. Recent advances in the polymerase chain reaction. *Curr. Appl. Sci.* 252: 1643-1651

Falconer, D.B. 1981. *Introduction to Quantitative Genetics*. Longman, New York, 340 p.

Faure, S., Noyer, J.L., Carreel, F., Horry, J.P. and Lanaud, C. 1993. Maternal inheritance of chloroplast genome and paternal inheritance of mitochontrial genome in bananas. *Curr. Genet.* 25: 265-269

Filippis, L.D., Hoffman, E. and Hampp, R. 1996. Identification of somatic hybrids of tobacco generated by electrofusion and culture of protoplasts using RAPD-PCR. *Pl. Sci.* 121: 39-46

Ford, R. and Taylor, P.W.J. 1997. The application of RAPD markers for potato cultivar identification. *Aust. J. agric. Res.* 48: 1213-1217

Fridbory, G., Pedersen, M., Landstorm, L.E. and Eriksson, T. 1978 The effect of metabolites inhibiting morphogenesis. *Physiol. Pl.* 43: 104-106

Fukuoka, S., Hosaka, K. and Kamijima, O. 1992. Use of random amplified polymorphic DNAs (RAPD) for identification of rice accessories. *Jpn. J. Genet.* 67: 243-252

Galderisi, U., Cipollaro, M., De Bernado, G., De Masi, L., Galano, G. and Cascino, A. 1999. Identification of hazelnut (*Corylus avellana*) cultivars by RAPD analysis. *Pl. Cell Rep.* 18: 652-655

Gawel, N.J. and Jarret, R.L. 1991. Cytoplasmic genetic diversity in banana and plantain. *Euphytica* 52: 19-23

Gawel, N.J., Jarret, R.L. and Whittemoe, A.P. 1992. Restriction Fragment Length Polymorphism (RFLP) based polygenetic analysis of *Musa. Theor. Appl. Genet.* 81: 783-786

Geertsen, V. 1988. Development of new cut-flower crops. Udvikling af nye snitblomster.*Gron Viden, Havebrug* 12: 4-5 (Translated from the French).

Geertsen, V. 1989. Effect of photoperiod and temperature on the growth and flower production of *Heliconia psittacorum* 'Tay'. *Acta Hort.* 252: 117-122

Geertsen, V.1990.Influence of photoperiod and temperature on the growth and flowering of *Heliconia aurantiaca* . *HortScience* 25: 646-648

Gehrig, H.H., Rosid, H. and Kluge, J.M. 1997. Detection of DNA Polymorphism in the genus *Kalanchoe* by RAPD-PCR finger print and its relationships to intergeneric taxonomic position and ecophysiological photosynthetic behaviour of the species. *Pl. Sci.* 125: 41-51

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

Gidoni, D., Rom, M., Kanik, T., Zur, M., Izhar, S. and Firon, N. 1994. Strawberry cultivar identification using randomly amplified polymorphic DNA (RAPD) markers. *Pl. Breed.* 113: 339-342

Godwin, I.D., Sangduen, N., Kunanvatchaidach, R., Piperids, G. and Adkins, S.W. 1997. RAPD Polymorphisms among variant and phenotypically normal rice somaconal progenies. *Pl. Cell Rep.* 16: 320-324

Goel, A.K. 2004.Heliconias:nature wonders from neotropical region. *J. Indian Hort.* 11: 20-21

Goh, C.J., Kumar, P.P. and Yau, C.K. 1995. Genetic variations detected with RAPD markers in Heliconia. *Acta Hort.* 420: 72-74

Gosal, S.S. and Grewal, H.S. 1991. Tissue culture propagation: Problems and potentials. *Horticulture – New Technologies and Applications* (eds. Praka, J. and Perik, R.L.M.). Kluwer Academic Publishers, Netherlands, pp. 197-200

Graham, G. C., Henry, R. J. and Redden, R. J. 1994. Identification of navy bean varieties using RAPD. *Aust. J. Exp. Agric.* 34:1173-1176

Graham, J. and Mc Nicol, R.J. 1995. An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* species. *Theor. Appl. Genet.* 90: 1128-1132

Gupta, P.K., Balyan, H.S., Sharma, P.C. and Ramesh, B. 1996. Microsatellites in plants – A new class of molecular markers. *Curr. Sci.* 70: 45-53

Gupta, P.K., Rajeev, K. and Varshney, M. 1999. Molecular markers for genetic fidelity during micropropagation and germplasm conservation. *Curr. Sci.* 76: 1308-1310

Haesun, C., Kyungsu, K., Kyung, G. and Youn-Su, L. 1999. Classification of lilium using random amplified polymorphic DNA (RAPD). *Korean J. Hort. Sci. Technol.* 17: 144-147

Halward, T., Stalker, T., La Rue, E. and Kochert, G. 1992. Use of single primer DNA amplifications in genetic studies of pea nut (*Arachis hypogea* L.). *Pl. Mol. Biol.* 18: 315-325

Hang, A., Hoffman, D.L. and Burton, C.S. 1996. DNA variation and genetic relationships among hullers, barley accessions as detected by random amplified polymorphic DNA (RAPD). *Euphytica* 87:41-52

Harvey, M. and Botha, F.C. 1996. Use of PCR based method logics for the determination of DNA diversity between *Saccharum* varieties. *Euphytica* 89: 257-265

Hashmi, G., Huettel, R., Meyer, R., Krusberg, L. and Hammerschlag, F. 1997. RAPD analysis of somaclonal variants derived from embryo callus cultures of peach. *Pl. Cell Rep.* 16: 624-627

He, S., Ohm, H. and Mackenzi, C.S. 1992. Detection of DNA sequence polymorphism among wheat varieties. *Thoer. Appl. Genet.* 84: 573-578

Heinze, B. and Schemidt, J. 1995. Monitoring genetic fidelity vs somaclonal variation in Norway spruce (*Picea alies*) somatic embryogenesis by RAPD analysis. *Euphytica* 85: 341-345

Hillis, B. and Moritz, C. 1990. Molecular Systematics. *J. trop. Subtrop. Bot.* 7: 308-312

Hong, Y.Y., Schugler, S.K., Jutta, K. and Hanna, S. 1997. An RAPD marker tightly linked to the scab resistance gene. *J. Am. Soc. hort. Sci.* 122: 47-52

Howell, E.C., Newbury, H.J., Swennen, R.L., Withers, L.A. and Ford, L.B.V. 1994. The use of RAPD for identifying and classifying *Musa* germplasm. *Genome* 37: 328-332

Hu, J. and Quiros, C.F. 1992. Identification of broccoli and cauliflower cultivars with RAPD markers. *Pl. Cell Rep.* 10: 505-511

Huang, S.Z., Xie, M.Y., Tong, H.Y., Han, Y.L., Gu, Y. and Ju, L. 1999. The tissue culture of *Iris xiphium* L. var. hybridium. *J. Pl. Resour. Environ.* 8: 48-52

Hussain, S.C.T. 1995. Response of Gladiolus to rapid cloning through *in vitro* techniques. M.Sc. thesis, Kerala Agricultural University, Thrissur, p. 37-196

Hussain, S.C.T., Geetha, C.K., Rajeevan, P.K. and Valsalakumari, P.K. 1995. Plant regeneration from root derived callus in gladiolus. *J. Ornam. Hort.* 2: 46-50

Hussey, G. 1976a. *In vitro* release of axillary shoots from apical dominance in monocotyledonous plantlets. *Ann. Bot.* 40: 1323-1325

Hussey, G. 1976b. Propagation of Dutch Iris by tissue culture. *Scient. Hort.* 3: 21-28

Hussey, G. 1977. *In vitro* propagation of gladiolus by precocious axillary shoot formation. *Scient. Hort.* 6: 287-296

Huy Bao, P., Granata, S., Castiglione, S., Gejiao, C., Goprdamo. C., Cuzzoni, E. and Phan, H.B. 1996. Evidence for genomic changes in transgenic rice recovered from protoplasts. *Transgenic Res.* 5: 97-103

IARI, 1999. *Research Report 1998-99*. Indian Agricultural Research Institute. Indian Council of Agricultural Research, New Delhi, pp. 124-132

Ibiapaba, M. V. B., Da-Luz, J. M.Q. and Innecco, R. 2000. Evaluation of spacing in *Heliconia psittacorum* L. cv. Sassy and Andromeda. *Ciencia Agrotecnologia* 24: 181-186

Ibiapaba, M.V.B., Da-Luz, J.M.Q. and Innecco, R. 1997. Performance of two heliconia species at different spacings in Fortaleza. *Revista Brasileira de Horticultura Ornam.* 3: 74-79

Iqbal, M.J., Asad, S. and Zafer, Y. 1995. DNA polymorphisms in banana and sugarcane varieties revealed by RAPD analysis. *Proc. Induced Mutations Mol. Tech. Crop. Improv.* Vienna, Austria, pp. 309-317

Iqbal, M.J., Aziz, N., Saeed, N.A., Safer, Y. and Malik, K.A. 1997. Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. *Theor. Appl. Genet.* 94: 139-144

*Jaccard, P. 1908. Nouvelles rescherches suv la distribution florale. *Bull. Soc. Vandoise des Sciences Neturelles* 44: 223-270

Jack, E.S., Danin, P.Y., Fazio, G., Horejsi, T., Reis, N. and Katzir, N. 2000. Comparative analysis of cultivated melon groups (*Cucumis melo* L.) by using RAPD and SSR. *Euphytica* 115: 225-241

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

Jau-Yueh, W., Keng-Chang, C. and Ming-Jen, F. 1999. Randomly amplified polymorphic DNA (RAPD) markers for identification and genetic diversity analysis of Anthurium cultivars. *J. agric. Res.* 48: 52-63

Jeffreys, A.J., Wilson, V. and Thein, S.L. 1985. Hypervariable minisatellite regions in human DNA. *Nature* 316: 67-73

Jie He., Lay Pheng, T. and Chong-JinGoh. 2000. Alleviation of photoinhibition in Heliconia grown under tropical natural conditions after release from nutrient stress. *J. Pl. Nutr.* 23: 181-196

Jie-He, S., Chee, C. W.and Goh, C. J.1996. 'Photoinhibition' of Heliconia under natural tropical conditions: the importance of leaf orientation for light interception and leaf temperature. *Pl. Cell Environ.*19: 1238-1248

Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimates of genetic and environmental variability in soybean. *Agron. J.* 47: 314-318

Johnston, J.A., Wesselingh, R.A., Bouck, A.C., Donovan, L.A. and Butlin, R. 2001. Phylogeography Hybridization and Speciation. *Mol. Ecol.* 10: 673-681

Jong-Hwa, K., Yeol, S.C., Hei, Y. and Young, J.K. 1999. Comparative studies on the *Hibiscus syriacus* and its allied species based on RAPD analysis. *J. Korean Soc. Hort. Sci.* 40: 241-244

Jongsuk, L., Prongok, L. and Roh, M.S. 1994. Classification of lilies using random amplified polymorphic DNA (RAPD) analysis. *Acta Hort.* 414: 137-144

Joshi, S.P., Prabhakar, K.R. and Vidhya, S.C. 1999. Molecular markers in plant genome analysis. *Curr. Sci.* 97: 230-240

Juan, A.R. 1997. Notes on heliconias. http://www.gardenweb.com/heliconia-psittacorum

Karihaloo, J.L., Brauner, S. and Gottileb, D.L. 1995. Random amplified polymorphic DNA variation in the egg plant (*Solanum melongena* S.). *Theor. Appl. Genet.* 90: 767-770

Kaundun, S.S., Zyvoloup, A. and Park, Y.G. 2000. Evaluation of the genetic diversity among elite tea (*Camellia sinensis*) accessions using RAPD markers. *Euphytica* 115: 7-16

Keil, M. and Griffin, A.R. 1994. Use of RAPD markers in the discrimination and verification of genotypes in eucalyptus. *Theor. Appl. Genet.* 89: 442-450

Khanuja, S.P.S., Shasany, A.K., Dhawan, S. and Sushilkumar, S. 1998. A rapid procedure for isolating somaclones of attended genotypes in *Mentha arvensis*. *J. Med. Arom. Pl. Sci.* 20: 359-361

Kim, Y. and Byrne, D.H. 1994. Biosysstemical classification of genus *Rosa* using isozyme polymorphisms. *HortScience* 29: 483

Kim, Y.J. and Byrne, D.H. 1996. Intraspecific hybrid verification of rosa with isozymes. *HortScience* 29: 483

Kochieva, E.Z., Suprunova, T.V., Semenova, S.K. and Kinach, T. 1999. Using RAPD analysis for cultivar identification in aubergine. *Genetika* 35: 1165-1168

Koes, R.E., Spelt, C.E., Mol, J.N.M. and Geratz, G.M. 1987. The chalcone synthase multigene family of *Petunia hybrida. J. Herb.* 81: 341-346

Kokaeva, Z.V., Bobrova, V.K., Walekho, R.K.M., Gostimskii, S.A. and Troitskii, A.V. 1997. RAPD analysis of the somacloncal and intercultivar variability in peas. *Doklady Biol. Sci.* 355: 369-371

Koller, B., Lehmann, A., Mc Dermot, J.M. and Gessler, C. 1993. Identification of apple cultivars using RAPD markers. *Theor. Appl. Genet.* 85: 901-904

Konoshima, H. 1980. Diffusible growth regulators from Gladiolus. *J. Jap. Soc. hort. Sci.* 49 : 397-402

Kozyrenko, M.M., Artiukova, E.V., Lauve, L.S. and Boltenkov, E.V. 2002. The analysis of genetic variability of callus cultures of several species of the genus Iris. *Biotechnologiya* 18: 38-48

Kress, W. J.1983. Crossability barriers in neotropical Heliconia. *Ann. Bot.* 52: 131-147

Krishnamurthy, K.B., Mythili, J.B. and Meenakshi, S. 2001. Micropropagation studies in single and double types of tuberose (*Polianthes tuberosa* L.). *J. Appl. Hort.* 3: 82-84

*Kumar, M.B., Barker, R.E. and Reed, B.M. 1995. Genetic stability of micropropagated strawberries. *In vitro* 31: 52-53

Kumar, P.P., Nathan, M. and Goh, C. 1996. Involvement of ethylene on growth and plant regeneration in callus cultures of Heliconia (*Heliconia psittacorum* L.). *Pl. Growth Regulation* 19: 145-156

Kumar, P.P., Yau, J.C.K. and Goh, C.J. 1998. Genetic analysis of *Heliconia* species and cultivars with randomly amplified polymorphic DNA (RAPD) makers. *J. Am. Soc. Hort. Sci.* 123 : 91-97

Kunisaki, J.T. 1975. *In vitro* propagation of cordyline (*Cordyline terminalis* L.). *Hort Science* 78: 365-371

Kuriakose, S. 1997. Standardization of *in vitro* techniques for mass multiplication of *Aranthera* and *Dendrobium*. Ph.D. thesis, Kerala Agricultural University, Thrissur, 207 p.

Lalrinawmi, C. and Talukdar, M. C. 2000 a. Effect of spacing and size of rhizome on the flower production of Heliconia (*Heliconia psittacorum* L.). *J. agric. Sci. Soc. N. E. India* 13: 48-51

Lalrinawmi, C. and Talukdar, M.C. 2000b. Heliconia: an unusual cut flower for tropics. *Indian Hort.* 75: 22-23

Lalrinawmi,C. and Talukdar, M. C. 1999. Effect of spacing and size of rhizome on the rhizome production of heliconia (*Heliconia psittacorum* L.). *Hort. J.* 12: 45-49

Lanham, P.G. and Brennen, R.M. 1999. Genetic characterization of gooseberry (*Ribus grossularia*) germplasm using RAPD, ISSR and AFLP markers. *J. hort. Sci. Biotech.* 74: 361-366

Lashermes, P., Tronslot, P., Anthony, F., Comber, M.C. and Charrier, A. 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffee arabica*. *Euphytica* 87: 59-64

Lazaro, A. and Aguinagalde, I. 1998. Genetic diversity in *Brassica Oleracea* L. and wild relatives using RAPD markers. *Ann. Bot.* 82: 829-833

Lee, Y.H., Nig, N.Y. and Goh, C.J. 1994. Pollen formation and fruit set in some cultivars of *Heliconia psittacorum*. *Scient. Hort.* 60: 167-172

Lekawatana, S.and Criley, R. A.1989. Pot culture of *Heliconia stricta* 'Dwarf Jamaican'. *Acta Hort.* 252: 123-128

Lesur, C., Boury, S., Wolff, K., Becher, A., Weising, K., Kahl, G. and Peltier, D. 2000. Comparison of seven molecular techniques for *Pelargonium* cultivar identification. *Acta Hort.* 508: 297-299

Levi, A. and Roveland, L.J. 1997. Identifying blueberry cultivars and evaluating their genetic relationships using RAPD and SSR anchored primers. *J. Am. hort. Sci.* 122: 74-78

Li, M. and Midmore, D. J. 1999. Estimating the genetic relationship of Chinese water Chestnut (*Eleocharis dulcis* H.) cultivated in Australia, using RAPDs. *J. hort. Sci. Biotech.* 74: 224-231

Lilien-Kipnis and Kochba, M. 1987. Mass propagation of new gladiolus hybrids. *Acta Hort.* 212: 631-638

Lim, S.H., Teng, P.C.R., Lee, Y.H. and Goh, C.J. 1999. RAPD analysis of some species in the genus *Vanda*. *Ann. Bot.* 83: 193-196

*Linde, P.C.G. and Hol, G.M. 1988. Tissue culture of Iris propagation too much improved. *Bloembodlencultuur* 99: 36-37

Linde, P.C.G., Hol, G.M., Blom, B.G.J., Aartrijk, J. and Clerk, G.J. 1988. *In vitro* propagation of *Iris hollandica* Tub cv. Prof. Blaauw. 1. Regeneration of bulb scale explants. *Acta Hort.* 1: 121-128

Litt, M. and Lutty, J.A. 1989. A hyper variable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.* 44: 397-401

Logan, A.E. and Zettler, F.W. 1985. Rapid *in vitro* propagation of virus indexed gladioli. *Acta Hort.* 164: 169-180

Loh, C.S., Goh, C.J. and Rao, A.N. 1978. Some factors affecting morphogenesis of *Aranda* orchid tissue culture. *Proc. Symp. Orchidology, April 16-19,1978 (eds.Gein,K.L.and Vone,A.S.). Orchid Soc.* South East Asia, Singapore, pp. 43-45

Lopez, G.A., Sanabria, E.M. and Davilla, P.M. 2001. Comparative morphology of vegetative structures of cultivars 'Distance' and 'Red yellow' of *Heliconia latispatha* Bentham. *Proc. Interam. Soc. trop. Hort.* 45: 82-86

Lu, Z., Reighard, G.I., Baird, W.V., Abbott, A.G. and Rajapakse, S. 1996. Identification of peach root stock cultivars by RAPD markers. *HortScience* 3: 127-129

Lucretti, S., Novak, F.J., Laneri, U. and Dolezel, J. 1984. *In vitro* propagation of tall bearded Iris species by somatic embryogenesis. *Vakblad-voor-de-Bloemisterij* 34: 40-41 (Translated from the Dutch).

Lundergan, C.A. and Janick, J. 1980. Regulation of apple shoot proliferation and growth *in vitro*. *Hort. Res.* 20 : 19-24

Maciel, N. and Rojas, E. 1994. Growth and development of *Heliconia bihai* and *H. latispatha* under different levels of shade. *Proc. Interamerican Soc. trop. Hort.* 38 : 257-263

Makarevitch, I., Golovnina, K., Scherbik, S. and Blinov, A. 2000. Phylogenetic relationships of the Siberian *Iris* species inferred from non coding chloroplast DNA sequences. *Int. J. Pl. Sci.* 164: 229-237

Mandal, P.K., Santha, M. and Mehta, S.L. 1996. RAPD analysis of *Lathyrus sativus* somaclones. *J. Pl. Biochem. Biotech.* 5: 83-86

Marsolais, P.C., Pringle, J.S. and White, B.N. 1993. Assessment of randomly amplified polymorphic DNA (RAPD) as genetic markers

for determining the origin of interspecific *Lilac* hybrids. *Taxonomy* 42: 531-537

Martin, G.B., Williams, J.G.K. and Tang, V.S. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance gene to tomato by using random primers and near isogenic lines. *J.S.W. agric. Univ.* 88: 25-28

Mc Creight, J.D. 2000. Molecular and phenotypic variation in melon PI 313970. *Acta Hort.* 570: 235-239

Mc Lean, M., Geratz, A.G.M., Baird, W.V. and Meagher, R.B. 1990. Six actin gene subfamilies map to five chromosomes of *Petunia hybrida*. *J. Hered.* 81: 341-346

Mechado, M.A., Coletta, F., Targon, M.L. and Pompen, J. 1996. Genetic relationship of Mediterranean mandarins (*Citrus deliciosa* T.) using RAPD markers. *Euphytica* 92: 321-328

Millan, T., Osuna, F., Cobos, S. and Torres, A.M. 1996. Using RAPD's to study phytogenetic relationship in rosa. *Theor. Appl. Genet.* 92: 273-277

Miller, P. A., William, V. C., Robinson, H. P. and Comstock, R. E. 1958. Estimates of genotypic and environmental variance and covariance in upland cotton and the implication in selection. *Agron. J.* 126-131

Misra, S. and Singh, R. 1999. *In vitro* propagation of gladiolus cv. American Beauty. *J. Ornam. Hort.* 2: 67-70

Mondal, T.K., Singh, H.P. and Ahuja, P.S. 2000. Isolation of genomic DNA from tea and other phenol rich plants. *J. Plantn. Crops* 28: 30-34

Moreno, M.T., Torres, A.M., Millan, T., Armada, J., Cubero, J. I., Morisot, A. and Ricci, P. 1996. Use of molecular markers in taxonomic studies of *Rosa* spp. *Acta Hort.* 424: 293-295

Moretzsolm, M.C., Nunes, C.D.M., Ferreira, M.E. and Grattapaglia, D.M. 2000. RAPD linkage mapping of shell thickness locus in oil palm. *Theor. Appl. Genet.* 100: 63-70

Mori, M., Hosaka, K., Umemura, Y. and Kaneda, C. 1993. Rapid identification of Japanese potato cultivars by RAPDs. *Jpn. J. Genet.* 68: 167-174

Moriera, L.A., Mollemma, C. and Heuden, S.V. 1999. Search for molecular markers linked to *Liriomyza trifoli* resistance in tomato. *Euphytica* 109: 149-156

Mukundan, C. 2000. Characterization of important cultivars of *Jasminum* species by using molecular markers. M.Sc. (Hort.) thesis, University of Agricultural Sciences, Bangalore, 146 p.

Mulachy, O.L., Cresti, M., Snasavini, S., Douglas, G.C., Linskens, H.F., Mulachy, B.G., Vignani, R. and Pancaldi, M. 1993. The use of random amplified polymorphic DNAs to fingerprint apple genotypes. *Scient. Hort.* 54: 89-96

Munthali, M.T., Newburry, J.J. and Ford, L.B.V. 1996. The detection of somaclonal variants of beet using RAPD. *Pl. Cell Rep.* 15: 474-478

Murashige, I. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Pl.* 15: 473-497

Murashige, T. 1974. Plant propagation through tissue culture. *A. Rev. Pl. Physiol.* 25: 135-166

Murashige, T., Serpa, M. and Jones, J. 1974. Clonal multiplication of Gerbera through tissue culture. *Hort. Sci.* 9: 175-180

Murray, M.G. and Thompson W.F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acid Res.* 8: 4321-4325

N'goran, J.A.K., Laurent, V., Risterucci, A.M. and Lanand, C. 1994. Comparative genetic diversity studies of *Theobroma cacao* L. using RFLP and RAPD markers. *Heredity* 73: 589-597

Nagaraju, V. and Parthasarathy, V.A. 1995. Effect of growth regulators on *in vitro* shoots of gladiolus hybrids. *Folia-Horticulturae* 7: 93-100

Nair, P.K.K. 1970. *Pollen Morphology of Angiosperms – A Historical and Phytogenetic Study.* Scholar Publishing House, Lucknow, pp. 67-73

Nathan, M.J., Goh, C.J. and Kumar, P. 1992. *In vitro* propagation of *Heliconia psittacorum* by bud culture. *HortScience* 27: 450-452

Nathan, M.J., Kumar, P.P. and Goh, C. 1993. High frequency plant regeneration in *Heliconia pstittacorum* L. *Pl. Sci.* 90: 63-71

Nayar, A. S. 2001. Molecular evaluation of genomic stability of banana plants developed by *in vitro* clonal propagation. M. Sc (Hort) thesis, Kerala Agricultural University, Thrissur 68p.

Newbury, H.J., Howell, E.C., Crouch, J.H. and Lloyd, F.B.V. 2000. Natural and Culture induced genetic variation in plantains. *Aust. J. Bot.* 48: 493-500

Ngo, A. and Hoult, S., 2000. *Growing Heliconias in NT, Australia- Cost and returns.* Agnote- Northern territory of Australia. 435: 11-12

Noli, E., Salvi, S. and Rosa, R. 1997. Comparative analysis of genetic relationships in barley based on RFLP and RAPD markers. *Genome* 40: 607-616

Okeyo, O.P. and Kako, S. 1998. Genetic diversity and identification of *Cymbidium* cultivars as measured by RAPD markers. *Euphytica* 99: 95-101

Oropeza, M., Guevara, P., Gareia, E.D., Ramirez, J.L. and De-Garcia, E. 1995. Identification of somaclonal variants of sugarcane resistant to

sugarcane mosaic virus via RAPD markers. *Pl. Mol. Biol. Rep.* 13: 182-191

Padmesh, P., Sabu, K.K., Seeni, S. and Pushpangadan, P. 1999. The use of RAPD in assessing genetic variability in *Andrographis paniculata,* a hepatoprotective drug. *Curr. Sci.* 76: 833-835

Panse, V. G. 1957. Genetics of quantitative characters in relation to plant breeding. *Indian J. Genet.* 17:310-328

Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers.* 4th Edition. Indian Council of Agricultural Research, New Delhi, 347 p.

Parani, M., Anana, A. and Parida, A. 1997. Application of RAPD fingerprinting in selection of micropropagated plants of *Piper longum* for conservation. *Curr. Sci.* 73: 81-83

Parent, J.G. and Page, D. 1998. Identification of raspberry cultivars by sequence characterized amplified region DNA analysis. *HortScience* 33: 140-142

Pathania, N. S. and Misra, R. L. 2001. Characterisation of gladiolus mutants using RAPD markers. *J. Ornam. Hort.* 4: 65-68.

Pathania, N.S., Misra, R.L. and Raghava, S.P.S. 2001. Precocious shoot proliferation and microsome production in gladiolus through tissue culture. *J. Ornam. Hort.* 4: 69-73

Paull, R. E. and Chantrachit,T. 2001. Benzyladenine and the vase life of tropical ornamentals. *Postharvest Biol. Technol.* 21: 303-310

Pedersen, C. and Brandt, K. 1992. A method for disinfection of underground rhizome tips of Alstroemeria and Heliconia. *Acta Hort.*325: 499-504

Peltier, D., Farcy, E., Dulieu, H. and Berville, A. 1994. Origin, distribution and mapping of RAPD markers from wild *Petunia* species in *Petunia hybrida. Theor. Appl. Genet.* 88: 637-645

Philipp, S., Banerjee, N.S. and Das, M.R. 2000. Genetic variation and micropropagation in varieties of *Piper longum* L. *Curr. Sci.* 78: 169-172

Piccioni, E., Barcaccia, G., Falcineth, M. and Standardi, A. 1997. Estimating alfa-alfa somaclonal variation in axillary branching propagation and indirect somatic embryogenesis by RAPD fingerprinting. *Int. J. Pl. Sci.* 158: 556-562

Pillai, P.N. 2003. Morpho-Anatomical and Molecular Characterization of *Dendrobium* Sw. cultivars. Ph.D. thesis, Kerala Agricultural University, Thrissur, 84 p.

Pillay, M., Nwakanma, D.C. and Tenkoyano, A. 2000. Identification of RAPD markers linked to A and B genome sequences in banana. *Genome* 43: 763-767

Prasad, R.N., Sharma, A.K. and Chaturvedi, H.C. 1993. *In vitro* rapid cloning of *Gladiolus*. *Proc. 26th A. Meeting Pl. Tiss. Cult. Ass. India Symp. Pl. Tiss. Cult. Biotech. Appl. Univ.*, Rajasthan, Jaipur, pp. 15-16

Priyakumari, I. 2001. *In vitro* clonal propagation of two promising *Gladiolus* varieties. MSc. Thesis. K. A. U. Thrissur. P 40-72

Rajamanickam, C. 2003. Random Amplified Polmorphic DNA (RAPD) analysis of banana (*Musa* spp.). Ph.D. thesis, Kerala Agricultural University, Thrissur, 229 p.

Ram, A.S. and Sreenath, H. L. 1999. A method for isolation of coffee DNA using rapd octamer and decamer primers. *J. Plantain. Crops* 27: 125-130

Ranamukhanachchi, D.G., Henny, R.J. and Guy, C.L. 2001. DNA fingerprinting to identify nine Anthurium pot plant cultivars and examine their genetic relationship. *HortScience* 36: 758-760

Rani, V., Parida, A. and Raina, S.N. 1995. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoids* M. *Pl. Cell Rep.* 14: 459-462

Rao, T.M., Negi, S.S. and Swancy, R.D. 1991. Micropropagation of gladiolus cv. Friendship. *Indian J. Hort.* 57: 360-365

Ratnaparkhe, M.B., Gupta, V.S. and Moorthy, V.M.R. 1995. Genetic fingerprinting of pigeon pea and its wild relatives using RAPD markers. *Theor. Appl. Genet.* 91: 893-894

Razdan, M.K. 1993. *An Introduction to Plant Tissue Culture*. Oxford and IBH Publishing Co. Pvt. Ltd., Calcutta, pp. 172-189

Renning, C.M., Schnell, R.J. and Gazit,F.R. 1995. Using RAPD markers to identify *Annona* cultivars. *J. Am. Soc. hort. Sci.* 120: 726-729

Riek, J. De, Dendauw, J., Leus, L., Loose, M. De, Bock Stacle, E. Van. 1999. Variety protection by use of molecular markers; some case studies on ornamentals. Development of integrated systems for large-scale propagation of elite plants using *in vitro* techniques. Contribution presented at the work group meeting, Ancona, Italy, October, 1999, Plant Breeding Abstracts, 1530 p.

Rival, A., Bertrand, L., Beule, T., Combes, M.C., Tronslot, P. and Lashermes, P. 1998. Suitability of RAPD analysis for the detection of somaclonal variants in oil palm. *Pl. Breed.* 117: 73-76

*Robinson, H.F., Comstock, R.E. and Harvey, P.H. 1965. Estimation of heritability and the degree of dominance in corn. *Agron. J.* 14 : 352-359

Rose, M.J. and Barthlott, W. 1995.Pollen-connecting threads in Heliconia (Heliconiaceae). *Pl. Syst. Evolution*195: 61-65

Rout, G.R., Das, P., Goel, S. and Raina, S.N. 1998. Determination of genetic stability of micropropagated plants of ginger using random amplified polymorphic DNA (RAPD) markers. *Bot. Bull. Acad. Sinica* 39: 23-27

*Ryuk, H.M., Ji-Yong, C., Jung, N., Insup, S. and Jong, S.K. 1999. Isozyme and randomly amplified polymorphic DNA (RAPD) analysis for genetic relationship among *Calanthe discolor, C. sieboldii* and *C. bicolor* native to Cheju Island. *J. Chinese Soc. Hort. Sci.*45-271-277

Samec, P. and Nasinec, V. 1996. The use of RAPD technique for the identification and classification of *Pisum sativum* L. genotypes. *Euphytica* 89: 229-234

Sanchez, I., Gaviria, D., Gallego, G., Fagardo, D., Valencia, J.A., Lobo, M., Thome, J., Roca, W. and Crane, J.H. 1998. Molecular characterization for the management of Columbian collection of Musaceae. *Proc. Intermer. Soc. trop. Hort.* 42: 252-259

Sangwan, R.S., Konan, N.K., Pawlicki, N. and Sangwan, N.B.S. 1995. Analysis of *in vitro* regenerated cassava plants using RAPD technology. *Bot. Bull. Acadamia Sinica* 39: 21-23

Santalla, M., Power, I.B. and Davey, M.R. 1998. Genetic diversity among mungbean germplasm revealed by RAPD markers. *Pl. Breed.* 117: 473-478

Sato. A.Y., Carvalho, N, Pinto, D. and Blant, M. 1999. Application of AMF to micropropagated heliconia and gerbera plants during acclimatization period. *Horticutlura Brasileira* 17: 25-28

Schenk, R. G. and Hilderbrandt, A. C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can. J. Bot.* 29:199-204

Schneider, S., Reustle, G. and Zyprian, E. 1996. Detection of somaclonal variation in grapevine regenerants from protoplasts by RAPD-PCR. *Vitis* 35: 99-100

Schnell, R.J., Ronnin, C.M. and Knight, R.J. Jr. 1995. Identification of cultivars and validation of genetic relationships in *Mangifera indica* using RAPD markers. *Theor. Appl. Genet.* 90: 269-274

Scott, M.C., Anolles, G.C. and Trigiano, R.N. 1996. DNA amplification fingerprinting identifies closely related *Chrysanthemum* cultivars. *J. Am. Soc. Hort. Sci.* 121: 1043-1044

Scovel, G., Ben-Meir, H., Ovadis, M. and Vain, S.A. 1998. RAPD and RFLP markers tightly linked to the locus controlling carnation (*Dianthus caryophyllus*) flower type. *Theor. Appl. Genet.* 96: 117-122

Sharma, R., Mohapatra, T., Mukherjee, A.K., Pal, K. and Sharma, O.P. 1999. Molecular markers for seed oil content in Indian mustard. *J. Pl. Biochem. Biotech.* 8: 99-102

Shen, T.M., Cowen, R.D. and Meyer, M.M. 1991. *In vitro* propagation of tuberose (*Potianthes tuberosa*). *HortScience* 26: 756-757

Shiau, Y.J., Hseu, S.H., Wang, T.Y. and Tsag, H.S. 1998. Studies on tissue culture *Heliconia psittacorum* Rhizomatosa. Identification and control of bacterial contamination of explants cultural *in vitro*. *J. agric. Res. China* 47: 346-363

Shiau, Y.J., Wang, T.Y. and Tsay, H.S. 1999. Studies on tissue culture of *Heliconia psittacorum* cv. Rhizomatosa. *J. agric. Res. China* 48: 42-51

Shibata, M., Kishimoto, S., Hirai, M., Aida, R., Ikeda, I., Considine, J.A. and Gibbs, H. 1998. Analysis of the perclinial chimeric structure of chrysamthemum sports by randomly amplified polymorphic DNA. *Acta Hort.* 454: 347-353

Shibu, M.P., Ravishankar, K.V., Anand, L., Ganeshaiah, K.N. and Shanker, R.U. 2000. Identification of specific DNA markers in the dioecious tree, nutmeg (*Myristica fragrans* H.). *Pl. Genet. Res. Newsl.* 12: 59-61

Shimizu,K., Miyabe,Y., Nagaike,H., Yabuya,T. and Adachi,T. 1999. Production of somatic hybrid plants between *Iris ensata* and *Iris germanica*. *Euphytica* 107: 105-113

Shoyama, Y., Zhu, X.X., Nakai, R. and Shiraishi, S. 1997. Micropropagation of *Panax ginseng* by somatic embryogenesis and RAPD analysis of regenerated plantlets. *Pl. Cell Rep.* 16: 450-453

Simi, S. 2001. Molecular characterization of banana (*Musa* AAB plantain subgroup) clones. M.Sc. thesis, Kerala Agricultural University, Thrissur, 69 p.

Smulders, M.J.M., Rus-Kortekaas, W. and Vosman, B. 2000. Microsatellite markers useful throughout the genus *Dianthus*. *Genome* 43: 208-210

Solivia, M., Gautsci, B., Salzmann, C., Tenzer, I. and Widmer, A. 2000. Isolation and characterization of microsatellite loci in the orchid *Ophrys araneola* (Orchidaceae) and a test of cross-species amplification. *Mol. Ecol.* 9: 2178-2179

Somri, S. 1998. Improvement of papaya (*Carica papaya* L.) for south eastern Queensland investigations on type and fruit quality. Ph.D thesis, University of Galton College, Queensland, p. 125

Stavrakakis, M.N., Biniari, K. and Hatzopoulose, P. 1997. Identification and discrimination of eight Greek group cultivars by random amplified polymorphic DNA markers. *Vitis* 36: 175-178

Steinitz, B., Cohen, A., Goldberg, Z. and Kochba, M. 1991. Precocious gladiolus corm formation in liquid shake cultures. *Pl. Cell Tiss. Org. Cult.* 26: 63-70

Stockinger, E.J., Mulinix, C.A., Long, C.M. and Lezzoni, A.I. 1996. A linkage map of sweet cherry based on RAPD analysis of a microspore derived callus culture population. *Euphytica* 106: 201-205

Subramanian, J., Litz, R.E. and Schnell, R.J. 1996. Selection and characterization of resistance in mango embryogenic cultures to *Colletotrichum gloeosporoides*. *HortScience* 31: 695-696

Sutton, J. 1978. The production of virus free *Gladiolus* using meristem culture. *Proc. Int. Hort. Cong.,* Sydney, Australia, pp. 349-352

Takatsu, I. 1982. A method of apical meristem culture for the production and practical use of virus free gladiolus corms. *Bull. Ibraki-Ken hort. Exp. Stn.* 1: 11-19

Takatsu,Y., Miyamoto,M., Inoue,E., Yamada,T. and Niwa, M.2001. Interspecific hybridization among wild Gladiolus sp. based on RAPD markers. *Scient. Hort.* 9: 3-4

Takemori, N., Shinoda, K. and Kadotani, N. 1994. RAPD markers for confirmation of somatic hybrids in the dihaploid breeding of potato (*Solanum tuberosum* L.). *Pl. Cell Rep.* 13: 367-371

Talukdar, M.C., Lalrinawmi, C. and Singh, S. 2002. *In vitro* propagation of heliconia. *J. Ornam. Hort.* 1: 45-46

Tantz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucl. Acid Res.* 17: 6463-6471

Tartarini, S. 1996. RAPD markers linked to the Vf gene scab resistance in apple. *Theor. Appl. Genet.* 92: 803-810

Tatineni, V., Cantrell, R.G. and Davis, D.D. 1996. Genetic diversity in elite cotton germplasm determined by morphological characteristics and RAPDs. *Crop Sci.* 36: 186-192

Thomas, A.S. 1996. Micropropagation in selected varieties of *Anthurium andreanum* Lind. M.Sc. Thesis, Kerala Agricultural University, Thrissur, 100 p.

Timothy, C. 1996. Heliconia book: A guide to selection and cultivation. http://www.agrotropicalandes.com/heliconia-humilis

Tom, W. 1997. Stokes tropical : A source for heliconia plants. http: // www. heliconia - amazon.com / heliconia- gen- des

Torandore, N., Bonomi, M., Marcurci, R., Barcaccia, G., Parrini, P. and Lucchin, M. 2003. Morphological, karyological and molecular analysis in *Aeter* spp. (Asteraceae). *Israel J. Plant Sci.* 51 (2): 109-116

Torres, A.M., Millan, T. and Cubero, J.I. 1993. Identifying rose cultivars using random amplified polymorphic DNA markers. *HortScience* 28: 323-334

Triplett, J. K.and Kirchoff, B. K. 1991. Lamina architecture and anatomy in the Heliconiaceae and Musaceae. *Can. J. Bot.* 69: 887-900

*Truksa, M. and Prochazka, S. 1996. Potential use of RAPD markers in verification of cucumber hybrids. *Rostlinna Vyroba* 42: 241-244

Vainstein, A. and Ben-Meir, H. 1994. DNA fingerprinting analysis of roses. *J. Am. Soc. Hort. Sci.* 199: 1099-1103

Valles, M.P., Wang, Z.Y., Montaron, P., Potrykus, I. and Spangenberg, G. 1993. Analysis of genetic stability of plants regenerated from suspension culture and protoplasts of meadow fescue (*Festuca patensis* H.). *Pl. Cell Rep.* 12: 101-106

Varghese, Y.A., Kanaak, C., Sethuraj, M.R. and Ecke, W. 1997. Evaluation of random amplified polymorphic DNA (RAPD) markers in *Hevea brasiliensis*. *Pl. Breed.* 116: 47-52

Verma, S.K., Khanna, V. and Singh, N. 1999. Random amplified polymorphic DNA analysis of Indian scented basmati rice, germplasm for identification of variability and duplicate accessions, if any. *Electrophoresis* 20: 1786-1789

Vidal, M.C. and Garcia, E. 2000. Analysis of a *Musa* species. Somaclonal variant resistant to yellow Sigatoka. *Pl. Mol. Biol. Rep.* 18: 23-31

Virk, P.S., Ford, L.B.V., Jackson, M.T. and Newburg, H.J. 1995. Use of RAPD for the study of diversity within plant germplasm collections. *Heredity* 74: 170-179

Vornam, B. and Gebhardt, K. 2000. PCR based markers reveal genetic identity and diversity in subset collections of wild and cultivated apple. *Acta Hort.* 530: 463-467

Vos, P., Hogers, R., Blecker, M., Lee, V.T., Pot, J. and Kliper, M. 1995. AFLP : A new technique for DNA fingerprinting. *Nucl. Acid Res.* 3: 4407-4417

Walker, C.A. and Werner, D.J. 1997. Isozyme and randomly amplified polymorhic DNA (RAPD) analysis of Cherokee rose and its putative hybrids, 'Silver Moon' and 'Anemone'. *J. Am. Soc. Hort. Sci.* 122: 659-664

Walther, R., Han, A., Lerer, A., Durdevani, A., Khayat, E. and Altman, A. 1997. Analysis of somaclonal variation in tissue cultural banana plants. *Acta Hort.* 447: 379-383

Walton, M. 1990. Application of RFLP technology to applied plant breeding. *Proc. hort. Biotech. Symp., 2-9, 1990* (eds. Benett, A.B. and Neil, S.D.O.). University of California, Davis, Wiley, Liss, New York, pp. 99-102

Wang Y.L.Y., Zhang, S. and Cheng, C.T. 2003. Phylogenetic relationship among several sub genus Yulania species based on RAPD markers. *Acta Horticulturae Sinica* 30 (3): 299-302

Wang, G., Castiglione, S., Zhang, J., Fu, R., Ma, J., Li, W.S.Y. and Sala, F. 1994. Hybrid rice (*Oryza sativa* L.). Identification and parentage determination by RAPD finger printing. *Pl. Cell Rep.* 14: 112-115

Wang, J.Y., Chuang, K.C. and Ming, J. 2001. Comparative analysis of genetic similarity among anthurium cultivars by using ISSR and RAPD markers. *J. agric. Res. China* 50: 54-67

Wang, J.Y., Chuang, K.C. and Rim, C.K. 1999. Randomly amplified polymorphic DNA (RAPD) markers for identification and genetic diversity analysis of Anthurium cultivars. *J. agric. Res. China* 48: 52-63

Wang, Y. Chen, J., Lu,.J. Lmikarna, O. 1998. RAPD analysis of *Vitis* sp. and Florida bunch grapes. *Scient. Hort.* 82: 85-94

Watson, L. and Dallwitz, M.J. 1991. The families of flowering plants: Description, illustrations and identifications. http: // www. biodiversity. uno.edu-delta- heliconia

Waugh, R. and Powel, W. 1992. Using RAPD markers for crop improvement. *Trends Biotech.* 10: 186-191

Weber, J.L. and May, P.E. 1989. Abundant class of human polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Human Genet.* 44: 388-396

Weising, K., Nybom, H., Wolff, K. and Meyer, W. 1995. *DNA Fingerprinting in Plants and Fungi.* CRC Press, Boca Raton, Florida, p. 1-3

Welsh, J. and Mc Clelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids Res.* 18: 7213-7218

Wen, C.S. and Hsiano, J.Y. 1999. A study on the taxonomic relationship of *Lilium longiflorum* Thunb. var. formosanum Baker based on

isozyme, RAPD and morphological characters. *J. Chinese Soc. Hort. Sci.* 45: 293-302

Wen, X., Ming, P.X, and Xin, D.X. 2003. Genetic diversity in wild accessions of *Rosa roxburghii* Tratt from four provinces as revealed by RAPD analysis. *Scientia Agricultura Sinica* 36 (7): 823-828

White, P. R. 1934. Potentially unlimited growth of excised tomato root tips in liquid medium. *Physiol. Pl.* 9: 585-600

Wickson, M.E. and Thimann, K.V. 1958. The antagonism of auxin and kinetin in apical dominance. *Physiol. Pl.* 11: 62-74

Wilde, J., Waugh, R. and Powell, W. 1992. Genetic finger printing of *Theobroma* clones using randomly amplified polymorphic DNA markers. *Theor. Appl. Genet.* 83: 871-877

Williams, J.G.K., Kubelit, A.R., Livat, R.J., Rafalski, J.A. and Tingsey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531-6535

Wing, R.A., Zhang, H.B. and Tanksley, S.D. 1994. Map based cloning in crop plants. Tomato as a model system. Genetic and physical mapping of jointless. *Mol. Gen. Genet.* 242: 681-688

Wolff, K. and Van-Riju, J.P. 1993. Rapid detection of genetic variability in chrysanthemum using random primers. *Heredity* 71: 335-341

Wolff, K., Zietkiewicz, E. and Hofstra, H. 1995. Identification of *Chrysanthemum* cultivars and stability of DNA fingerprint patterns. *Theor. Appl. Genet.* 91: 439-447

Wolff, K.,Van-Riju, J.P.and Illofstva, I. 1994. RFLP analysis in chrysanthemum. *Theor. Appl. Genet.* 88: 472-478

Wroblewska, A., Brzosko, E., Czernecka, B. and Nowosielski, J. 2003. High levels of genetic diversity in populations of *Iris aphylla* L. on endangered species in Poland. *Bot. J. Linnean Soc.* 142: 65-72

Xu, Y., Chark, M.S. and Pehu, E. 1993. Use of RAPD markers to screen somatic hybrids between *Solanum tuberosum* and *S. brevidens*. *Pl. Cell Rep.* 12: 107-109

Yang, H., Tabei, Y., Kamada, H., Kayano, T. and Takaiwa, T. 1999. Detection of somaclonal variation in cultural rice cells using diagoxygenin based random amplified polymorphic DNA. *Pl. Cell Rep.* 18: 520-526

Yu, L.X. and Nguyen, H.T. 1994. Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). *Theor. Appl. Genet.* 87: 668-672

Zhang, D., Germain, E., Aloisi, S.R. and Gandelin, M.H. 2000. Development of amplified fragment length polymorphism markers for variety identification in rose. *Acta Hort.* 508: 113-119

Zhuravlev, V.N., Kozyrenko, M.M., Artyukova, E.V., Reunova, G.D. and Hyshko, M.V. 1998.Genetic studies in Iris. *Genetika-Moskva* 34: 368-372

Zimmer, K.and Carow, B.1977. Possibilities of partial vacuum storage. Grundlagen und Moglichkeiten der "Unterdrucklagerung". *Deutscher Gartenbau* 31: 87-89 (Translated from the French).

Zirkle, C. 1937. Acetocarmine Mounting Media. *Science* 85: 528

Ziv, M. 1979. Transplanting Gladiolus plants propagated *in vitro*. *S. Indian Hort.* 11 : 257-260

*Original not seen

Appendices

APPENDIX – I

Composition of MS, SH and White basal media

Nutrients	Quantity (mg l^{-1})		
	MS	SH	White
Macronutrients			
NH_4NO_3	1650.00	-	-
$(NH_4)_2SO_4$	-	-	-
$NH_4H_2PO_4$	-	300.00	-
KCl	-	-	65.00
KNO_3	1900.00	2500.00	80.00
KH_2PO_4	170.00	-	-
$MgSO_4.7H_2O$	370.00	400.00	720.00
$NaH_2PO_4.H_2O$	-	-	16.50
Na_2SO_4	-	-	200.00
$CaCl_2.2H_2O$	440.00	200.00	-
$Ca(NO_3)_2 4H_2O$	-	-	300.00
Micronutrients			
H_3BO_3	6.20	5.00	1.50
$MnSO_4.4H_2O$	22.30	13.20	7.00
$ZnSO_4.7H_2O$	8.60	1.00	3.00
KI	0.83	1.00	0.75
$Na_2MoO_4.2H_2O$	0.25	0.10	-
$CuSO_4.5H_2O$	0.025	0.20	-
$CoCl_2.6H_2O$	0.025	0.10	-
$FeSO_4.7H_2O$	27.85	15.00	-
$Fe_2(SO_4)_3$	-	-	2.50
$Na_2EDTA.2H_2O$	37.25	20.00	-

APPENDIX – I Continued

Nutrients	Quantity (mg l^{-1})		
	MS	SH	White
Vitamins			
Thiamine, HCl	0.10	5.00	0.10
Pyridoxine, HCl	0.50	0.50	0.10
Nicotinic acid	0.50	5.00	0.50
Amino acid			
Glycine	2.00	-	3.00
Others			
Inositol	100.00	1000.00	-
Sucrose *	30.00	30.00	30.00
Agar *	8.00	8.00	8.00

EVALUATION, MOLECULAR CHARACTERISATION AND IN *VITRO* PROPAGATION OF HELICONIAS

SMITHA BABU

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY IN HORTICULTURE

Faculty of Agriculture
Kerala Agricultural University, Thrissur

2005

DEPARTMENT OF POMOLOGY AND FLORICULTURE
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM - 695 522

ABSTRACT

Heliconias are newly introduced cut flowers in Kerala, which is an ideal choice for commercial cultivation as intercrops in coconut gardens. Objectives of the study were to evaluate *Heliconia* species and varieties as cut flowers, undertake molecular characterization of *Heliconia* species/ varieties using RAPD (Random amplified polymorphic DNA) markers and to standardise *in vitro* propagation procedures in *H. psittacorum* cv. Deep Orange.

There was wide variation in the morphological and inflorescence characters such as plant height, number of leaves, leaf area, number of shoots, days taken from planting to flowering, flowering duration, number of flowering shoots, length of petiole, length of spike, number of bracts, size of bracts and vase life.

The less robust *H. psittacorum* varieties recorded lowest plant height, highest number of shoots and inflorescence of lesser dimension. They were earlier in flowering, with more number of flowering shoots. The robust varieties such as Pedro Ortiz, Wagneriana Red and Wagneriana Yellow recorded higher plant height, lower number of shoots and inflorescence of higher dimension. They were late bloomers with lower number of flowering shoots.

Highest pollen size was recorded in Wagneriana Yellow (360 μ) and highest pollen fertility was recorded in *H. latispatha*.

The variability was high both at genotypic and phenotypic level (GCV and PCV) for most of the vegetative and inflorescence characters studied. PCV values were slightly higher than GCV values for all the characters. All the characters showed high heritability and genetic advance except number of leaves. Bract size had significant positive correlation both at genotypic and phenotypic levels with plant height, leaf area, number of bracts, spike length, days to flowering and vase life. Number of flowering shoots had significant positive correlation both at genotypic and phenotypic levels with number of shoots and petiole length.

Petra Orange recorded highest net returns and cost : benefit ratio (Rs. 6,28,900 and 1.54, respectively). Lower net returns and cost : benefit ratio was recorded in Wagneriana Yellow (Rs. 25,000 and 1.02, respectively).

The 12 species and varieties of *Heliconia* were distinctly differentiated by the eight primers *viz.,* OPA-01, OPA-14, OPA-17, OPA-18, OPB- 07, OPB-12, OPB-18 and OPB-20. Jaccard's similarity coefficient value ranged from 0.083 to 0.556. The least similarity coefficient (0.083) values were those of Golden Torch and *Heliconia humilis*. The highest value for similarity index (0.556) was obtained for Deep Orange and Petra Orange. UPGMA based dendrogram constructed using similarity matrix data grouped the 12 species and varieties into five cluster. Each cluster consisted of similar morphotypes except that of *H. latispatha* and De Rooij

Shoot apex, bits of rhizome, leaf segments and root segments were used for standardising the explant. Shoot apex was found to be the most effective. Highest survival percentage (45) was observed in the treatment with absolute alcohol one minute wash + mercuric chloride (0.10 %) for 10 minutes. For culture establishment, treatment with BA and its various combination with auxin and gibberellins yielded early bud initiation. For culture establishment, among the various media studied (MS, SH and White), MS medium was standardized for further studies since it had recorded early bud initiation. For the induction of axillary buds the treatment combination with SP_9 [MS + BA (2.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})], SP_{39} [MS + 2ip (2.0 mg l^{-1}) + IAA (0.2 mg l^{-1})] and SP_{45} [(MS +2ip (3.5 mg l^{-1}) + IAA (0.2 mg l^{-1}) + GA_3 (2.5 mg l^{-1})] was considered as the best treatment since it produced the highest number of shoots. For *in vitro* rooting minimum number of days (25) was observed in treatment R_{11} where MS medium was supplemented with IAA (1.0 mg l^{-1}) + NAA (0.5 mg l^{-1}). Similarly this treatment recorded highest number of roots (12.00).